Prospective evaluation of the saline infusion test for excluding primary aldosteronism due to aldosterone-producing adenoma

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Background **Data on the performance of the tests used to confirm the diagnosis of primary aldosteronism (PA) are limited.**

Objective **To prospectively investigate the accuracy of the saline infusion test (SIT).**

Methods **Three hundred and seventeen (26.9%) out of 1125 patients screened in the PAPY study underwent measurement of plasma aldosterone, cortisol and renin activity after infusion of 2 l of isotonic saline intravenously over 4 h. They comprised patients with a baseline aldosterone/renin ratio (ARR) >40 and one every four patients not fulfilling such criterion. The area under the receiver-operator characteristic curves (AUC) of aldosterone values after SIT was used as a measure of accuracy for diagnosing PA, aldosterone-producing adenoma (APA) or idiopathic hyperaldosteronism (IHA).**

Results **One hundred and twenty (37.9%) patients had PA that was due to an APA in 46 (38.3%) and to IHA in 74 (61.7%). No untoward effect occurred with the SIT. The AUC (0.811 ± 0.026, 0.878 ± 0.040 and 0.784 ± 0.034 for identification of PA, APA and IHA, respectively) was higher ($P < 0.0001$) than that under the diagonal. By sensitivity/specificity versus criterion values plot, the best aldosterone cut-off values for identifying APA and IHA were 6.75 and 6.91 ng/dl, respectively. However, even at these optimal cut-offs, sensitivity and specificity were moderate because of values overlapping between patients with and without the disease. Moreover, there were no differences of AUC and aldosterone cut-offs between APA and IHA.**

Conclusion **In a multicenter study the SIT was safe and specific for excluding PA, but had no place for discriminating between an APA and IHA. *J Hypertens* 25:1433–1442**

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Introduction

Primary aldosteronism [1] (PA) is more common [2–6] than previously held [7], as shown by the largest prospective investigation of 1125 newly-diagnosed hypertensive patients referred to hypertension centers heretofore performed [8]. In that study, the overall prevalence of PA was 11.2% and 4.8% of patients who had an aldosterone-producing adenoma (APA) removed with cure or amelioration of hypertension [8]. Potential curability and the high prevalence rate emphasize the need for developing accurate strategies for timely diagnosing APA.

Despite being sensitive, the aldosterone/renin ratio (ARR) and the multivariable approaches based on the biochemical hallmarks of PA have a limited specificity, which translates into a moderate accuracy [2,5,6,9]. The initial claim that the ARR alone provides complete separation of patients with PA from those with primary (essential) hypertension (PH) [10] was not confirmed by the general experience. Calculation of the ARR only in patients with increased aldosterone secretion, under carefully controlled sodium intake, and with arbitrary setting of the lowest value of the denominator of the ratio [e.g. plasma renin activity (PRA)] to 0.2 ng/ml per h,

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might circumvent the problem of over-inflating the ARR, thus increasing its specificity. However, this was difficult to implement in practice and hampered by overlooking PA patients with only mild elevation of aldosterone [11,12]. Moreover, calculation of the ARR under antihypertensive medications, of which some (e.g. β-adrenergic blockers) blunt renin secretion but have little effect on aldosterone levels [13–15], can generate a false-positive ARR, thus further lowering its specificity [11,16]. Of further concern, neither the ARR nor the multivariate score allow distinction of patients with idiopathic hyperaldosteronism (IHA) from low-renin PH, who can have a high ARR [17–19].

Adrenal vein sampling (AVS), is invasive and carries a low risk of complications [20,21] but is required to identify the surgically curable forms of PA [22]. Hence, a confirmatory test is performed after a positive screening test to select patients for AVS [23,24]. However, with the exception of one study [25], these tests, which comprise oral sodium loading [26], saline infusion (SIT) [27,28], captopril [2,29] or the fludrocortisone suppression test, have been evaluated retrospectively in few selected patients with a high prior (pre-test) probability of PA [24,30], mostly using another test as a referent rather than a conclusive diagnosis of APA [12,13,31]. For example, in the first report on the SIT, only five patients had an APA [27] whereas, in the largest study, only eight and 14 referred hypertensives had an APA and IHA, respectively [32]. In a further study, which excluded patients with severe hypertension, a plasma aldosterone value after the SIT >11 ng/dl allowed the identification of 28 PA patients [25]. More recently, in a study comparing the SIT with the fludrocortisone test in 39 patients with low-renin hypertension, only five were conclusively diagnosed with an APA [28]. It was contended that the aldosterone/cortisol ratio after the SIT may allow differentiation of APA and IHA [33], and a plasma aldosterone value after the SIT higher than 10 ng/dl can identify APA. However, further investigation was deemed to be necessary to gain confidence in the diagnosis of IHA in those with a plasma aldosterone after the SIT between 5 and 10 ng/dl [28]. The optimal aldosterone value after the SIT therefore remained undefined.

Thus, the Steering Committee of the PAPY (PA Prevalence in Hypertensives) study [8] aimed to assess the performance of confirmatory tests in a large series of hypertensive patients in whom a conclusive diagnosis of APA was eventually made.

Patients and methods

Overview

The protocol of the PAPY study [8] was approved by the institutional ethical committees and followed the Statement for reporting Studies of Diagnostic Accuracy (STARD) recommendations [34] and the requirements of the Declaration of Helsinki. Briefly, consecutive patients with a new diagnosis of hypertension referred to specialized centers for the diagnosis and treatment of hypertension nationwide in Italy were enrolled after informed consent was obtained [8]. A prior diagnosis of secondary hypertension and the refusal to participate in the study were the sole exclusion criteria.

