Comparison of Confirmatory Tests for the Diagnosis of Primary Aldosteronism

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Context: Primary aldosteronism (PA) is the most frequent form of secondary hypertension, accounting for up to 5–10% of all hypertensive patients, and the diagnosis of PA can present an important challenge for the clinician. After a positive screening test, the diagnosis is confirmed by a suppression test, often an iv saline load test (SLT) or a fludrocortisone suppression test (FST). The FST is considered by many to be the most reliable but is more complex and expensive.

Objective and Design: Our objective was to compare the specificity of SLT with FST for the diagnosis of PA.

Patients and Setting: The study included 100 hypertensive patients referred to hypertension units with suspected PA after the screening test.

Intervention: All patients underwent FST and SLT.

Main Outcome Measures: We assessed plasma aldosterone concentrations (PAC) before and after FST and SLT.

Results: After iv SLT, 10.4% of the PA patients were negative and 16.1% of patients with essential hypertension were positive after SLT; that is, a correct diagnosis with SLT was obtained in 88% of patients compared with FST. PAC after SLT and PAC after FST were highly correlated ($P < 0.0001$). Receiver operator characteristic curve analysis demonstrated that the best cutoff for PAC after SLT was 5 ng/dl. Patients with aldosterone-producing adenoma displayed a smaller reduction of PAC compared with patients with bilateral adrenal hyperplasia; a PAC after SLT greater than 6 ng/dl identified all patients eventually diagnosed as having aldosterone-producing adenoma.

Conclusions: This study demonstrates that the iv SLT is a reasonably good alternative to the more expensive and complex FST for the diagnosis of PA after a positive screening test. (*J Clin Endocrinol Metab* 91: 2618–2623, 2006)

PRIMARY ALDOSTERONISM (PA) is the most frequent form of secondary hypertension, accounting for up to 5–10% of all hypertensive patients (1). The rate of diagnosis of PA has dramatically increased after the widespread use of the plasma aldosterone concentration (PAC)/plasma renin activity (PRA) ratio as a screening test (2). The diagnosis of PA is an important challenge for the clinician because it has been recently demonstrated that patients with PA exhibit a higher rate of cardiovascular complications compared with matched essential hypertensives (3). It should be emphasized that an increased PAC/PRA ratio is not by itself a diagnosis of PA; a suppression test should always be performed to demonstrate that the aldosterone secretion is inappropriate for a high-salt diet and not normally suppressible; in fact,

between 30 and 50% of patients with a positive PAC/PRA ratio will display aldosterone levels that are normally suppressed after confirmatory testing (1, 4, 5). The most widely used tests are the saline load (either oral or iv) and the fludrocortisone suppression test (FST), the latter of which is considered by some authors the most reliable test for the confirmation of PA (1, 6, 7). A third possibility is the oral saline load, which consists of the administration of salt supplementation for 3 d followed by urinary aldosterone and sodium measurements on the third day (patients are considered positive if urinary aldosterone is >12 μg/d and sodium is >200 mmol/d) (1, 6). The iv saline load test (SLT) is generally preferred to FST because it does not require hospitalization and therefore costs less and is more easily performed; in fact, FST requires 4-d hospitalization and the consumption of fludrocortisone tablets together with salt and potassium supplementation (1, 6, 7). Hypokalemia is frequent during FST, and so potassium levels need to be checked at least twice per day during the test and the doses of potassium supplementation adjusted to maintain normokalemia. Fludrocortisone with salt has been reported by Lim *et al.* (8) to be potentially responsible for adverse cardiac effects; however, that study used a much higher dose of fludrocortisone (1.5 mg/d) and salt (480 mmol/d) compared with the widely accepted protocols for FST (0.4 mg/d and 90

mmol/d, respectively). Finally, only small doses of potassium supplements were used, which may have been insufficient in many patients to prevent potassium loss.

Saline load requires only a 4-h infusion of 0.9% NaCl solution (1, 2, 9) but carries a potential risk for acute volume overload, especially in individuals rendered predisposed by preexisting left ventricular or renal dysfunction. Furthermore, SLT can potentially promote potassium wasting, which is usually not monitored during this test. To the best of our knowledge, only one study over 20 yr ago compared the two tests in patients with suspected PA (10). However, in that study, the FST was performed using more than double the dose that is currently suggested as ideal for the correct application of the test (1 mg/d instead of 0.4 mg/d) (1, 6, 10).

