MANAGEMENT OF ENDOCRINE DISEASE

The role of confirmatory tests in the diagnosis of primary aldosteronism

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Abstract

The strategy for diagnosis of primary aldosteronism (PA) in the hypertensive population includes firstly a screening step, based on the measurement of plasma aldosterone-to-renin ratio (ARR), a test which must have high sensitivity, and secondly a confirmatory step based on the demonstration of excessive aldosterone production independent of the renin-angiotensin-aldosterone system (RAAS) activity. The high proportion of false-positive ARR results and conversely of actual PA without a persistent elevation in baseline plasma aldosterone concentration necessitates the addition of a confirmatory step in the work-up of PA diagnosis. The present review focuses on the description of the different dynamic tests available for demonstrating autonomy of aldosterone secretion, on the performance and limitations of confirmatory tests and on possible strategies for PA diagnosis which may either include or avoid the confirmatory step for PA diagnosis. Large prospective studies comparing different strategies with and without dynamic testing are mandatory to delineate clearly the role and limits of confirmatory tests in the work-up of PA.

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Introduction

Primary hyperaldosteronism (PA) is an autonomous disease characterized by an excess of aldosterone production leading to arterial hypertension and hypokalemia driven by high potassium urinary excretion. The prevalence of PA ranges from 5 to 10% of patients

with arterial hypertension, 13% in stage 3 and 17–23% in resistant hypertension (1, 2, 3). A recent prospective study has confirmed a 5.9% prevalence of PA in 1672 unselected hypertensive patients from primary care centers (4). The diagnosis of PA is of paramount importance due to the

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increased associated morbidities, including altered renal function, atrial fibrillation, stroke, myocardial infarction and lastly cardiovascular mortality, in PA patients when compared to patients with essential hypertension (5, 6, 7, 8). PA diagnosis should be raised in a number of conditions including: grade 2 and 3 hypertension, resistant hypertension, hypokalemia-associated hypertension, hypertension with obstructive sleep apnea, adrenal incidentaloma in hypertensive subjects, hypertension in subjects under 40 years of age and subjects with a family history of first-degree PA (3, 9). Investigation of PA should include a screening step based on the measurement of plasma renin activity (PRA) or direct renin concentration (DRC), assays for aldosterone and the calculation of the aldosterone-to-renin ratio (ARR). These assay results are effective in screening for PA with a sensitivity of 68–94% (2, 10, 11, 12). However, retrospective analyses suggest that 30–50% patients with an elevated ARR at screening may indeed correspond to false-positive PA. Such lack of specificity of the screening step for PA diagnosis has led expert centers to perform dynamic testing for confirmation of the PA diagnosis.

This review will examine the rationale for including a confirmatory step in the work-up of PA after the initial ARR screening step. Thereafter, the methodological conditions and a description of the different confirmatory tests will be outlined and the comparative performance of confirmatory tests will be reviewed. Lastly, the question of whether, and under what conditions, confirmatory tests should be avoided in the work-up for diagnosing PA will also be discussed.

Rationale for performing or bypassing dynamic tests for confirmation of PA

Screening tests for PA diagnosis need to be highly sensitive to enable diagnosis of PA at a high rate. As a consequence, such a strategy also produces a percentage of false positives at this screening step. False-positive cases then need to be identified and excluded in order to avoid unnecessary invasive procedures and/or adrenal surgery. Confirmatory tests have been developed to demonstrate a non-suppressible aldosterone secretion using maneuvers that are designed to bring about suppression of circulating renin. Such confirmatory tests should principally exhibit a high negative predictive value so that false positives selected by ARR screening can be eliminated. Indeed about 40% of patients with a positive ARR screening test actually display a plasma aldosterone concentration that

is adequately suppressed by an inhibitory procedure, possibly corresponding to false-positive PA detection by the ARR or alternatively to actual angiotensin II-responsive PA (10). ARR may be elevated due to non-specific low renin levels in spite of no elevation in aldosterone levels, leading to a false-positive PA screening. Conversely, sodium restriction, which is recommended to hypertensive patients, may falsely raise renin levels and therefore normalize ARR due to the responsiveness of many PA to salt restriction, therefore leading to false interpretation of PA screening. This has been demonstrated in a recent study showing a 52% rate of false-negative PA screening in patients with confirmed PA, who were on a low sodium diet at the time of the screening test (13). A retrospective evaluation of the ARR profile in 71 patients having repeated screening tests, in outpatient and then inpatient conditions, showed that 31% of patients with PA, due to a unilateral aldosteronoma, exhibited at least one false-negative ARR value on repeated screening (14). The introduction of an elevated baseline aldosterone level as an additional criterion to ARR for positive screening may lead to a false-negative screening rate of between 36 and 43% (10).

In opposition, the assumption that in PA, aldosterone secretion is autonomous from angiotensin II secretion, which is the rationale basis of dynamic tests, has never been demonstrated in studies using a gold standard reference for the definitive diagnosis of aldosterone-producing adenoma. Moreover, a large proportion of PA remains dependent on angiotensin II as firstly observed by Streten *et al.* (15) and then by others (16, 17, 18, 19). The commitment of performing a confirmatory test systematically involves the risk of failing to catch PA which is responsive to angiotensin II, and to increase the complexity of the diagnostic work-up and finally its costs. Recently, an extensive analysis performed in two large dataset of prospectively collected patients with the same predefined protocol for the diagnosis assessment of aldosterone-producing adenoma has shown that a frank elevation of the ARR may allow to avoid dynamic testing (20). These important data give strength to the assumption that confirmatory tests are not mandatory for all patients in the work-up for PA diagnosis.

Conditions for performing a confirmatory test in the work-up for PA diagnosis

Similar to the screening step for PA diagnosis by ARR, the confirmatory step using a dynamic test of aldosterone

Figure 1

Regulation of the renin-angiotensin-aldosterone system and interaction of antihypertensive drugs.

secretion requires standardized conditions in order to ensure optimal reproducibility and to limit functional imbalance of the RAAS induced by any exogenous influence. Most antihypertensive drugs have an influence on the RAAS and should be withdrawn before static or dynamic measurements of aldosterone and renin concentrations are performed (Fig. 1). Mineralocorticoid antagonists should be withdrawn for at least 4 weeks while other antihypertensive drugs should be withdrawn for at least 2 weeks. These include β -blockers which decrease renin secretion, diuretics which increase aldosterone and renin concentrations by decreasing blood volume, angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists which stimulate renin secretion by suppressing angiotensin II negative feedback on renal juxta-glomerular cells (Fig. 1). In situations where antihypertensive drug withdrawal is not possible for reasons of safety, drugs should be maintained and the cutoff levels for plasma aldosterone concentration should be redefined (21). Products containing liquorice and non-steroidal anti-inflammatory drugs should also be withdrawn at least 3 weeks prior to the test, and estrogen-progestin compounds should ideally be withdrawn for 6 weeks. It is recommended that during the 3 days preceding the confirmatory test, patients consume a normal sodium diet which would be reflected by urinary sodium levels of 100–200 mmol/L and also receive potassium supplementation in order to correct prior hypokalemia and to thus ensure a normal serum potassium concentration on the day of the test. Aldosterone measurement in dynamic testing should be performed under standardized conditions, i.e. more than

2h after waking and then being in an upright position but in a seated position for the 15–60 min immediately prior to the test (3, 12). Several conditions may affect the level of circulating renin and therefore the ARR, including renal impairment which decreases renin and cardiac impairment which increases renin concentrations (3, 12).

Description and interpretation of the current confirmatory tests

The four common confirmatory tests include the Fludrocortisone Suppression Test, which is considered which is considered the most effective for complete RAAS suppression, the Saline Infusion Test which is the most widely used test, the Captopril Challenge Test, which is simple and innocuous, and the Oral Sodium Loading Test which is one of the tests used historically but is at present rarely used in expert centers. Other tests including the Losartan Suppression Test, the Furosemide Upright Test and the Captopril/Valsartan/Dexamethasone Combination Test have also been described. The procedures and interpretation of confirmatory tests are described in Table 1.