Screening and saline infusion test

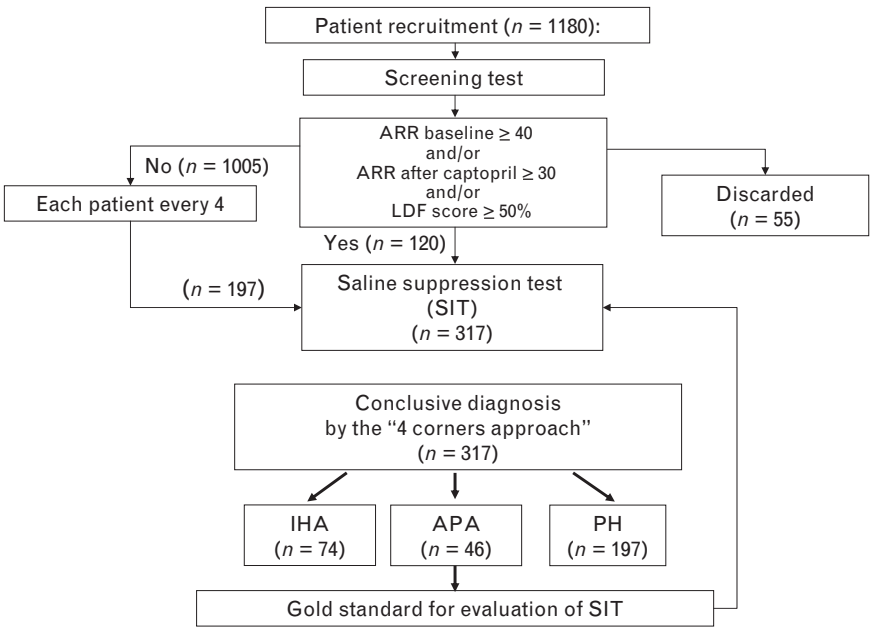
In the PAPY study, all patients underwent the screening test [8]; those who underwent the SIT were selected for the present investigation. By protocol, the SIT was required in the patients who had an ARR ≥ 40 baseline with a plasma aldosterone value ≥ 8 ng/dl, and/or ≥ 30 after captopril with a plasma aldosterone value ≥ 6 ng/dl, and/or with a logistic discriminant function score ≥ 0.50, and also in one in every four consecutive patients not fulfilling such criteria (Fig. 1). For the screening test and the SIT, treatment with mineralocorticoid receptor antagonists was withdrawn for at least 6 weeks; diuretics, β-blockers, angiotensin-converting enzyme inhibitors and AT-1 receptor blockers were stopped for at least 2 weeks. Only a long-acting calcium channel blocker (CCB) and/or doxazosin was allowed, if necessary, for minimizing the risks of uncontrolled hypertension.

Because hypokalemia blunts aldosterone secretion, and therefore might hamper the detection of suppressibility of aldosterone with volume expansion, the SIT was performed only when serum K^+ levels were ≥ 3.0. To achieve this goal, oral potassium supplementation was allowed before the SIT. The SIT entailed infusion of 2 l of 0.9% saline i.v. over 4 h [27], beginning between 0800 and 0930 h, when subjects remained recumbent. Before and after 4 h of infusion, blood was drawn from a forearm vein for the measurement of PRA, plasma aldosterone, cortisol and serum K^+.

Further tests

By protocol, the results of this test should not affect the further diagnostic work-up. To avoid a bias when evaluating the SIT performance, high-resolution computed tomography (CT) and/or magnetic resonance imaging (MRI) [8] was required in all patients who had a positive screening test; nodules were identified and measured as described [8]. Regardless of the CT or MRI findings, patients with positive screening tests who were willing to eventually undergo adrenalectomy were submitted to AVS [20] at five of the centers or to dexamethasone-suppressed adrenocortical scintigraphy at the other nine centers, to identify a lateralized excess production of aldosterone [8]. Adrenocorticotrophic hormone (ACTH) stimulation was not systematically used during AVS because it does not improve the diagnostic accuracy [35,36]. AVS was deemed to provide a lateralization diagnosis by the criteria previously detailed only if bilaterally selective [20].

Fig. 1



Flow chart indicating the study design. After enrolment, 55 patients were discarded from further analysis because of incomplete data, protocol violations or unwillingness to undergo further testing. ARR, aldosterone-renin ratio; APA, aldosterone-producing adenoma; IHA, idiopathic hyperaldosteronism; LDF, logistic discriminant function; PH, primary hypertension; SIT, saline infusion test.

Biochemical measurements

Serum and urine Na^+ and K^+ levels were measured by standard methods; hypokalemia was defined as serum $K^+ \leq 3.5$ mEq/l. PRA was measured by radioimmunoassay using commercial kits (RenCTK; Sorin Biomedica, Saluggia, Italy in 10 centers; Angiotensin I RIA CT; Radim, Pomezia, Italy in the remainders). The normal range when sitting at rest and on a normal Na^+ diet is 0.51–2.64 ng/ml per h; intra- and inter-assay coefficients of variation were within 8 and 10% for both kits. The assay for aldosterone was performed with a commercially available diagnostic kit (Aldosterone Mirya, Technogenetics, Cassina de Pecchi, Italy). The normal range is 2.0–15.0 ng/dl supine, 3.0–32.0 ng/dl upright on a normal Na^+ diet; both intra- and inter-assay coefficients of variation assay were less than 5.6%; the cross-reactivity of the antibody for aldosterone for the other adrenal steroids was $< 0.001\%$.