In this study, we compared FST and iv SLT as a confirmatory test in 100 patients with suspected PA after the screening test.

Patients and Methods

One hundred patients were positive for the PA screening test performed in the six units participating in the study in the period from January to December 2004. Patients were enrolled after written informed consent and approval of the study protocol by the local ethics committees. The reasons for patient referral were onset of hypertension at a young age, resistance of hypertension to conventional antihypertensive therapy, hypertension with unexplained spontaneous or diuretic-induced hypokalemia, high plasma aldosterone, low PRA, and/or adrenal incidentaloma. The screening tests were performed according to the conditions and cutoffs considered to be optimal in the single centers and as published previously; in particular, in the centers from Torino and Padova, the screening test was considered to be positive when the PAC/PRA ratio was higher than 40 (PAC in ng/dl and PRA in ng/ml·h) together with PAC higher than 15 ng/dl (2), whereas the centers from Reggio Emilia and Santiago considered the screening test to be positive when the PAC/PRA ratio was higher than 35 and 25, respectively, without a cutoff for PAC (5, 11), and finally, the center from Verona considered a PAC/renin ratio higher than 32 pg/ml as positive, because in their hands, this corresponded to a PAC/PRA ratio of 50 (12). In all centers, unless otherwise specified, PAC and PRA were determined by RIA (Sorin Biomedical Diagnostics, Vercelli, Italy). The intra- and interassay coefficients of variation (CV) for aldosterone were 7.9 and 9.6%, respectively; the normal range is 2–12 ng/dl supine and 5–0 ng/dl upright. The intra- and interassay CV for PRA were 5.4 and 9.1%, respectively; the normal range is 0.4–3 ng/ml·h supine and 1.5–6 ng/ml·h upright. In Verona, instead of PRA, renin was measured as direct active renin (DAR) by the Nichols Diagnostics (San Juan Capistrano, CA) chemiluminescent immune assay performed on the automated Nichols Advantage System. The intra- and interassay CV were less than 5 and 8%, respectively. The cutoff of the aldosterone/DAR ratio was chosen according to the data of a previous study where DAR was compared with PRA (12). In that study, PRA and DAR were closely correlated (r = 0.87; $P < 0.0001$). In Santiago, PAC was measured by RIA using a commercial kit from Diagnostic Products Corp. (Los Angeles, CA). The intra- and interassay CV for PAC were 5.1 and 5.9%, respectively. The PRA was determined as previously described by Kreft *et al.* (13). The intra- and interassay CV for PRA were 7.5 and 9.1%, respectively. Both methods demonstrated a high correlation with the PAC and PRA RIAs used by the Italian groups.

In all centers, blood samples were obtained in the sitting position between 0800 and 1000 h. All antihypertensive drugs were stopped at least 3 wk before the PAC and PRA measurements (at least 6 wk before for diuretics and at least 8 wk before for spironolactone). Patients were advised to maintain a diet with normal and constant sodium intake (120 mmol sodium and 60 mmol potassium per day). All patients underwent both iv SLT and FST. Patients not in washout during the screening period and therefore taking an α-blocker (doxazosin) and/or a calcium channel blocker (verapamil or amlodipine) maintained the same therapy during and for the period between the two tests.