Fludrocortisone suppression test (FST)

The FST is regarded by some authors as the most effective confirmatory test for the demonstration of angiotensin II independency of aldosterone secretion, but demands intensive work-up, must be done as an inpatient procedure, and includes special dietary and posture requirements and monitoring of blood pressure and plasma potassium levels (22). The test consists of the oral administration of 0.1 mg fludrocortisone every 6h over 4 days, with slow-release potassium chloride supplementation every 6h in order to maintain plasma potassium level, which is checked every 6h, as close as possible to 4 mmol/L and of controlled sodium intake of 6g/day, in order to maintain 24-h urine sodium content, from day 3 to day 4, greater than 200 mEq/day. An alternative procedure named Fludrocortisone Dexamethasone Suppression test (FDST) includes the administration of 1 mg dexamethasone on the last day of the FST in order to eliminate any stimulatory ACTH effect on aldosterone secretion (23). Plasma sampling on day 4 may include assays for plasma cortisol at 07:00 and 10:00h, and plasma aldosterone and DRC at 10:00h (3, 22, 24). The test is considered to be valid when DRC is below 8.4 mU/L on day 4 and plasma cortisol level is lower at 10:00h than at 07:00h, thereby

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Table 1 Procedures and interpretation of confirmatory tests.

Test	Procedure	Interpretation	Concerns
Fludrocortisone suppression test (FST) (3, 15, 16, 17)	Oral Intake of 0.1 mg/6 h fludrocortisone during 4 days, potassium chloride and sodium chloride (6 g/day) Blood sampling for plasma potassium/6-h (kalemia >4 mmol/L) and 24-h urinary sodium from day 3 to day 4 (>200 mEq/24 h) On day 4, plasma cortisol at 07:00 h and PAC, PRA and plasma cortisol are at 10:00 h	PAC >6 ng/dL (170 pmol/L) confirms PA Test validity if PRA <1 ng/mL/h and plasma cortisol 10:00 h < cortisol 07:00 h (3, 16)	4-day hospitalization, monitoring of kalemia/6 h
Saline infusion test (SIT) (3, 16, 17, 18)	Performed between 08:00 and 09:00 h, in supine* position for at least 1 h before and during the test. Infusion of 2 L of 0.9% saline over 4 h. Blood sampling for PAC and PRA or DRC at T0 and T + 4 h *Alternatively in seated position for at least 30 min before and during the test	At T + 4 h, PAC <5 ng/dL (140 pmol/L) excludes PA, PAC >10 ng/dL (280 pmol/L) confirms PA, 5 ng/dL < PAC < 10 ng/dL is a 'gray zone' (3, 16) PAC >6.8 ng/dL (190 pmol/L) proposed as the most accurate threshold (19, 20, 21, 22) In the seated test, PAC >6 ng/dL (170 pmol/L) confirms PA (18)	Hourly BP monitoring is recommended
Oral sodium loading test (OSLT) (3, 16, 17, 23, 24)	Oral intake of 6 g/day sodium chloride for 3 days and potassium chloride to correct hypokalemia 24-h urinary sodium (urinary sodium >200 mEq/24 h) and urinary aldosterone sampling from day 3 at 08:00 h until day 4 at 08:00 h	Urinary aldosterone concentration >12 or 14 µg/24 h (33 or 39 nmol/24 h) confirms PA, urinary aldosterone concentration <10 µg/24 h (28 nmol/24 h) excludes PA (16, 23, 24)	Contraindicated if severe hypertension, kidney failure, cardiac arrhythmia, severe hypokalemia
Captopril challenge test (CCT) (3, 16, 17)	Oral intake of 25–50 mg captopril after 1 h in seated position Blood sampling for PAC, PRA or DRC and plasma cortisol at T0 and T + 2 h	At T + 2-h PAC decrease <30% and suppressed PRA confirms PA (3, 16) PAC cutoff thresholds between 8.5 and 13.9 ng/dL (246–390 pmol/L) were proposed (27, 28, 29) An ARR >25 or 35 ng/dL per ng/mL/h confirms PA (39, 28)	No contraindication. Monitor BP if angioedema and renovascular hypertension
Losartan test (30, 31).	Oral intake of 50 mg losartan after 1 h in seated position Blood sampling for PAC, PRA at T0 and T + 2 h and T + 4-h Wu <i>et al.</i> (2009, Japan guidelines)	An ARR >25 or 35 ng/dL per ng/mL/h confirms PA (39, 28)	
Furosemide upright test (FUT) (29, 46, 50)	After 30 min in supine position, intravenous injection of 40 mg furosemide in upright position maintained for 2-h Blood sampling for PRA at T0, T + 1 h and T + 2 h	PRA <2 ng/mL/h at T + 1 h and T + 2 h confirm PA (29, 46)	Contraindicated if advanced atherosclerosis at risk for cerebrovascular events, or arrhythmia
Dexamethasone-Captopril-Valsartan test (DCVT) (33)	Oral intake of 2 mg dexamethasone plus 50 mg captopril plus 320 mg valsartan at 12:00 h, and 50 mg captopril the next morning at 07:30 h Blood sampling for PAC, DRC and cortisol the next morning at 08:30 h	ARR >0.3 ng/dL/µU/mL (9 pmol/IU) and PAC >3.1 ng/dL (85 pmol/L) confirms PA (33)	Monitor plasma potassium level Contraindicated if severe kidney failure (Cl _{creat} <30 mL/min)

excluding an inadvertent rise in ACTH which may prevent aldosterone suppression. In these strict conditions, failure of standing 10:00 h plasma aldosterone to be suppressed to less than 6 ng/dL (165 pmol/L) confirms the diagnosis of PA (3, 22, 24).

Saline infusion test (SIT)

The SIT is widely used internationally and consists of a sodium load delivered by acute administration of a venous infusion of saline in order to suppress RAAS activity and ultimately suppress aldosterone secretion. To perform the test, the patient should remain in a supine, or alternatively seated, position for 30–60 min before and during the test. Commencing between 08:00 and 09:00 h, 2 L of 0.9% saline is infused over 4 h and blood samples are drawn at time 0 and 4 h for plasma aldosterone and renin measurements (3, 25, 26, 27). Hourly monitoring of arterial blood pressure is recommended during the test (3). At the 4-h time point, a plasma aldosterone level, measured in the supine position, of less than 5 ng/dL (140 pmol/L) makes the diagnosis of PA unlikely, whereas a level greater than 10 ng/dL (280 pmol/L) confirms PA. Aldosterone levels between 5 and 10 ng/dL (140–280 pmol/L) are considered to be in the ‘gray zone’ and therefore inconclusive (3, 24). However, the diagnostic cutoff has been the subject of extensive discussion by expert teams, and a cutoff of 6.8 ng/dL (190 pmol/L) provides the best diagnostic performance for several authors (19, 27, 28, 29, 30) including the PAPY study which is the sole study using a multiple criteria diagnosis of aldosterone-producing adenoma (29). Researchers from Brisbane, Australia, have proposed a cutoff for plasma aldosterone of 6 ng/dL (165 pmol/L), after a SIT performed in seated posture, for confirmation of PA (26).

Oral sodium loading test (OSLT)

The OSLT consists of the assessment of urinary aldosterone concentration following oral salt loading to diagnose PA. The test can be performed in an outpatient setting except in those patients at risk of eventual adverse side effects (see below). The test consists of the oral administration of 6 g sodium chloride/day for 3 days, in order to achieve a 24-h urine sodium excretion of greater than 200 mmol/day. Adequate potassium chloride supplementation is given to maintain plasma potassium level above 4 mmol/L. Urine sodium and aldosterone measurements are performed on the third day of the test and a 24-h urinary aldosterone concentration greater than 12 or 14 µg/24 h (33 or 39 nmol)

is regarded as diagnostic for PA, whereas a level below 10 µg/24 h (28 nmol) excludes PA (31, 32).