Cortisol (normal range 50–250 ng/ml) was measured with a commercial kit (Cortisol Bridge; Adaltis, Casalecchio di Reno, Italy); the intra- and inter-assay coefficients of variation of this assay were less than 6 and 10%, respectively. The cross-reactivity of the antibody for the other adrenal steroids was 18% for 11-desoxycortisol, 7.5% for corticosterone, 7.5% for 21-desoxycortisol; 7.3% for desoxycorticosterone, 6% for 17α-progesterone, and less than 0.1% for aldosterone and most other known steroids. Hormone values showed no differences across centers; nonetheless, samples are being re-assayed in a single laboratory (Padua) for a quality control project.

Diagnostic criteria

The diagnosis of PA required evidence of autonomous excess aldosterone production based on an ARR ≥ 40 baseline, and/or ≥ 30 after captopril, and/or with a logistic discriminant function score ≥ 0.50 as described above [8]. A keynote feature of the PAPY study entails the recognition that the cause of PA can be unequivocally identified only in APA, albeit with the strict diagnostic criteria described in Table 1. Cure of hypertension was defined as a systolic blood pressure < 140 mmHg and diastolic blood pressure < 90 mmHg without medications; improvement as a systolic and diastolic blood pressure $< 140/90$ mmHg, respectively, or a fall by at least 10% from baseline on the same or reduced number of medications and/or reduced number of defined daily doses, as described previously [8]. Thus, on completion of the diagnostic work-up, the leading investigator of each hypertension

Table 1 The 'four corners' criteria for the diagnosis of aldosterone-producing adenoma

Four corners criteria
1. Biochemical diagnosis of primary aldosteronism
2. Unequivocal evidence of lateralized aldosterone secretion at:
bilaterally selective adrenal vein sampling or
mineralocorticoid adrenal scintigraphy.
3. Evidence of adrenocortical nodule on computed tomography
and/or magnetic resonance imaging and/or surgery and/or histopathology
4. Post-adrenalectomy follow-up data:
Cure or improvement of hypertension
Correction of the biochemical picture of primary aldosteronism

Note that all four items must be satisfied.

center determined whether the patient had PA or not. The diagnosis of APA thereafter had to stand on the aforementioned criteria. Patients with a biochemical evidence of PA but without conclusive evidence for a lateralized excess of aldosterone, were presumed to have IHA.

Statistical analysis

Quantitative variables, including PRA, aldosterone and cortisol, that showed a skewed distribution were transformed to achieve a normal distribution. One-way analysis of variance with Bonferroni's test post-hoc was used to compare quantitative variables between groups. The distribution of categorical variables was investigated by chi-squared analysis; correlation was assessed by a non-parametric Spearman test. $P < 0.05$ was considered statistically significant.

The cut-off values that gave the highest accuracy (e.g. the best combination of sensitivity and false-positive rate) were determined by the sensitivity/specificity versus criterion value plot [37]. The accuracy of the SIT for identifying PA, APA and IHA was estimated by the area under the receiver operator characteristics (ROC) curve (AUC) which can be interpreted as: (i) the average value of sensitivity for all possible values of specificity and (ii) the average value of specificity. When the variable under study cannot distinguish between groups, the AUC is 0.5 (e.g. the ROC curve will coincide with the diagonal). When there are no values overlapping between the groups, the AUC equals 1 and the ROC curve will reach the upper left corner of the plot.

To investigate the performance of the test in patient cohorts with a different prior probability (prevalence) of PA and its subtypes, the ROC curves were determined in all patients, and then in those with a positive screening test [8]. Comparison of the ROC curves [37] was performed with MedCalc software, version 9.2.0.0 (MedCalc Software, Mariakerke, Belgium).

Power of the study

Power calculation (with MedCalc) showed that a study with 67 patients with and without PA, or any of its subtypes, had a 90% statistical power to show a statistical difference (at a type I error of 0.05) of 0.350 between the AUC under the ROC curve for the SIT and the identity line.

Results

Baseline anthropometric characteristics

Of the 1180 patients originally enrolled, 55 were excluded from further analysis because of incomplete data, protocol violations or unwillingness to undergo additional tests (Fig. 1). Overall, the anthropometric and biochemical features of these patients did not differ from the remaining 1125 patients. There were no patients with renal failure or heart failure.

One hundred and ninety-seven (75.5%) of the eligible patients with a negative screening test and 120 PA patients underwent the SIT. The baseline anthropometric and hormonal features of these 317 patients divided by diagnosis are shown in Table 2. The APA patients were older and had a higher systolic blood pressure (BP) than those with PH. They did not differ from the patients with IHA, except for lower serum K^+. By definition, in both APA and IHA patients, plasma aldosterone and ARR were higher, and renin and potassium levels were lower than in the PH patients, whereas plasma cortisol levels were similar.

The SIT was performed, on average, 4 weeks after the screening; however, there were no significant differences between the PRA, aldosterone, cortisol and ARR baseline values measured at the screening test and at the SIT.