For the period between the screening and the end of the second confirmatory test, patients were advised to maintain the same diet and sodium intake, as described above. The second test was performed after at least 4 wk from the first. The two tests were performed as described previously (1, 2): in particular, iv SLT was performed by infusing 2 liters of 0.9% NaCl over 4 h, and the test was considered positive if posttest PAC levels were more than 5 ng/dl (1, 9, 10). In the FST, upright PAC was measured at 1000 h after 4 d fludrocortisone acetate (0.1 mg every 6 h) administration and sodium chloride supplementation (slow-release sodium chloride given after meals, 30 mmol thrice daily) and with patients consuming sufficient dietary salt to achieve a urinary excretion rate of 3 mmol/kg·d. The FST was considered positive if the posttest upright (1000 h) PAC levels were more than 5 ng/dl (1, 2, 6, 7). Potassium levels were carefully monitored during FST to minimize changes of plasma potassium levels, and potassium supplementation was modified accordingly. Plasma potassium levels were monitored during SLT in a subgroup of 61 patients after which we did not perform this assay anymore because of the small variation observed during this test (potassium variations during the test were −0.05 ± 0.2 mmol/liter). All patients underwent a computed tomography (CT) scan with fine cuts (2.5–3 mm) of the adrenal. Adrenal venous sampling (AVS) was not available in all centers and was performed in 37 (55%) of 67 patients with confirmed PA. Criteria for cannulation and for lateralization were considered as described previously (2). Two patients were excluded from the final evaluation, because they had PRA more than 1 ng/ml·h at the end of the FST. All adenomas were surgically removed and confirmed by histological examination. All patients with PA were screened for glucocorticoid remediable aldosteronism (GRA) using a long-PCR technique, as described previously (14–16).

Statistical analysis

All evaluated parameters are expressed as mean ± sd or median (25–75th percentile) where appropriate. The normal distribution of the various parameters was investigated using the Kolmogorov-Smirnov test. Values between groups were compared by the Student's *t* test and the Mann-Whitney *U* test. Receiver operator characteristic (ROC) analysis was used to determine the test characteristics of the different variables predicting the diagnosis.

Correlations were evaluated by Spearman's correlation coefficient.

The positive predictive value of the test was defined as the ratio between subjects that were true positives (defined by FST) and all subjects that were positive for the test.

The negative predictive value was defined as the ratio between subjects that were true negatives (defined by FST) and all the subjects that were negative for the test. The different ROC curves were compared by the area under the curves (AUC) and by the method of Hanley and McNeil (17) A value of z above the critical level of 1.96 was used to accept the hypothesis that the two areas were different. A *P* value of <0.05 was considered statistically significant.

Results

The characteristics of the population studied are shown in Table 1. Seven (10.4%) of 67 patients with PA, as confirmed by FST, were negative after iv SLT and 5 (16.1%) of 31 patients with essential hypertension (EH) after FST were positive after iv SLT; that is, SLT gave a correct diagnosis in 86 of 98 patients (88%). Therefore, the iv SLT resulted in 90% sensitivity and 84% specificity with a positive predictive value of 92% and a negative predictive value of 79% (Table 2). Of the seven false-negative diagnoses of PA with SLT, one displayed a nodule on the CT scan, but AVS revealed bilateral adrenal hyperplasia (BAH), and the remaining six displayed normal adrenals on CT scan but did not undergo AVS because it was unavailable in the centers of diagnosis. Interestingly, the patients who were positive with iv SLT and negative with FST also displayed normal adrenals on CT scan [none underwent AVS and therefore the presence of micro-

TABLE 1. Characteristics of the population studied after subdivision into EH and PA according to the results of FST

	EH (range)	PA (range)	P
n	31	67	
Age (yr)	50.8 ± 8.2	50.5 ± 10.2	0.85
SBP (mm Hg)	151 ± 16	163 ± 16	0.0007
DBP (mm Hg)	94 ± 8	103 ± 9	<0.0001
Serum K (mEq/liter)	4.1 ± 0.3	3.7 ± 0.6	0.0009
Upright PRA (ng ml^{-1}h^{-1})	0.3 (0.2–0.4)	0.2 (0.2–0.3)	0.001
Upright PAC (ng/dl)	16.9 ± 5.1	30.8 ± 16.5	<0.0001
Upright PAC/PRA	51.7 (38–65)	120.6 (71–194.3)	<0.0001
PAC before SLT (ng/dl)	15.3 ± 5.2	27.7 ± 13.5	<0.0001
PAC after SLT (ng/dl)	3.7 ± 0.2^a	13 ± 10.3^a	<0.0001
SBP before SLT (mm Hg)	144 ± 18	154 ± 17	0.009
DBP before SLT (mm Hg)	88 ± 10	96 ± 9	0.0001
SBP after SLT (mm Hg)	154 ± 14^b	160 ± 19^c	0.12
DBP after SLT (mm Hg)	93 ± 9^d	98 ± 8	0.01
PAC before FST (ng/dl)	14.9 ± 3.8	28 ± 12.5	<0.0001
PAC after FST (ng/dl)	4 ± 1^e	17 ± 10.9^a	<0.0001
SBP before FST (mm Hg)	144 ± 15	156 ± 18	0.002
DBP before FST (mm Hg)	90 ± 8	96 ± 8	0.003
SBP after FST (mm Hg)	158 ± 12	164 ± 2^c	0.10
DBP after FST (mm Hg)	98 ± 7	99 ± 0.1^f	0.69