Captopril challenge test (CCT)

The CCT consists of the acute blockade of the RAAS by the angiotensin-converting enzyme inhibitor captopril. Patients receive 25–50 mg captopril orally after sitting for at least 1 h with blood samples drawn at time zero and after 2 h for PRA/DRC, plasma aldosterone and plasma cortisol measurements (33, 34). Plasma aldosterone is normally suppressed by more than 30% but remains elevated in PA, with cutoff values ranging from 8.5 ng/dL (246 pmol/L) to 13.9 ng/dL (390 pmol/L) reported by different expert teams (35, 36, 37). Another criterion of PA includes a post-captopril plasma aldosterone-to-PRA (pg/mL per ng/mL/h) greater than 200 or plasma aldosterone-to-DRC above 40 (38).

Losartan test (LT)

Blockade of the RAAS has also been proposed for the diagnosis of PA, using the angiotensin receptor antagonist losartan. Patients receive 50 mg losartan after sitting for at least 1 h with blood samples taken at time zero and 2 h or 4 h later for PRA and plasma aldosterone measurements. A plasma aldosterone-to-PRA ratio (ng/dL per ng/mL/h) greater than 25 or 35 provides better accuracy for PA diagnosis (28, 38, 39).

Furosemide upright test (FUT)

The FUT is based on the ability of an acute infusion of furosemide to physiologically enhance PRA, a response that is lacking in the case of PA. After 30 min in the supine position, patients receive an *i.v.* bolus of 40 mg furosemide and blood samples are drawn after 2-h upright posture for PRA measurement. A PRA value below 2 ng/mL/h is diagnostic for PA (39, 40).

Dexamethasone-Captopril-Valsartan Test (DCVT)

The rationale of the DCVT is to pharmacologically eliminate aldosterone-stimulating factors including the RAAS and ACTH by a combination of an angiotensin-converting enzyme inhibitor, angiotensin receptor antagonist and a glucocorticoid. A default response to DCVT is diagnostic for PA. Patients receive orally a combination of 2 mg Dexamethasone, 50 mg Captopril and 320 mg Valsartan at 12:00 h, followed by 50 mg

of Captopril the next morning at 07:30h, with blood samples taken at 08:30h for plasma aldosterone, DRC and plasma cortisol measurements. A plasma aldosterone-to-DRC greater than 0.3ng/dL/ μ U/mL (9pmol/L/ μ U/mL) and a plasma aldosterone concentration above 3.1ng/dL (85pmol/L) are diagnostic for PA. Monitoring of plasma potassium is advisable due to the demonstration of possible plasma potassium increase of 0.4mmol/L in hypertensive subjects without PA (41).

Side effects and precautions associated with confirmatory tests

Confirmatory tests based on sodium overload for RAAS suppression (i.e. SIT, OSLT and FST) share the same side effects and require the same precautions to be taken during their administration. The FST is burdensome and requires hospital admission and both sodium chloride and potassium chloride monitoring. The FST may actually induce profound hypokalemia and cardiac arrhythmia and therefore requires close monitoring of potassium levels at 6-hourly intervals for the duration of the test. The SIT should be performed on an inpatient basis since it may increase arterial blood pressure and even trigger acute pulmonary edema. The OSLT should be performed on an outpatient basis, though it is sometimes performed as an inpatient (38) or should possibly be avoided particularly in cases of heart failure or serious cardiac arrhythmia (3, 25). The SIT and the OSLT share the same contraindications, i.e. severe uncontrolled hypertension, kidney failure and profound uncorrected hypokalemia (3, 25). The FUT is contraindicated in subjects with advanced atherosclerosis at high risk of cerebrovascular events and in those at risk of diuretic-induced arrhythmia (38). It may also evoke orthostatic hypotension and worsen pre-existing hypokalemia. Conversely, the CCT is almost devoid of side effects and may be performed in subjects with severe hypertension or heart failure (3, 25). Careful monitoring of blood pressure is recommended in the case of renovascular hypertension or angioedema due to possible captopril-induced excessive decrease in blood pressure (38). The DCVT is contraindicated in severe kidney failure where creatinine clearance is less than 30mL/min (41).

Evaluation of performance of confirmatory tests for the diagnosis of PA

Four testing procedures, namely FST, SIT, OSLT and CCT, are commonly used, while the FUT is mostly used in Japan. Few studies have challenged the performances of these

tests, with most of these being retrospective and including small series of hypertensive subjects with suspected PA. In most studies, evaluation of a confirmatory test was based either on comparison with another dynamic test, one of which was arbitrarily considered as the reference test. Alternatively, the diagnosis of PA relied on either one or several criteria including adrenal imaging, adrenal scintigraphy, adrenal venous sampling (AVS) or the clinical response to adrenal surgery. Most studies did not fulfill the STARD Statement for Reporting Diagnostic Accuracy Studies according to which diagnostic tests should be evaluated against a clinical reference standard (42). Most studies consisted of face-to-face comparison of two distinct tests or more rarely, comparison of three tests. Threshold values of post-test aldosterone concentrations or ARR that were used were also heterogeneous and defined by each study investigator. In view of all of these limitations, there is actually no evidence favoring one test above others for confirmation of PA. The performances of the different confirmatory tests are described in Table 2.

Fludrocortisone suppression test (FST)

The FST has been considered by several authors as the reference test for the confirmatory step of PA diagnosis (43, 44). Gordon and Stowasser, in 1999, reported the superiority of the FST in a series of 97 patients positive using FST while only 17/97 were positive when tested with the SIT (44). Mulatero *et al.* also compared both tests, in a series of 100 hypertensive patients, and found that the SIT failed to detect 12% of PA patients who tested positive with the FST (11). In a recent pilot study, Ahmed *et al.* found that the FST was superior to the SIT when the latter was performed in the classic recumbent position, with only 33% of the 24 FST-positive patients also testing positive with SIT (26). In both of the latter studies, the reference criteria for PA diagnosis was FST positivity itself, so that it is not possible to determine the actual sensitivity of the test (11, 26). Willenberg *et al.* showed that the FST diagnosed PA in 87% of patients from a cohort of 33 hypertensive subjects with proven PA (45). Nevertheless, all studies mentioned above are based on the assumption that the FST is the gold standard for proving the autonomy of aldosterone secretion in PA while such assumption has never been validated against a clinical reference standard (42).

Saline infusion test (SIT)

The SIT is the most widely used dynamic test for PA diagnosis. The test was first introduced in the 80s by

Table 2 Diagnostic performances of confirmatory tests.

Test	Reference	Number of subjects	Post-test aldosterone threshold (pmol/L)	Diagnostic accuracy	Diagnostic criteria
Fludrocortisone suppression test (FST)	(11)	98	140	Se 100%	FST positivity
	(45)	33	150	Se 87% Spe 95%	AVS/surgery outcome
Saline infusion test (SIT)	(26)	66	165	Se 100%	FST positivity
	(35)	44	246	Se 100%	CT/scintigraphy/AVS/surgery
	(28)	118	196	Se 88% Spe 100%	AVS/surgery outcome
	(11)	98	139	Se 88% Spe 88%	FST positivity
	(19)	120	196	Se 82% Spe 75%	CT/AVS
	(37)	57	170	Se 60%	Scintigraphy/AVS/surgery
	(45)	33	88	Se 82% Spe 92%	AVS/surgery outcome
	(26)	66	165 seated 140 supine	Se 96% seated Se 33% supine	FST positivity
	(57)	199	139	29% of false negative	AVS/surgery outcome
	(46)	236	222	Se 85% Spe 92%	FST positivity
	(47)	164	310	Se 90.4% Spe 95.9%	AVS/Surgery outcome
Oral sodium loading test (OSLT)	(35)	44	240	Se 100%	CT/scintigraphy/AVS/surgery
	(32)	80	Urinary aldosterone 12 µg/dL/day	Se 96% Spe 93%	CT/scintigraphy/AVS
Captopril challenge test (CCT)	(35)	44	246	Se 97%	CT/scintigraphy/AVS/surgery
	(19)	317	385	Se 69.6% Spe 74%	CT/AVS
	(37)	57	336	Se 96%	Scintigraphy/AVS/surgery
	(28)	82	ARR >30 ng/ng ARP	Se 70% Spe 100%	AVS/Surgery outcome/SIT
Losartan test	(47)	164	460	Se 87% Spe 91.8%	AVS/surgery outcome and FST positivity
	(46)	236	305	Se 90% Spe 90%	FST positivity
	(39)	135	277 and ARR >35	Se 84.5% Spe 93.8%	SIT positivity
	(28)	54	ARR >25	Se 80% Spe 77%	AVS/surgery outcome/SIT
Furosemide upright test (FUT)	(37)	57	PRA ≥2 ng/mL/h	Se 93% Se 100%	Scintigraphy/AVS/surgery
	(41)	148	83 ARR 0.32 ng/dL/µU/mL	Se 82% Spe 98% Spe 89%	Dexamethasone-FST positivity