At the time of the SIT, 41% of the patients were untreated, 35% were on a CCB or doxazosin, and 24% were on both agents. The APA patients required a combination of the two antihypertensive drugs more often (42% of cases) than the other two groups. However,

Table 2 Anthropometric and biochemical characteristics of the patients with primary hypertension (PH) and primary aldosteronism (PA) caused by an aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism (IHA)

	PH ($n=197$)	P	APA ($n=46$)	P	IHA ($n=74$)	P (IHA versus PH)
Age (years, range)	46 (20–76)	$P=0.031$	51 (27–77)	NS	48 (22–73)	NS
Body mass index (kg/m^2)	27.4 ± 4.7	NS	27.4 ± 4.1	NS	26.9 ± 4.1	NS
Systolic blood pressure (mmHg)	147 ± 18	$P=0.002$	158 ± 23	NS	153 ± 16	$P=0.066$
Diastolic blood pressure (mmHg)	97 ± 11	NS	97 ± 10	NS	100 ± 10	$P=0.035$
Serum K^+ (mEq/l)	4.1 ± 0.4	$P<0.0001$	3.4 ± 0.5	$P<0.0001$	3.9 ± 0.4	$P=0.028$
Na^+_uV (mEq/day)	145 (135–155)	NS	131 (110–153)	NS	136 (122–151)	NS
Serum creatinine (μmol/l)	79 (44–195)	NS	83 (53–120)	NS	80 (50–120)	NS
PRA (ng/ml/h)	1.31 (1.00–1.63)	$P=0.002$	0.64 (0.31–0.98)	NS	0.52 (0.37–0.68)	$P<0.001$
Plasma aldosterone (ng/dl)	17.9 (16.3–19.6)	$P<0.0001$	32.1 (26.0–38.2)	NS	25.6 (22.4–28.8)	$P<0.0001$
ARR (ng/dl)/(ng/ml per h)	13.7 (12.2–16.3)	$P<0.0001$	50.2 (26.5–123.2)	NS	49.2 (32.9–77.8)	$P<0.0001$
Plasma cortisol (nmol/l)	146 (137–154)	NS	131 (120–143)	NS	143 (130–156)	NS

Data are mean ± SD (95% confidence interval) for variables not normally distributed; range is reported for age and serum creatinine. PRA, Plasma renin activity; Na^+_uV, sodium urinary excretion; ARR, aldosterone (ng/dl)/PRA (ng/ml per h) ratio; NS, not significant.

it is unlikely that treatment altered the SIT results because there were no differences across treatment groups of PRA, aldosterone and cortisol, within the entire cohort, or in each diagnosis group, at baseline and after the SIT (see below).

Saline infusion test

A rise in blood pressure was occasionally seen during the SIT but no side-effects and no significant change of serum K^+ were recorded (Table 3). PRA, aldosterone and cortisol concentrations decreased in all groups, without differences across treatments. In the PH group PRA, aldosterone and cortisol concentrations fell by 66, 64 and 37%, respectively; in the APA group, they fell by 46, 47 and 44%; in the IHA, the corresponding decreases were 52, 54 and 42%. After the SIT, there were no significant differences of PRA and cortisol, although aldosterone was higher in APA and IHA than in PH group (both $P < 0.0001$) but there was an overlapping of values (Fig. 2).

The percentage fall of aldosterone from baseline correlated with that of PRA in the all cohort ($r = 0.177$, $P < 0.001$), PH ($r = 0.298$, $P = 0.001$) and APA ($r = 0.396$, $P = 0.030$) groups, but not in the IHA group ($r = 0.254$, NS). The fall of aldosterone also correlated with that of cortisol in the all cohort ($r = 0.451$, $P < 0.001$), PH ($r = 0.520$, $P < 0.001$), APA ($r = 0.489$, $P = 0.003$) and IHA ($r = 0.372$, $P = 0.005$) groups.

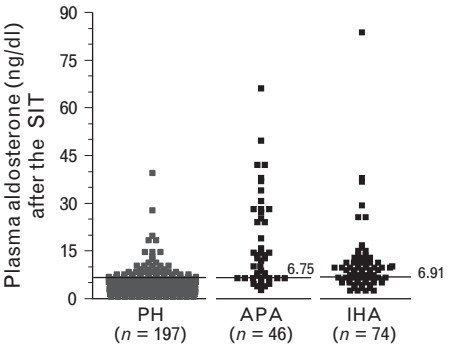
Power of the study

Based on power calculation with its sample size, this study had $> 90\%$ power to investigate the accuracy of the SIT for all diagnosis subgroups.

Diagnostic accuracy of the saline infusion test

Figure 3 illustrates the ROC curve of plasma aldosterone after the SIT (upper panel) and the sensitivity/specificity versus criterion plot (lower panel) for identification of APA. Table 4 shows the AUC of plasma aldosterone after the SIT in the diagnosis groups, along with point estimates of the optimal cut-off value. The AUC differed ($P < 0.0001$) from that under diagonal for diagnosing APA, IHA and PA at large, indicating the usefulness of the SIT. There were subtle differences of optimal cut-offs across diagnosis groups, but point estimate values fell in a narrow range (6.48–6.91 ng/dl).