Data are expressed as mean $\pm$ SD or median (25th–75th percentiles). SBP, Systolic blood pressure; DBP, diastolic blood pressure.
[a] Pre *vs.* post, $P < 0.0001$; [b] pre *vs.* post, $P = 0.0002$; [c] pre *vs.* post, $P = 0.0007$; [d] pre *vs.* post $P = 0.004$; [e] $P < 0.0001$; [f] pre *vs.* post $P = 0.02$.

aldosterone-producing adenoma (micro-APA) in some of these patients cannot be excluded]. We did not observe major variations of plasma potassium concentrations during FST (no more than 0.2 mmol/liter) because it was carefully monitored and promptly corrected with supplements. In a subgroup of 61 patients, we measured potassium during SLT (although is not currently done in the test), and we did not observe significant variation of the plasma potassium levels (mean reduction, -0.05 mmol/liter). Therefore, we did not measure potassium levels during SLT in the remaining patients.

Because not all the authors use the same cutoff for the PAC after iv SLT, we investigated the effect of choosing different cutoffs of PAC on the diagnosis of PA. A hypothetical choice of a higher cutoff for post-iv SLT PAC, *i.e.* 7.5 ng/dl, resulted in an increase of false-negative diagnoses of PA (21 of 67, 31.3%) and a decrease of the false-positive diagnoses of EH (one of 31, 3.2%). We also examined the effects of choosing different cutoff values of upright PAC, recumbent PAC, and post-iv SLT PAC on the relationship between sensitivity and specificity rates by ROC curve analysis (Fig. 1A). An upright PAC of 15 ng/dl displayed a sensitivity of 88% and a specificity of 32%; a sensitivity of 100% was obtained with PAC of 7 ng/dl and a specificity of 100% with a PAC of 28 ng/dl. The ROC curve for post-iv SLT PAC showed a sensitivity of 88% with PAC of 5 ng/dl and a specificity of 84%. A sensitivity of 100% was obtained with a PAC of 2 ng/dl, and a specificity of 100% was obtained with a PAC of 7.7 ng/dl. Recumbent PAC did not add significant information compared with upright PAC (sensitivity of 100% with PAC of 7

TABLE 2. Comparison of patients who resulted positive and negative to the FST and SLT

SLT	FST		
	Positive	Negative	Total
Positive	60	5	65
Negative	7	26	33
Total	67	31	98

ng/dl and specificity of 100% with PAC of 26 ng/dl). The AUC was significantly higher for PAC post SLT (AUC, 0.92) compared with upright PAC (AUC, 0.79; z = 4.34), upright PAC/PRA (AUC, 0.85; z = 2.26), and recumbent PAC (AUC, 0.84; z = 2.9).

We also investigated the level of PAC post SLT with the highest sensitivity and specificity, which was 5 ng/dl (88% for both) (Fig. 1B).

PAC were reduced significantly after both tests in patients with PA and EH; however, patients with EH displayed greater reduction of PAC compared with patients with PA, as expected (Table 1 and Fig. 2).