Streeten (15) and Holland (30). Thereafter, expert teams evaluated the accuracy of the test. In a case-control retrospective study, Giacchetti *et al.* evaluated 61 PA subjects compared to 157 essential hypertensive subjects and found that the SIT showed a sensitivity of 88% and 100% specificity for confirming PA after ARR screening, at a cutoff level for post-SIT plasma aldosterone of 195 pmol/L (7 ng/dL) (28). Mulatero *et al.*, in a prospective study including 100 hypertensive patients with suspected PA, found that the SIT identified PA with 88% sensitivity and 88% specificity using a plasma aldosterone cutoff of 139 pmol/L (5 ng/dL) (11). Rossi *et al.* carried out the PAPY study, a prospective evaluation of SIT accuracy in 317 patients of 1125 hypertensive patients screened by the ARR. This study is the only to fulfill the STARD statement (42) by providing a definitive diagnosis of an aldosterone-producing adenoma using the 'four corners criteria' which include (i) a biochemical diagnosis of PA, (ii) the demonstration of lateralization of aldosterone secretion, (iii) the pathology assessment of an adenoma and (iv) the evidence of the cure of hypertension and hypokalemia (29). In this large series, performance of the SIT was moderate in identifying an aldosterone-producing adenoma, with 82.6% sensitivity and 75.1% specificity, using a plasma aldosterone cutoff of 187 pmol/L (6.75 ng/dL), likely due to overlapping values between patients with or without the disease. Sensitivity was even lower (68.9%) in diagnosing idiopathic hyperaldosteronism; however, the test had a strong negative predictive value of 95% (29). Willemberg reported similar accuracy with 82% sensitivity and a higher specificity of 92%, using a lower aldosterone threshold of 88 pmol/L, in a cohort of 33 patients undergoing both SIT and FST (45). More recently, groups in Japan and China have questioned the accuracy of the SIT. In a retrospective study, Nanba *et al.* have found that among 120 Japanese hypertensive patients investigated for PA after ARR screening, 57 patients underwent three confirmatory tests with 60% positive by SIT (plasma aldosterone cutoff 166 pmol/L, 6 ng/dL) compared to 88% for the other two tests (i.e. CCT and FUT). Nevertheless, the positive rate of SIT was similar to that for both the other tests in the subgroup of surgically proven aldosterone-producing adenoma (37). Song *et al.* prospectively investigated 236 Chinese hypertensive patients separated by the FST into 135 PA and 101 essential hypertensive subjects. All patients performed three tests including SIT. The performance of the SIT was high, with 85% sensitivity and 92% specificity, using a plasma aldosterone cutoff of 222 pmol/L (8 ng/dL) (46). In another recent Chinese study, Meng *et al.* retrospectively

studied 115 PA and 49 essential hypertensive patients and found a high degree of accuracy for the SIT, with 90.4% sensitivity and 95.9% specificity, using a plasma aldosterone cutoff of 310 pmol/L (11.2 ng/dL), higher than that shown in previous studies (47). Of note, the prevalence of hypokalemia was high (74.8%), which may have influenced the rate of positive SIT, as highlighted in a study comparing the performance of the SIT when carried out in hypokalemic (91% sensitivity) or normokalemic conditions (57% sensitivity) (48).

Oral sodium loading test (OSLT)

The OSLT was proposed in 1983 by Bravo *et al.* and used to investigate PA in a series of 80 patients with an adrenal adenoma or hyperplasia, proven by non-invasive or invasive imaging or by AVS (32). In this prospective study, the OSLT exhibited 96% sensitivity and 93% specificity with a urinary aldosterone cutoff at 12 µg/24 h. Agharazii investigated 44 hypertensive subjects and found 100% sensitivity for detecting PA by the OSLT with a plasma aldosterone cutoff at 240 pmol/L (8.65 ng/dL) (35). No large series or comparative studies challenging the OSLT are available and its value has been questioned due to the lack of standardization, variable adherence to the protocol and the inaccuracy of 24-h urine collection. High urinary sodium concentrations may interfere with the determination of urinary aldosterone by decreasing its concentration by up to 30% (49). Head-to-head comparison of OSLT with other suppression tests are lacking but a recent comparison between SIT and OSLT, in a series of 44 hypertensive subjects, found poor repeatability of urinary aldosterone measurements after OSLT and inconclusive results of the latter in half of the patients tested (50).

Captopril challenge test (CCT)

The accuracy of the CCT remains controversial, with contrasting results reported in European and Asian studies. The CCT was first proposed for the diagnosis of PA, by Corvol *et al.* in 1983, in a series of 68 hypertensive patients including 18 PA, with a finding of 100% sensitivity and specificity of the test for diagnosing an aldosterone-producing adenoma (plasma aldosterone cutoff 676 pmol/L (24 ng/dL) (33). Small series performed in the 2000s also suggested the positive diagnostic value of the CCT (34, 35, 51), but these studies did not respond to the standards of high-quality methodology (42). Later, several authors have compared the CCT to other more cumbersome tests, i.e. SIT and FST. In the PAPY study, which provides a

firm diagnosis of an aldosterone-producing adenoma on multiple criteria, Rossi *et al.* evaluated the performance of the CCT in 317 hypertensive patients positive for ARR screening. At a plasma aldosterone cutoff of 385 pmol/L (13.9 ng/dL), the accuracy of the CCT was poor with only 69.6% sensitivity and 74% specificity overall, showing fair sensitivity of 86% when sodium intake was above 130 mEq per day, while sensitivity dropped to 56% with a lower sodium intake (36). Recent Asian studies have challenged the CCT as a tool for diagnosing PA. Nanba *et al.* found that 86% of patients who were positive for the ARR, and 96% of patients with unilateral or bilateral proven PA, exhibited an abnormal response (i.e. ARR cutoff >200) to the CCT (37). Meng *et al.* found sensitivity of 87% and 91.8% specificity of the CCT, using a plasma aldosterone cutoff of 460 pmol/L (16.7 ng/dL), for diagnosing PA in their study of 164 hypertensive patients based on an accurate diagnosis of an aldosterone-producing adenoma performed on multiple criteria (47). Song *et al.* also reported excellent accuracy for the CCT in their cohort of 236 hypertensive patients, including 135 PA proven by the FST, with 90% sensitivity and specificity of the CCT at a plasma aldosterone cutoff of 305 pmol/L (11 ng/dL) (46). In these studies, a high proportion of patients were hypokalemic (35, 37, 46, 47). The CCT has been criticized by European experts who emphasized a high rate of false-negative or false-positive CCT in 36% of subjects from a small series of normokalemic PA patients (18). Such misleading results could be explained by a lesser degree of response to inhibition of ACE by captopril in low renin essential hypertension or conversely to the preserved sensitivity to angiotensin II of some aldosterone-producing adenomas (52).