Fig. 2



The dot diagram shows the plasma levels of aldosterone after the saline infusion test (SIT) in the patients with primary (essential) hypertension (PH), aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism (IHA). On average, values were significantly ($P < 0.001$) higher in both the APA and IHA groups compared to the PH group, but there was a marked overlap of individual values across groups. The optimal cut-off values for discrimination, as determined by the sensitivity/specificity versus value criterion plot for identifying the two PA subtypes, is also shown.

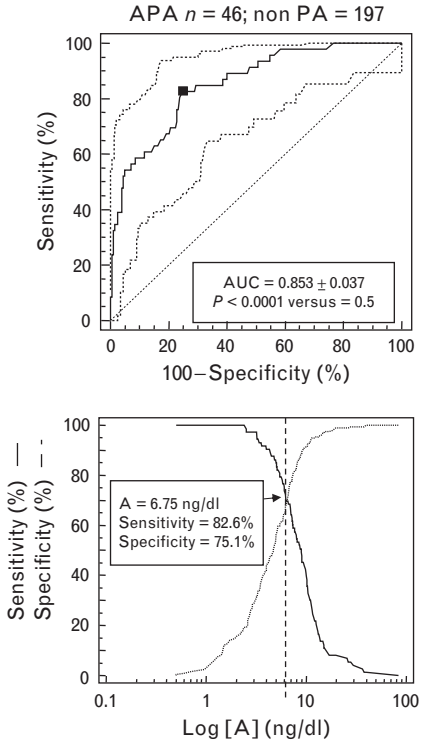
Table 4 shows the AUC and the optimal cut-off values of plasma aldosterone after the SIT for identification of APA, IHA and PA in the all cohort and in the patients with a positive screening test. Table 5 shows the SIT operative features (sensitivity, specificity, likelihood ratios and predictive values) at these optimal cut-off values. The highest diagnostic accuracy (AUC = 0.877, 0.806–0.948) was obtained for identification of APA in the patients with an ARR > 40, the lowest AUC (0.786, 0.727–0.844) for identification of IHA in the all cohort. Thus, a consistent improvement of the AUC was seen by applying the SIT to a pre-selected cohort with a higher prevalence of diseases. Figure 4 shows the SIT positive and negative predictive value as a function of the disease prevalence, at the sensitivity and specificity (82.6% and 75.1%) corresponding to the optimal aldosterone cut-off values for the identification of APA in the all cohort.

Table 3 Changes of plasma renin activity, aldosterone and cortisol observed after the saline infusion in patients with primary hypertension (PH) and primary aldosteronism caused by an aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism (IHA)

	PH ($n = 197$)		APA ($n = 46$)		IHA ($n = 74$)	
	Saline		Saline		Saline	
	Before	After	Before	After	Before	After
Serum K^+ (mEq/l)	4.0 ± 0.3	3.9 ± 0.4	3.5 ± 0.5	3.5 ± 0.3	3.9 ± 0.4	3.8 ± 0.4
Plasma renin activity (ng/ml per h)	1.07 (0.74–1.44)	0.36 (0.35–0.74)**	0.63 (0.32–0.95)	0.28 (0.23–0.49)*	0.64 (0.43–0.86)	0.31 (0.27–0.50)**
Plasma aldosterone (ng/dl)	15.1 (13.5–16.9)	5.5 (4.8–6.1)**	32.4 (25.6–39.1)	17.5 (13.3–21.6)**	24.2 (20.7–27.6)	11.1 (8.5–13.6)**
Plasma cortisol (nmol/l)	101 (91–111)	63 (56–70)**	93 (68–117)	52 (35–70)**	88 (63–112)	51 (36–67)**

* $P < 0.05$, ** $P < 0.0001$ versus before saline.

Fig. 3



The upper panel show the receiver-operating characteristics curve of plasma aldosterone (A) after the saline infusion test for the identification of aldosterone-producing adenoma (APA). The lower panel illustrates the sensitivity/specificity versus value criterion plot used for identifying the optimal cut-off value. In the upper panels, the black square identifies the optimal cut-off value (6.75 ng/dl). PA, Primary aldosteronism.

The saline infusion test end-points

Given the correlation between the SIT-induced changes of aldosterone and PRA, or cortisol, we wondered whether PRA- or cortisol-corrected aldosterone values could provide a better diagnostic accuracy over the raw plasma aldosterone values. Hence, we measured the AUC under the ROC curve of the PRA- and cortisol-corrected aldosterone values for identification of APA, IHA and PA. This analysis showed an AUC under the raw aldosterone values higher than the cortisol-adjusted aldosterone values (0.853, 0.794–0.912 versus 0.764, 0.698–0.822, $P < 0.05$); at variance, the AUC of the raw and the PRA-corrected aldosterone values were superimposed (Fig. 5).

Effect of hypokalemia

By investigating whether the aldosterone response to SIT differed between normokalemic and hypokalemic PA patients, we found no evidence for significant differences in their AUC (Table 6) for diagnosing APA, IHA or PA.

Discussion

A timely diagnosis of the PA subtypes is crucial because removal of a unilateral adrenal cause of excess aldosterone corrects the hyperaldosteronism and cures, or markedly ameliorates, hypertension in most patients, and induces regression of its detrimental consequences [38,39]. Identification of the surgically curable forms of PA requires AVS [22,40], which is costly [41], minimally invasive, and not without risks [20]. Therefore, it should be reserved for patients with confirmed PA who are candidates to adrenalectomy.