We also considered the effect of choosing different post-iv SLT PAC cutoffs on the diagnosis of APA in the subgroup of patients with the final diagnosis made by AVS; a post-iv SLT PAC of more than 10 ng/dl was observed in 17 (81%) of 21 of the APA diagnosed by AVS. A post-iv SLT PAC of more than 7.5 ng/dl was observed in 20 (95%) of 21 of the APA diagnosed by AVS. A post-iv SLT PAC of more than 5 ng/dl was observed in 21 (100%) of 21 of the APA diagnosed by AVS. This suggests that patients with APA display smaller reduction of PAC after the test compared patients with BAH; in fact, PAC were less suppressed in APA than in BAH ($P < 0.01$) (Fig. 3). We also performed a ROC curve analysis for the post-iv SLT PAC for the diagnosis of APA made by AVS (n = 36); a sensitivity of 100% was observed with PAC higher than 6 ng/dl (with a specificity of 49%).

The two tests did not show statistically significant differences in terms of both blood pressure variation and safety (Table 3). We also performed a linear regression analysis of PAC post iv SLT and PAC post FST; the two parameters were highly associated ($P < 0.0001$; r = 0.784) (Fig. 4).

Eighteen of 21 patients with APA confirmed by AVS have undergone unilateral adrenalectomy, and nine of these patients were cured (PA < 140/90 without treatment), whereas nine displayed a marked reduction of the number of drugs
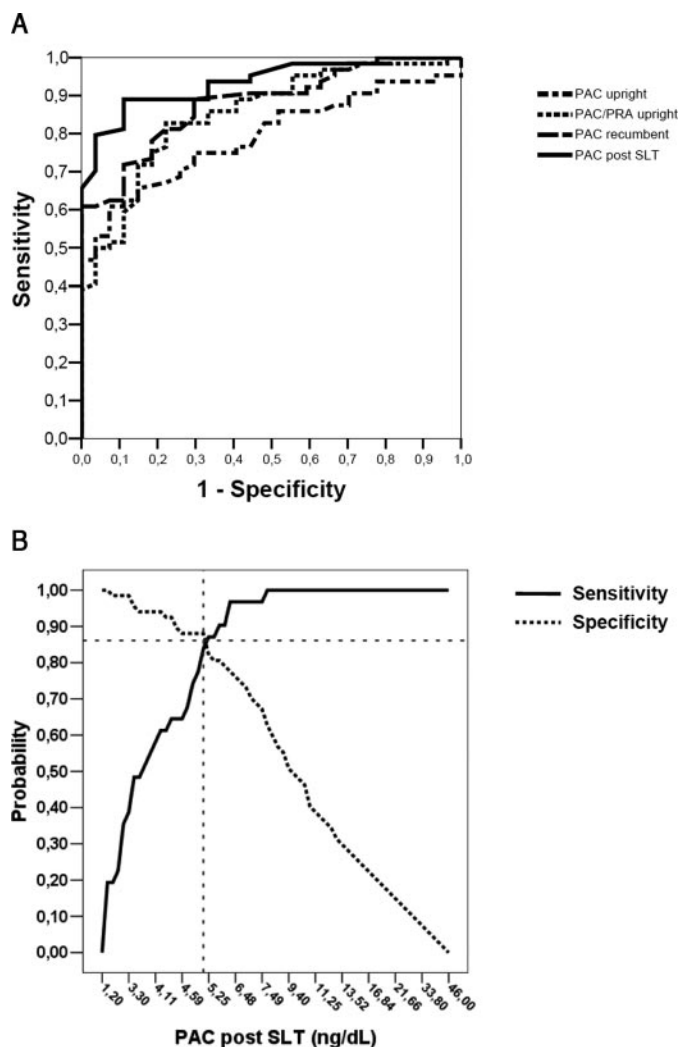
Fig. 1. A, ROC curve analysis of PAC, PAC/PRA, PAC recumbent, PAC post SLT. B, Sensitivity and specificity for PAC post SLT. A cutoff value of 5.0 ng/dl corresponded to specificity and sensitivity of 88%.

Fig. 2. PAC before and after FST and SLT. A clustering *box plot* summarizes the differences in PAC before and after FST and SLT in patients with EH and PA. Median value is indicated by the *thick line*. *Box plot* and *bar* indicate 75th and 95th percentiles, respectively. PAC is significantly higher in patients with PA compared with patients with EH before and after the two tests ($P < 0.0001$ for all comparisons). There is no statistically significant difference between PAC after FST and PAC after SLT in patients with EH and in patients with PA.

necessary to keep blood pressure under control. Furthermore, all patients displayed normalization of the PAC/PRA ratio.