Furosemide upright test (FUT)

The FUT was designed in 1976 by Kaplan *et al.* for identifying low renin hypertension in Caucasian and black patients with essential hypertension (53). FUT was introduced in Japan for the detection of secondary hypertension among hypertensive patients (54). The aldosterone-to-PRA ratio was evaluated retrospectively in 35 aldosterone-producing adenomas and in 79 non-secreting adenomas selected from a cohort of 159 hypertensive patients between 1989 and 1999. The test identified PA with 100% sensitivity but only 61% specificity (55). A recent study by Nanba *et al.*, retrospectively performed in 120 Japanese hypertensive patients, compared the FUT with other tests (i.e. SIT and CCT). In this study, the positive rates for the FUT, defined as a PRA below 2 ng/mL/h, were 87% in the 120 patients

detected positive for the ARR and 94% in the subset of 57 patients with unilateral or bilateral proven PA (37). The proportion of positive response with FUT was similar to that with CCT and significantly higher than that with SIT. The FUT has not been evaluated in non-Asian hypertensive patients.

Losartan suppression test (LST)

Wu *et al.* have suggested that angiotensin II receptor blockade might be of value for confirmation of PA after screening by ARR. In a prospective, cohort study, they compared the accuracy of the CCT and the losartan test, which consists of the administration of a single dose of 50 mg losartan, in 135 hypertensive patients from the Taiwan Primary Aldosteronism Investigation (TAIPAI) who passed both tests in which performances were evaluated against the SIT (considered as the reference test). Using an ARR >35 together with a plasma aldosterone cutoff of 277 pmol/L (10 ng/dL), the accuracy of the losartan test for the diagnosis of PA was superior to that of the CCT with sensitivities of 84.5 vs 66.2%, and specificities of 93.8 vs 89.1% respectively (39). Accuracy of the losartan test was recently confirmed in older hypertensive subjects from the same TAIPAI cohort (40). Giacchetti *et al.* performed a similar comparison between the CCT and the losartan test in 82 and 54 patients respectively and did not find the losartan test to be superior, when compared with the CCT, for the confirmation of PA (28).

Dexamethasone-Captopril-Valsartan Test (DCVT)

Recently, Tsiavos *et al.* proposed a new, sensitive test combining the screening and confirmatory steps and performed overnight. This test consists of the simultaneous blockade of the renin-angiotensin system, by captopril and valsartan, together with ACTH suppression by 2 mg dexamethasone. This DCVT rapid test was compared to the dexamethasone-FST, in a cohort of 45 PA patients and 103 patients with essential hypertension on the basis of the dexamethasone-FST. The DCVT exhibited 98% sensitivity and 89% specificity at an ARR cutoff 0.32 ng/dL/ μ U/mL (41).

Comparative performances of the confirmatory tests

A parallel evaluation of the different confirmatory tests has been performed in several studies. Mulatero *et al.* compared the performances of SIT with FST for the diagnosis of PA,

in 100 hypertensive patients, and found a strong correlation of plasma aldosterone concentrations after each of the two tests, together with a positive predictive value for SIT of 92% and a negative predictive value of 79% when the diagnosis of PA was based on the FST (11). Recently, a group from Brisbane, Australia investigated the accuracy of the SIT when compared to the FST as the gold standard for PA diagnosis. Ninety six percent of PA, confirmed by FST, exhibited a positive response to SIT when the latter was performed in the seated posture compared with only 33% positive response to SIT when performed in the recumbent position (26). Willenberg *et al.* in their study, which was performed in 156 hypertensive patients, found slightly better performance for the FST compared to SIT i.e. 87 vs 82% sensitivity and 97 vs 92% specificity respectively, using different plasma aldosterone cutoffs, and with a high proportion of false-negative and false-positive test results obtained with both tests (45).

Several studies have compared the SIT and CCT tests. In the PAPY prospective study, performed in 317 hypertensive patients positively screened for PA, Rossi *et al.* found a slightly higher, and borderline significant, accuracy of SIT compared to CCT, at an optimal plasma aldosterone cutoff that was 2-fold higher after CCT than after SIT, indicating a greater degree of suppression of aldosterone secretion with the latter test. These authors stressed the importance of sodium intake, which markedly affected the performance of the CCT when below 130 mEq per day, with CCT sensitivity dropping from 85.7 to 56% (36). In the COMPASS study, Song *et al.* examined 135 Chinese hypertensive patients with PA and 101 patients with essential hypertension, both CCT and SIT exhibited high areas under the receiver-operator characteristics (ROC) curves at 0.96, 85–90% sensitivity and 90–92% specificity (46). Similarly, Meng *et al.* found in 164 Chinese hypertensive patients, ROC areas under curve of 0.972

and 0.933 for post-SIT and post-CCT plasma aldosterone respectively (47). Nanba *et al.* investigated the CCT, SIT and FUT for PA diagnosis, in 120 Japanese hypertensive patients, and found 86–87% positive using CCT and FUT but only 63% positive with SIT. These contrasting results suggest a differential performance of confirmatory tests between Caucasian and Asian populations (37).

International guideline recommendations for the use of confirmatory tests for PA diagnosis

Between 2011 and 2016, three international guidelines and recommendations on PA management were released by US, French and Japanese expert teams (3, 25, 38). The main guidelines on confirmatory tests from these teams are summarized in Table 3. All of the guidelines recommend a work-up that includes a screening step by the ARR with different threshold values, and a confirmatory step by dynamic testing. Both US and French guidelines consider that the association of a high ARR together with an elevated plasma aldosterone concentration, i.e. above 550 pmol/L, with or without concomitant indirect criteria of aldosterone over-secretion, that is hypokalemia and non-detectable PRA, are sufficient for a definitive diagnosis of PA without the need for a confirmatory test. In contrast, the Japanese guidelines consider there to be a need for a confirmatory dynamic step without exception. The US guidelines also assess that confirmatory tests should be avoided in patients unwilling or unable to proceed further in the diagnosis and surgical cure of an aldosterone-producing adenoma (3). Conversely, a plasma aldosterone concentration below a definite threshold may exclude PA, with different cutoffs specified in the French and Japanese guidelines (Table 3). The procedure recommended by the

Table 3 International guidelines on confirmatory testing in the work-up for PA diagnosis.

Guidelines	Screening step	Need for a confirmatory step	Conditions allowing to obviate CT ¹	Recommended CT ¹
Endocrine Society Guidelines 2016 (3)	Positive if ARR >20 ¹	Yes	If spontaneous hypokalemia and ARR below detection level and PAC >550 pmol/L (20 ng/dL)	≥1 CT No sufficient evidence to recommend one test over others
French Endocrine Society Guidelines 2016 (11, 17)	Positive if ARR 30 ¹ and PAC >240 pmol/L (9 ng/dL)	Yes	If PAC >550 pmol/L (20 ng/dL)	≥1 CT SIT is recommended
Japan Endocrine Society Guidelines 2009 (30)	Positive if ARR >20 ¹ and PAC 333 pmol/L (12 ng/dL)	Yes	No exception	≥2 CT among CCT, FUT, SIT

¹PAC in ng/dL, ARR in ng/mL/h.

CT, confirmatory test; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

US and French guidelines is to perform at least one of the available tests, the SIT being favored by the latter, while the Japanese guidelines recommend performing at least two consecutive tests out of the three (i.e. SIT, CCT or FUT).

Is it feasible to eliminate the confirmatory step in PA work-up?

Data on confirmatory tests generally comes from studies with low levels of evidence due to small series of disease and control cases, the paucity of comparative studies simultaneously challenging different tests, the lack of one validated reference test for definitive PA diagnosis and therefore the tautological methodology consisting of arbitrarily choosing one test as the reference test, and the lack of consensus for positivity thresholds for confirmatory tests (25). Few studies have assessed the definitive diagnosis of an aldosterone-producing adenoma using a gold standard definition of APA (19, 29, 36, 47) which includes the so-called 'four corners criteria' to which may be added a fifth criteria namely the detection of a CYP11B2-positive adenoma in the resected adrenal cortex at immunohistochemistry (8). All confirmatory tests presuppose that aldosterone secretion in PA is independent and dissociated from renin secretion, which in reality is far from always being the case (15, 16, 17, 18, 19). Moreover, tests such as sodium loading or fludrocortisone suppression may be dangerous especially when there is profound hypokalemia, severe hypertension or heart failure.