Patients are commonly selected for AVS by the demonstration of non-suppressibility of aldosterone after captopril [42], oral sodium loading [26], acute saline infusion, or fludrocortisone and salt [26,43,44]. The latter might be the best confirmatory test [43], but the untrivial risk of severe hypokalemia implying the need of close monitoring through hospitalization lowers its usefulness in practice. Likewise, oral sodium loading has not gained wide acceptance in practice because of poor patient compliance and inconsistent results [26,44]. By contrast, the SIT can be easily performed on an outpatient basis and might be safe and accurate, thus approaching the features of an ideal test [27,33]. However, this test was investigated in a small series of highly selected patients retrospectively, and/or with reference to another test that was optimistically assumed to comprise a 'gold diagnostic' standard [2–6,14]. Instead, it should be evaluated with respect

Table 4 Area (AUC) under the receiver-operating characteristics curve and optimal cut-off values for plasma aldosterone concentration after the saline infusion test (SIT)

Diagnosis	Patients	AUC (95% CI)	*P*	Optimal cut-off (ng/dl)
Primary aldosteronism				
	All ($n = 317$)	0.811 (0.764–0.859)	< 0.0001	6.80
	Preselected ($n = 163$)	0.836 (0.774–0.898)	< 0.0001	6.91
Aldosterone-producing adenoma				
	All ($n = 243$)	0.853 (0.794–0.912)	< 0.0001	6.75
	Preselected ($n = 105$)	0.877 (0.806–0.948)	< 0.0001	6.48
Idiopathic hyperaldosteronism				
	All ($n = 271$)	0.786 (0.727–0.844)	< 0.0001	6.91
	Preselected ($n = 129$)	0.812 (0.738–0.886)	< 0.0001	6.91

Preselected patients were those with a positive screening test, as described in the Patients and Methods section. *P*-values denote the significant difference from the AUC of the identity line. The optimal cut-off was the value of plasma aldosterone after the SIT that provided the highest accuracy (e.g. the best trade-off between sensitivity and specificity). CI, Confidence interval.

Table 5 Operative feature of plasma aldosterone concentration after the saline infusion test (at the optimal cut-off values shown in Table 3) for identification of primary aldosteronism (PA), aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism (IHA) in all the patients and in those preselected based on the result of the screening test

Diagnosis	Patients (disease prevalence)	Sensitivity (95% CI)	Specificity (95% CI)	Positive likelihood ratio	Negative likelihood ratio	Positive predictive value	Negative predictive value
Primary aldosteronism							
	All (37.9%)	73.3 (64.5–81.0)	76.1 (69.6–81.9)	3.07	0.35	65.2	82.4
	Preselected (56.4%)	73.9 (63.7–82.5)	80.3 (69.1–88.8)	3.75	0.32	82.9	70.4
Aldosterone-producing adenoma							
	All (18.9%)	82.6 (68.6–92.2)	75.1 (68.5–81.0)	3.34	0.23	44.3	94.9
	Preselected (32.4%)	88.2 (72.5–96.6)	74.6 (62.9–84.2)	3.48	0.16	62.5	93.0
Idiopathic hyperaldosteronism							
	All (27.3%)	68.9 (57.1–79.2)	77.0 (70.7–82.8)	3.02	0.40	53.1	86.9
	Preselected (45%)	70.7 (57.3–81.9)	80.3 (69.1–88.8)	3.58	0.37	74.5	77.0

Preselected patients were those with a positive screening test, as described in the Patients and Methods section. Sensitivity: the probability that a test result will be positive when the disease is present (true positive rate). Specificity: the probability that a test result will be negative when the disease is not present (true negative rate). Positive likelihood ratio: the ratio between the probability of a positive test result given the presence of the disease and the probability of a positive test result given the absence of the disease. Negative likelihood ratio: the ratio between the probability of a negative test result given the presence of the disease and the probability of a negative test result given the absence of the disease. Positive predictive value: the probability that the disease is present when the test is positive. Negative predictive value: the probability that the disease is not present when the test is negative. CI, Confidence interval.

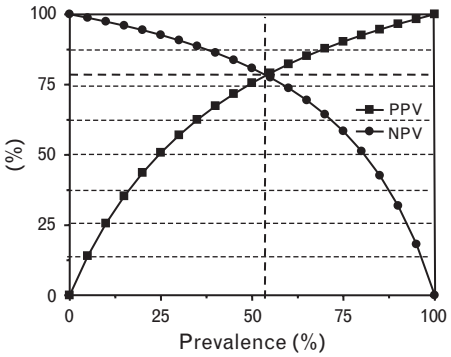
to a conclusive diagnosis [34]. Furthermore, most studies, with few exceptions [10,32], comprised only a few patients without PA, thus precluding calculation of the sensitivity and specificity and increasing the chances of overestimating the SIT performance.

Importantly, even though IHA does not benefit from surgery and mandates medical treatment, practically all studies were designed to confirm PA, rather than identify its surgically curable forms.

A major strength of the PAPY study consists of the use of strict criteria (Table 1) to diagnose APA [8], which provided the opportunity to evaluate the SIT for confirming this diagnosis, rather than that of IHA, and PA at large. Furthermore, the systematic use of the SIT in the patients with a positive screening test and in a large number of those who tested negative, allowed evaluation of its diagnostic performance with unprecedented accuracy under conditions similar to those encountered in daily practice.