Discussion

Over the last decade, several studies have demonstrated that PA is the most frequent cause of secondary hypertension (1, 5, 11, 16, 18–21). Hence, the diagnostic work-up for PA is highly relevant to current clinical practice. To date, the accepted approach for the diagnosis of PA is based on a screening test, the measure of the PAC/PRA ratio, followed by a confirmatory test in subjects who are positive by the screening test (1). The FST has been considered by some authors as the most reliable confirmatory test for PA (1, 6, 7). However, the FST requires hospitalization, which is time-consuming for both patients and healthcare providers and is highly costly. Although many centers use the iv SLT as an alternative to the FST, a direct comparison between these two tests has not been performed to date. Herein, we report a comparison between FST and iv SLT in a large population of
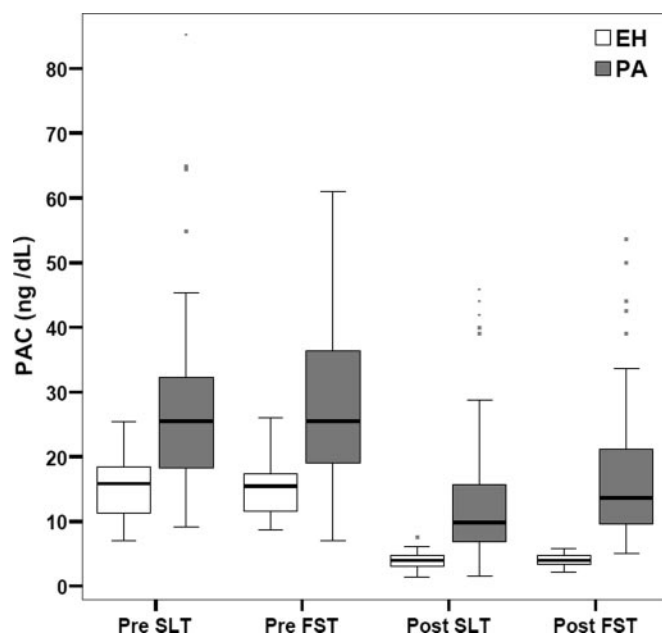
hypertensive subjects with a positive PA screening test. The results of the present study support the SLT test as a reasonably reliable alternative to the more expensive and complex FST for the diagnosis of PA.

In our study, both SLT and FST proved to be safe and generally well tolerated, with a small percentage of patients displaying a significant increase in blood pressure, which was controlled in all cases. The SLT resulted in a correct diagnosis in most cases (88%) and gave a high positive predictive value (92%) with high sensitivity and specificity (90% and 84%, respectively). Seven (10.4%) of 67 PA patients were overall misdiagnosed by SLT. One was shown to have BAH by AVS. The exact proportion of APA missed by SLT cannot be determined by this study because 45% of the patients with PA did not undergo AVS, and CT lacks reliability in differentiating APA from BAH. Hence, it can only be said that, at worst, SLT may have missed six of 27 (22%) of APA, and at best, it did not miss any. However, none of the patients with a final diagnosis of APA were misdiagnosed by SLT, which is of particular importance, because this subtype of PA benefits from and indicates unilateral adrenalectomy. Furthermore, our study shows that a few patients with BAH may be missed with the use of the SLT and thus considered as low-renin essential hypertensives. However, low-renin essential hypertension and BAH may in fact belong to a common pathophysiological continuum, and both conditions have been shown to benefit from low doses of spironolactone (22), which successfully controls hypertension in patients who are positive at the screening test for PA (23, 24).