Caveats of the strategy including a confirmatory test have been recently highlighted. Rossi *et al.* have demonstrated in a normoaldosteronemic hypertensive patient the immunochemical characterization of an aldosterone-producing adenoma (56). Cornu *et al.* have found, in a cohort of 199 hypertensive patients diagnosed with PA, that 29% of patients among those with a post-SIT aldosterone level below 139 pmol/L (5 ng/dL) had lateralized aldosterone secretion on AVS, and therefore, concluded that AVS should be considered for patients, eligible for surgery, who have an elevated basal aldosterone concentration even if their recumbent post-SIT aldosterone is low (57).

Some authors have proposed eliminating the confirmatory step after ARR screening and before performing AVS based on the presence of high probability features which include an adrenal mass, hypokalemia and an ARR greater than twice the cutoff level for positivity (58). Tirosh *et al.* found in a prospective observational study of 59 hypertensive subjects with a high suspicion

of PA that the sole ARR had a 100% accuracy for the case detection of PA in those with a BMI <30 kg/m² suggesting the possible avoidance of a confirmatory test in lean subjects (59). Investigators in the PAPY study have tested the hypothesis that a high ARR should make performing a confirmatory test unnecessary. For this purpose, they interrogated two large data sets of patients that were prospectively collected and studied with a protocol including the CCT. Criteria for the firm diagnosis of an aldosterone-producing adenoma used the 'four corners criteria'. The exploratory phase was performed on the historical PAPY cohort data set and the validation phase on the Padua hypertensive cohort, referred from 2012 to 2015. The final analysis concluded that the baseline ARR carries essential quantitative information for PA diagnosis and that increasing the ARR cutoff exponentially decreases the rate of false positives, therefore reducing the need for performing a confirming test, i.e. CCT in the present study. Interestingly, the CCT provided no diagnostic benefit over the baseline ARR, the latter allowing the false-positive risk of the ARR screening with high ARR values to be reduced to almost zero (20).

Conclusions

Dynamic testing of aldosterone secretion, independent from the RAAS, is currently performed in the work-up of PA diagnosis. Nevertheless, confirmatory tests exhibit a higher negative than positive predictive value and therefore work rather as exclusion tests than confirmatory tests (19, 36). Expert teams and international guidelines have tried to define different clinical and biochemical conditions, some of them giving sufficiently high probability for PA diagnosis by the sole baseline hormonal work-up, in order to avoid a confirmatory step, others providing hormone values 'in the gray zone' and therefore necessitating confirmation by a confirmatory test. The choice of one test over others is dependent on the investigator's experience and, in secondary or tertiary centers, one could recommend a retrospective or prospective evaluation of their data, performed on large hypertensive populations. This would allow them to define their own experimental conditions and hormone thresholds for optimal accuracy in their routine procedures for RAAS evaluation. The FST is probably the most efficient test for obtaining the most effective RAAS suppression; however, its management is cumbersome and costly which explains that a minority of expert centers perform this test routinely. The SIT is widely used and recent promising data suggest that

optimal conditions for its performance, including carrying out the test in the seated position, may provide excellent accuracy. The SIT has been proposed as the reference test by the French guidelines on PA management. The CCT has been extensively evaluated and recent large series, from Europe and Asia, suggest that it should be proposed as a first line test in standardized conditions. Investigators from Japan also favor the FUT as a simple and innocuous test. Finally, there is a need for large prospective studies comparing the different strategies 'with and without dynamic testing' with appropriate tests compared face to face and with accurate assays for aldosterone and steroid profile measurements being employed using liquid-chromatography-tandem mass determination (60, 61). Such studies should help authentify the best criteria for PA confirmation in situations where basal and dynamic aldosterone/renin levels remain in the gray zone.

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References

- Rossi GP, Bernini G, Caliumi C, Desideri G, Fabris B, Ferri C, Ganzaroli C, Giacchetti G, Letizia C, Maccario M *et al.* A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. *Journal of the American College of Cardiology* 2006 **48** 2293–2300. (<https://doi.org/10.1016/j.jacc.2006.07.059>)
- Fardella CE, Mosso L, Gómez-Sánchez C, Cortés P, Soto J, Gómez L, Pinto M, Huete A, Oestreicher E, Foradori A *et al.* Primary hyperaldosteronism in essential hypertensives: prevalence, biochemical profile, and molecular biology. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 1863–1867.
- Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, Stowasser M & Young WF Jr. The management of primary aldosteronism: case detection, diagnosis, and treatment: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* 2016 **101** 1889–1916. (<https://doi.org/10.1210/jc.2015-4061>)
- Monticone S, Burrello J, Tizzani D, Bertello C, Viola A, Buffolo F, Gabetti L, Mengozzi G, Williams TA, Rabbia F *et al.* Prevalence and clinical manifestations of primary aldosteronism encountered in primary care practice. *Journal of the American College of Cardiology* 2017 **69** 1811–1820. (<https://doi.org/10.1016/j.jacc.2017.01.052>)
- Rossi GP, Sechi LA, Giacchetti G, Ronconi V, Strazzullo P & Funder JW. Primary aldosteronism: cardiovascular, renal and metabolic implications. *Trends in Endocrinology and Metabolism* 2008 **19** 88–90. (<https://doi.org/10.1016/j.tem.2008.01.006>)
- Mulatero P, Monticone S, Bertello C, Viola A, Tizzani D, Iannaccone A, Crudo V, Burrello J, Milan A, Rabbia F *et al.* Long-term cardio- and cerebrovascular events in patients with primary aldosteronism. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 4826–4833. (<https://doi.org/10.1210/jc.2013-2805>)
- Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME & Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *Journal of the American College of Cardiology* 2005 **45** 1243–1248. (<https://doi.org/10.1016/j.jacc.2005.01.015>)
- Seccia TM, Caroccia B, Adler GK, Maiolino G, Cesari M & Rossi GP. Arterial hypertension, atrial fibrillation, and hyperaldosteronism: the triple trouble. *Hypertension* 2017 **69** 545–550. (<https://doi.org/10.1161/HYPERTENSIONAHA.116.08956>)
- Baguet JP, Steichen O, Mounier-Véhier C & Gosse P. SFE/SFHTA/AFCE consensus on primary aldosteronism, part 1: epidemiology of primary aldosteronism (PA), who should be screened for sporadic PA? *Annales d'Endocrinologie* 2016 **77** 196–191. (<https://doi.org/10.1016/j.ando.2016.01.006>)
- Mosso L, Carvajal C, González A, Barraza A, Avila F, Montero J, Huete A, Gederlini A & Fardella CE. Primary aldosteronism and hypertensive disease. *Hypertension* 2003 **42** 161–165. (<https://doi.org/10.1161/01.HYP.0000079505.25750.11>)
- Mulatero P, Milan A, Fallo F, Regolisti G, Pizzolo F, Fardella C, Mosso L, Marafetti L, Veglio F, Maccario M. Comparison of confirmatory tests for the diagnosis of primary aldosteronism. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 2618–2623. (<https://doi.org/10.1210/jc.2006-0078>)
- Douillard C, Houillier P, Nussberger J & Girerd X. SFE/SFHTA/AFCE Consensus on Primary Aldosteronism, Part 2: First diagnostic steps. *Annales d'Endocrinologie* 2016 **77** 192–201. (<https://doi.org/10.1016/j.ando.2016.02.003>)
- Baudrand R, Guarda FJ, Torrey J, Williams G & Vaidya A. Dietary sodium restriction increases the risk of misinterpreting mild cases of primary aldosteronism. *Journal of Clinical Endocrinology and Metabolism* 2016 **101** 3989–3996. (<https://doi.org/10.1210/jc.2016-1963>)
- Tanabe A, Naruse M, Takagi S, Tsuchiya K, Imaki T & Takano K. Variability in the renin/aldosterone profile under random and standardized sampling conditions in primary aldosteronism. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 2489–2494. (<https://doi.org/10.1210/jc.2002-021476>)
- Streeten DH, Tomycz N & Anderson GH. Reliability of screening methods for the diagnosis of primary aldosteronism. *American Journal of Medicine* 1979 **67** 403–413. ([https://doi.org/10.1016/0002-9343\(79\)90786-1](https://doi.org/10.1016/0002-9343(79)90786-1))
- Gordon RD, Gomez-Sanchez CE, Hamlet SM, Tunny TJ & Klemm SA. Angiotensin-responsive aldosterone-producing adenoma masquerades as idiopathic hyperaldosteronism (IHA: adrenal hyperplasia) or low-renin essential hypertension. *Journal of Hypertension* 1987 **5** S103–S106.
- Irony I, Kater CE, Biglieri EG & Shackleton CH. Correctable subsets of primary aldosteronism. Primary adrenal hyperplasia and renin responsive adenoma. *American Journal of Hypertension* 1990 **3** 576–582. (<https://doi.org/10.1093/ajh/3.3.576>)
- Mulatero P, Bertello C, Garrone C, Rossato D, Mengozzi G, Verhovez A, Fallo F & Veglio F. Captopril test can give misleading