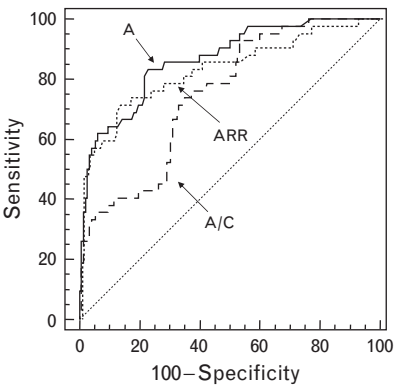
Moreover, patients in the PAPY study were carefully prepared from the pharmacological standpoint for both the screening and the SIT. It is noteworthy that we found no significant differences between the baseline hormonal values at the screening and at the SIT. As the latter was performed some weeks after the former, this finding rules out a carry-over effect of previous antihypertensive drug treatment on hormone levels.

Fig. 4



The plot shows the positive and negative predictive value as a function of the prevalence (pre-test or prior probability) of disease. Both were calculated at a sensitivity of 82.6 and a specificity of 75.1%, corresponding at the value of plasma aldosterone after the saline infusion test (SIT) of 6.75 ng/dl that furnished the highest accuracy for the identification of aldosterone-producing adenoma (APA). The plot shows that, under the most common conditions encountered in practice (e.g. with a disease prevalence $< 50\%$), the SIT test performs better at excluding rather than confirming the presence of the disease. The dashed vertical line identifies the APA prevalence at which the postive and negative predictive value curves cross. The dashed horizontal line identifies the corresponding predictive values.

Fig. 5



Comparison of the receiver-operating characteristics curve of plasma aldosterone, the aldosterone/renin ratio (ARR) and the aldosterone/cortisol ratio (A/C) after the saline infusion test for the identification of aldosterone-producing adenoma. Plasma aldosterone performed significantly better than the A/C ratio ($P < 0.05$) whereas no difference was found between aldosterone and the ARR.

Table 6 Results of the area under the receiver-operating characteristics (ROC) curve (AUC) analysis for plasma aldosterone concentration after the saline infusion test (SIT) in the different diagnosis group according to the presence or absence of normokalemia

Diagnosis	Patients (*n*)	AUC (95% CI)	*P*	Normokalemic versus hypokalemic
Primary aldosteronism				
Normokalemia	74	0. 805 (0. 751–0.858)	<0.0001	NS
Hypokalemia	33	0.763 (0.577–0.948)	0.033	
Aldosterone-producing adenoma[a]				
Normokalemia	209	0.821 (0.739–0.903)	<0.0001	NS
Hypokalemia	26	0.802 (0.628–0.976)	0.016	
Idiopathic hyperaldosteronism[a]				
Normokalemia	247	0.798 (0.737–0.859)	<0.0001	NS
Hypokalemia	15	0.661 (0.370–0.951)	NS	

Patients were classified as normokalemic or hypokalemic if they had serum K^+ before the SIT $\geq$ or $<$ 3.5 mEq/l, respectively. *P*-value denotes significant differences from the AUC of the identity line. [a] For identification of aldosterone-producing adenoma, cases with known idiopathic hyperaldosteronism were not included in the ROC analysis and vice versa. Hence, the AUC can overestimate the accuracy of the test in this subgroup analysis. CI, Confidence interval; NS, not significant.

The saline infusion test for confirming aldosterone-producing adenoma, idiopathic hyperaldosteronism and primary aldosteronism

In this series of hypertensive patients without renal and heart failure, the test was well tolerated and did not change serum K^+, in keeping with a recent report [31]. However, a modest rise in blood pressure was occasionally observed. The patients with PH, APA, and IHA showed a similar decrease of aldosterone following volume expansion, but the hormone remained significantly higher in the APA or IHA patients than in PH patients (Tables 3 and 5). We found no evidence for any treatment effect on hormone values (either the baseline or after the SIT), which is strong evidence against a long-acting calcium entry blocker and/or doxazosin influencing the aldosterone response to volume loading. From the practical standpoint, this implies that these treatments can be allowed during the SIT.

Accuracy and predictive values of the saline infusion test

The diagnostic accuracy (AUC) of the SIT for identification of APA, the only forms of PA that can be conclusively diagnosed, was higher ($P < 0.0001$) than that under the diagonal (Table 1; and also for IHA and PA, Fig. 3 and Table 4). However, at the plasma aldosterone value of 6.75 ng/dl, which corresponds to the highest point estimate of accuracy, the sensitivity and the specificity were only moderate (Tables 4 and 5).

Clinicians are more interested in the positive predictive value (e.g. the probability that the disease is present when the test is positive) and the negative predictive value (e.g. the probability that the disease is absent when the test is negative), rather than in knowing the test sensitivity and specificity. Hence, we calculated these values as a function of the APA prevalence (Fig. 3) and found that, at the relatively low ($< 50\%$) prevalence of APA that can be expected at most centers, the SIT performed better at excluding rather than confirming the presence of APA. At the high (37.9%) PA prevalence of our selected cohort, the positive and negative predictive values were 65.2 and 82.4%, respectively. Thus, even when the disease prevalence is more than three-fold greater than that (11.2%) found in the all PAPY study [8], the SIT was only moderately sensitive for identifying PA. At this prevalence rate, due to a slightly higher specificity, the SIT showed a high negative predictive value, indicating that it performs better with respect to exclusion rather than as a confirmation test. As predicted from Fig. 3, the negative predictive value increased further in all groups when the subgroup with a lower disease prevalence was examined. Conversely, a lower negative, but a higher positive predictive value, were obtained only when patients with a positive screening test (e.g. with a 56.4% PA prevalence) were evaluated (Table 4).

Accordingly, the SIT should be applied to populations with an enriched prevalence of PA, as in those pre-selected based on a screening test. However, even under these conditions, its accuracy is moderate and false-positive and negative results are to be expected. Finally, under the most common conditions of APA and PA prevalence, the SIT is more useful at ruling out than confirming the presence of the disease.

Our results are difficult to place in the context of the available data because of the lack of a conclusive diagnosis of PA subtypes in most studies as a result of the questionable criteria for diagnosing APA and PA [12,31]. For example, in one of the most rigorous studies available, the diagnosis of PA, but not of APA, was based on the SIT result itself, thus introducing a tautological bias [25]. Moreover, the patients with severe hypertension, who comprise a substantial proportion of the APA patients, were excluded. Notwithstanding the high accuracy found in that study [25], recent experiences with the SIT using other tests as a referent reported a moderate accuracy of the SIT in accordance with our results [28,31,43].

Is plasma aldosterone the best end-point of the saline infusion test?

The significant correlations of aldosterone and PRA or cortisol changes during the SIT indicate that the fall of aldosterone was likely induced by the acute volume

expansion and decrease of the ACTH drive during the morning of the SIT. Hence, the hypothesis arises that the diagnostic accuracy of the SIT for identifying APA, IHA and PA increased by correcting the percentage change of aldosterone for those of either PRA or cortisol. By contrast, we found no improvement of the AUC under the ROC curve by using the ARR (0.838, 0.777–0.887) or the aldosterone/cortisol ratio (0.747, 0.679–0.808) compared to aldosterone alone (Fig. 5). Thus, measuring PRA and/or cortisol does not improve the diagnostic accuracy performance of the SIT.

Accuracy of the saline infusion test according to serum K^+ levels

Studies supporting the use of the SIT for discriminating APA or IHA from PH were performed mainly in hypokalemic patients [28,33,45], but it was recently suggested that the SIT would fail to provide discrimination between PH and normokalemic PA [12]. Moreover, a low sensitivity of the SIT was reported in normokalemic PA patients [43]. However, because hypokalemia blunts and normokalemia increases aldosterone secretion, normokalemia should restore the responsiveness of aldosterone to volume expansion. Most of our patients underwent the SIT when normokalemic, but some remained hypokalemic, thus allowing investigation of whether the response of aldosterone differs between normokalemic and hypokalemic PA patients. The AUC did not differ between the normokalemic and the hypokalemic patients (Table 6), for identification of PA or its major subtypes. Thus, we found no support for the contention that the SIT may be inaccurate in normokalemic patients [12].

Limitations of the study

By protocol, we used a relatively high cut-off (≥ 40), which was higher than the optimal cut-off (≥ 25.86) determined experimentally [8]. Hence, it might be that milder forms of APA (and PA) were overlooked in patients without aldosterone suppressibility after the SIT (Fig. 1).

Moreover, few small APA, undetectable by CT, might have been mislabelled as IHA at centers where AVS was not available. This possibility is supported by the finding that, when the results were analysed according to the availability of AVS, not only the estimates of accuracy widened because of the smaller sample size, but also a clear-cut increase of the AUC for APA, and PA was seen (Table 7). This was likely because the relative proportion of APA increased and that of IHA decreased at centers with AVS availability, and vice versa [8].

Finally, caution is advised before applying inflexibly the post-SIT aldosterone cut-offs identified in this study because some variability in the estimate of the optimal cut-offs might occur at other centers with the use of different methods for aldosterone assay.

Table 7 Results of the receiver-operating characteristics curve (AUC) analysis of plasma aldosterone concentration after the saline infusion test (SIT) for identification of the aldosterone-producing adenoma, idiopathic hyperaldosteronism and primary aldosteronism in the five centers with availability of adrenal vein sampling (AVS) and at the nine without AVS

Diagnosis	Patients	AUC (95% CI)	*P*
Primary aldosteronism			
	At centers with AVS ($n=112$)	0.846 (0.763–0.929)	<0.0001
	At centers without AVS ($n=205$)	0.725 (0.725–0.849)	<0.0001
Aldosterone-producing adenoma			
	At centers with AVS ($n=104$)	0.889 (0.810–0.968)	0.023
	At centers without AVS ($n=139$)	0.824 (0.701–0.842)	<0.0001
Idiopathic hyperaldosteronism			
	At centers with AVS ($n=93$)	0.784 (0.575–0.915)	<0.0001
	At centers without AVS ($n=178$)	0.810 (0.731–0.874)	<0.0001

P-value denotes significant difference from the AUC of the identity line.

Conclusions

This multicenter study shows that the SIT: (i) is moderately sensitive for the identification of APA, the major surgically curable subtype of PA, in both hypokalemic and the normokalemic patients and (ii) performs better for excluding rather than confirming the presence of the disease, particularly when the prevalence of APA is less than 50%.

We also identified the optimal plasma aldosterone cut-offs for the diagnosis of APA, IHA and PA in cohorts with different disease prevalence. Further research is ongoing to determine whether the SIT offers any advantage over the more simple captopril test. The pathophysiologic mechanisms underlying the incomplete suppression of aldosterone in several hypertensive patients without overt PA are also worthy of further investigation.

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