- results in patients with suspect primary aldosteronism. *Hypertension* 2007 **50** e26–e27.
- 19 Rossi GP, Belfiore A, Bernini G, Desideri G, Fabris B, Ferri C, Giacchetti G, Letizia C, Maccario M, Mallamaci F *et al.* Comparison of the captopril and the saline infusion test for excluding aldosterone-producing adenoma. *Hypertension* 2007 **50** 424–431. (<https://doi.org/10.1161/HYPERTENSIONAHA.107.091827>)
 - 20 Maiolino G, Rossitto G, Bisogni V, Cesari M, Seccia TM, Plebani M, Rossi GP & PAPY Study Investigators. Quantitative value of aldosterone-renin ratio for detection of aldosterone-producing adenoma: the aldosterone-renin ratio for primary aldosteronism (AQUARR) study. *Journal of the American Heart Association* 2017 **6** e005574. (<https://doi.org/10.1161/JAHA.117.005574>)
 - 21 Solar M, Malirova E, Ballon M, Pelouch R & Ceral J. Confirmatory testing in primary aldosteronism: extensive medication switching is not needed in all patients. *European Journal of Endocrinology* 2012 **166** 679–686. (<https://doi.org/10.1530/EJE-11-0914>)
 - 22 Stowasser M, Taylor PJ, Pimenta E, Ahmed AH & Gordon RD. Laboratory investigation of primary aldosteronism. *Clinical Biochemist*. Reviews 2010 **31** 39–56.
 - 23 Gouli A, Kaltsas G, Tzonou A, Markou A, Androulakis II, Ragkou D, Vamvakidis K, Zografos G, Kontogeorgos G, Chrousos GP *et al.* High prevalence of autonomous aldosterone secretion among patients with essential hypertension. *European Journal of Clinical Investigation* 2011 **41** 1227–1236. (<https://doi.org/10.1111/j.1365-2362.2011.02531.x>)
 - 24 Chao CT, Wu VC, Kuo CC, Lin YH, Chang CC, Chueh SJ, Wu KD, Pimenta E & Stowasser M. Diagnosis and management of primary aldosteronism: an updated review. *Annals of Medicine* 2013 **45** 375–383. (<https://doi.org/10.3109/07853890.2013.785234>)
 - 25 Reznik Y, Amar L & Tabarin A. SFE/SFHTA/AFCE consensus on primary aldosteronism, part 3. Confirmatory testing. *Annales d'Endocrinologie* 2016 **77** 202–207. (<https://doi.org/10.1016/j.ando.2016.01.007>)
 - 26 Ahmed AH, Cowley D, Wolley M, Gordon RD, Xu S, Taylor PJ & Stowasser M. Seated saline suppression testing for the diagnosis of primary aldosteronism: a preliminary study. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 2745–2753. (<https://doi.org/10.1210/jc.2014-1153>)
 - 27 Kem DC, Weinberger MH, Mayes DM & Nugent CA. Saline suppression of plasma aldosterone in hypertension. *Archives of Internal Medicine* 1971 **128** 380–386. (<https://doi.org/10.1001/archinte.1971.00310210056004>)
 - 28 Giacchetti G, Ronconi V, Lucarelli G, Boscaro M & Mantero F. Analysis of screening and confirmatory tests in the diagnosis of primary aldosteronism: need for a standardized protocol. *Journal of Hypertension* 2006 **24** 737–745. (<https://doi.org/10.1097/01.hjh.0000217857.20241.0f>)
 - 29 Rossi GP, Belfiore A, Bernini G, Desideri G, Fabris B, Ferri C, Giacchetti G, Letizia C, Maccario M, Mallamaci F *et al.* Prospective evaluation of the saline infusion test for excluding primary aldosteronism due to aldosterone-producing adenoma. *Journal of Hypertension* 2007 **25** 1433–1442. (<https://doi.org/10.1097/HJH.0b013e328126856e>)
 - 30 Holland OB, Brown H, Kuhnert L, Fairchild C, Risk M & Gomez-Sanchez CE. Further evaluation of saline infusion for the diagnosis of primary aldosteronism. *Hypertension* 1984 **6** 717–723. (<https://doi.org/10.1161/01.HYP.6.5.717>)
 - 31 Young WF Jr. Primary aldosteronism: management issues. *Annals of the New York Academy of Sciences* 2002 **970** 61–76. (<https://doi.org/10.1111/j.1749-6632.2002.tb04413.x>)
 - 32 Bravo EL, Tarazi RC, Dustan HP, Fouad FM, Textor SC, Gifford RW & Vidt DG. The changing clinical spectrum of primary aldosteronism. *American Journal of Medicine* 1983 **74** 641–651. ([https://doi.org/10.1016/0002-9343\(83\)91022-7](https://doi.org/10.1016/0002-9343(83)91022-7))
 - 33 Thibonnier M, Plouin PF, Ménard J & Corvol P. Primary hyperaldosteronism: diagnostic value of the administration of a single dose of captopril. *Annali Medicae Internationa* 1983 **134** 251–255.
 - 34 Lyons DE, Kem DC, Brown RD, Hanson CS & Carollo ML. Single dose captopril as a diagnostic test for primary aldosteronism. *Journal of Clinical Endocrinology and Metabolism* 1983 **57** 892–896. (<https://doi.org/10.1210/jcem-57-5-892>)
 - 35 Agharazii M, Douville P, Grose JH & Lebel M. Captopril suppression versus salt loading in confirming primary aldosteronism. *Hypertension* 2001 **37** 1440–1443. (<https://doi.org/10.1161/01.HYP.37.6.1440>)
 - 36 Rossi GP, Belfiore A, Bernini G, Desideri G, Fabris B, Ferri C, Giacchetti G, Letizia C, Maccario M & Mallamaci F. Comparison of the captopril and the saline infusion test for excluding aldosterone-producing adenoma. *Hypertension* 2007 **50** 424–431. (<https://doi.org/10.1161/HYPERTENSIONAHA.107.091827>)
 - 37 Nanba K, Tamanaha T, Nakao K, Kawashima ST, Usui T, Tagami T, Okuno H, Shimatsu A, Suzuki T & Naruse M. Confirmatory testing in primary aldosteronism. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 1688–1694. (<https://doi.org/10.1210/jc.2011-2504>)
 - 38 Nishikawa T, Omura M, Satoh F, Shibata H, Takahashi K, Tamura N, Tanabe A & Task Force Committee on Primary Aldosteronism, The Japan Endocrine Society. Guidelines for the diagnosis and treatment of primary aldosteronism – the Japan Endocrine Society 2009. *Endocrine Journal* 2011 **58** 711–721. (<https://doi.org/10.1507/endocrj.EJ11-0133>)
 - 39 Wu VC, Chang HW, Liu KL, Lin YH, Chueh SC, Lin WC, Ho YL, Huang JW, Chiang CK, Yang SY *et al.* Primary aldosteronism: diagnostic accuracy of the losartan and captopril tests. *American Journal of Hypertension* 2009 **22** 821–827. (<https://doi.org/10.1038/ajh.2009.89>)
 - 40 Kuo CC, Balakrishnan P, Hsein YC, Wu VC, Chueh SC, Chen YM, Wu KD, Wang MJ & TAIPAI group. The value of losartan suppression test in the confirmatory diagnosis of primary aldosteronism in patients over 50 years old. *Journal of the Renin-Angiotensin-Aldosterone System* 2015 **16** 587–598. (<https://doi.org/10.1177/1470320313498632>)
 - 41 Tsiavos V, Markou A, Papanastasiou L, Kounadi T, Androulakis II, Voulgaris N, Zachaki A, Kassi E, Kaltsas G & Chrousos GP. A new highly sensitive and specific overnight combined screening and diagnostic test for primary aldosteronism. *European Journal of Endocrinology* 2016 **175** 21–28. (<https://doi.org/10.1530/EJE-16-0003>)
 - 42 Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, Lijmer JG, Moher D, Rennie D, de Vet HC *et al.* STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. *BMJ* 2015 **351** h5527. (<https://doi.org/10.1136/bmj.h5527>)
 - 43 Mulatero P, Dluhy RG, Giacchetti G, Boscaro M, Veglio F & Stewart PM. Diagnosis of primary aldosteronism: from screening to subtype differentiation. *Trends in Endocrinology and Metabolism* 2005 **16** 114–119. (<https://doi.org/10.1016/j.tem.2005.02.007>)
 - 44 Stowasser M, Gordon RD, Rutherford JC, Nikwan NZ, Daunt N & Slater GJ. Diagnosis and management of primary aldosteronism. *Journal of the Renin-Angiotensin-Aldosterone System* 2001 **2** 156–169. (<https://doi.org/10.3317/jraas.2001.022>)
 - 45 Willenberg HS, Vonend O, Schott M, Gao X, Blondin D, Saleh A, Rump LC & Scherbaum WA. Comparison of the saline infusion test and the fludrocortisone suppression test for the diagnosis of primary aldosteronism. *Hormone and Metabolic Research* 2012 **44** 527–532. (<https://doi.org/10.1055/s-0032-1314786>)
 - 46 Song Y, Yang S, He W, Hu J, Cheng Q, Wang Y, Luo T, Ma L, Zhen Q, Zhang S *et al.* Confirmatory tests for the diagnosis of primary aldosteronism: A Prospective Diagnostic Accuracy Study. *Hypertension* 2018 **71** 118–124. (<https://doi.org/10.1161/HYPERTENSIONAHA.117.10197>)
 - 47 Meng X, Li Y, Wang X, Li J, Liu Y & Yu Y. Evaluation of the saline infusion test and the captopril challenge test in Chinese patients

- with primary aldosteronism. *Journal of Clinical Endocrinology and Metabolism* 2018 **103** 853–860. (<https://doi.org/10.1210/jc.2017-01530>)
- 48 Schirpenbach C, Seiler L, Maser-Gluth C, Rüdiger F, Nickel C, Beuschlein F & Reincke M. Confirmatory testing in normokalaemic primary aldosteronism: the value of the saline infusion test and urinary aldosterone metabolites. *European Journal of Endocrinology* 2006 **154** 865–873. (<https://doi.org/10.1530/eje.1.02164>)
 - 49 Aldea ML, Barallat J, Martin MA, Rosas I, Pastor MC & Granada ML. Sodium interference in the determination of urinary aldosterone. *Clinical Biochemistry* 2016 **49** 295–297. (<https://doi.org/10.1016/j.clinbiochem.2015.11.004>)
 - 50 Ceral J, Malirova E, Ballon M & Solar M. The role of urinary aldosterone for the diagnosis of primary aldosteronism. *Hormone and Metabolic Research* 2014 **46** 663–667. (<https://doi.org/10.1055/s-0034-1374638>)
 - 51 Castro OL, Yu X & Kem DC. Diagnostic value of the post-captopril test in primary aldosteronism. *Hypertension* 2002 **39** 935–938. (<https://doi.org/10.1161/01.HYP.0000014324.68506.CA>)
 - 52 Westerdahl C, Bergenfelz A, Isaksson A & Valdemarsson S. Captopril suppression: limitations for confirmation of primary aldosteronism. *Journal of the Renin-Angiotensin-Aldosterone System* 2011 **12** 326–332. (<https://doi.org/10.1177/1470320310390405>)
 - 53 Kaplan NM, Kem DC, Holland OB, Kramer NJ, Higgins J & Gomez-Sanchez C. The intravenous furosemide test: a simple way to evaluate renin responsiveness. *Annals of Internal Medicine* 1976 **84** 639–645. (<https://doi.org/10.7326/0003-4819-84-6-639>)
 - 54 Nishikawa T & Omura M. Clinical characteristics of primary aldosteronism: its prevalence and comparative studies on various causes of primary aldosteronism in Yokohama Rosai Hospital. *Biomedicine and Pharmacotherapy* 2000 **54** (Supplement 1) 83s–85s. ([https://doi.org/10.1016/S0753-3322\(00\)80019-0](https://doi.org/10.1016/S0753-3322(00)80019-0))
 - 55 Hirohara D, Nomura K, Okamoto T, Ujihara M & Takano K. Performance of the basal aldosterone to renin ratio and of the renin stimulation test by furosemide and upright posture in screening for aldosterone-producing adenoma in low renin hypertensives. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 4292–4298. (<https://doi.org/10.1210/jcem.86.9.7867>)
 - 56 Rossi GP, Gioco F, Fassina A & Gomez-Sanchez CE. Normoaldosteronemic aldosterone-producing adenoma: immunochemical characterization and diagnostic implications. *Journal of Hypertension* 2015 **33** 2546–2549. (<https://doi.org/10.1097/HUH.0000000000000748>)
 - 57 Cornu E, Steichen O, Nogueira-Silva L, Küpers E, Pagny JY, Grataloup C, Baron S, Zinzindohoue F, Plouin PF & Amar L. Suppression of aldosterone secretion After recumbent saline infusion does not exclude lateralized primary aldosteronism. *Hypertension* 2016 **68** 989–994. (<https://doi.org/10.1161/HYPERTENSIONAHA.116.07214>)
 - 58 Kline GA, Pasięka JL, Harvey A, So B & Dias VC. High-probability features of primary aldosteronism may obviate the need for confirmatory testing without increasing false-positive diagnoses. *Journal of Clinical Hypertension* 2014 **16** 488–496. (<https://doi.org/10.1111/jch.12425>)
 - 59 Tirosh A, Hannah-Shmouni F, Lyssikatos C, Belyavskaya E, Zilbermint M, Abraham SB, Lodish MB & Stratakis CA. Obesity and the diagnostic accuracy for primary aldosteronism. *Journal of Clinical Hypertension* 2017 **19** 790–797. (<https://doi.org/10.1111/jch.13041>)
 - 60 Baron S, Amar L, Faucon AL, Blanchard A, Baffalieu L, Faucard C, Travers S, Pagny JY, Azizi M, Houillier P. Criteria for diagnosing primary aldosteronism on the basis of liquid chromatography-tandem mass spectrometry determinations of plasma aldosterone concentration. *Journal of Hypertension* 2018 **36** 1592–1601. (<https://doi.org/10.1097/HJH.0000000000001735>)
 - 61 Lenders JWM, Williams TA, Reincke M & Gomez-Sanchez CE. DIAGNOSIS of ENDOCRINE DISEASE: 18-Oxocortisol and 18-hydroxycortisol: is there clinical utility of these steroids? *European Journal of Endocrinology* 2018 **178** R1–R9. (<https://doi.org/10.1530/EJE-17-0563>)

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