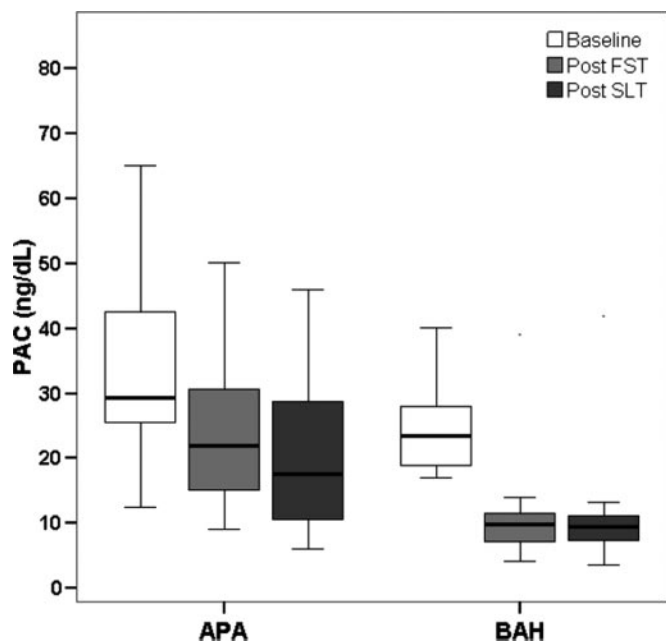The difference between FST and SLT may be because of the

FIG. 3. PAC before and after FST and SLT in patients with APA and BAH. A clustering *box plot* summarizes the differences in PAC before and after FST and SLT in patients with APA and BAH in whom the diagnosis has been made by AVS. Median value is indicated by the *thick line. Box plot* and *bar* indicate 75th and 95th percentiles, respectively. There is no statistically significant difference between PAC after FST and PAC after SLT in patients with APA and in patients with BAH. PAC was significantly higher both after FST and SLT in patients with APA compared with patients with BAH ($P < 0.01$ for both comparisons).
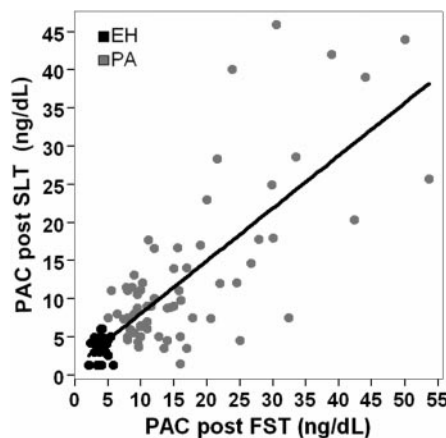

FIG. 4. Linear regression analysis of PAC post iv SLT and PAC post FST. There was a strong association between PAC after SLT and PAC after FST both in patients with EH and PA ($P < 0.0001$; r=0.784). *Gray dots* indicate patients with PA, *black dots* patients with EH.

different types of volume expansion, *i.e.* acute in the iv SLT and subacute in the FST, and/or to the different effects of posture or ACTH rhythm (25). In fact, patients were steadily recumbent during the iv SLT, whereas patients were in the upright position during the FST. This may increase the likelihood of SLT missing angiotensin II (AII)-responsive forms of PA, such as BAH and AII-responsive APA (26). On the other hand, in patients who underwent the iv SLT, the final measurement of aldosterone was performed around noon, when ACTH is lower than the morning baseline; this may have increased the chance of SLT missing forms of PA in which aldosterone is primarily regulated by ACTH and not responsive to AII (*e.g.* GRA and AII-unresponsive APA) (27).

A potential limitation of our study is that the different centers used slightly different screening cutoffs before the confirmatory test because we preferred to maintain the diagnostic screening strategy optimized in each center. How-

ever, the use of a relatively high PAC/PRA cutoff in some centers could have resulted in a lower detection of PA and possibly in decreased false-negative SLT results. Furthermore, because calcium channel blockers can rarely be responsible for a false-negative screening test (1.8% in a previous study) (4), we cannot rule out the possibility that a few patients with a mild form of PA could have been excluded from the study or have had a false-negative result with FST and SLT. Finally, because we did not perform a systematic study of the reproducibility of the two tests, we cannot rule out the possibility that a test would give different results if repeated.

Since the demonstration of a high prevalence of PA in the hypertensive population and especially in patients with grade 3 and resistant hypertension (28), the wide application of the screening test has resulted in a large increase in the diagnosis of PA (2). Consequently, this has also determined an increase in the costs related to the necessary diagnostic procedures. In this respect, when carefully performed, the iv SLT appears to be a reasonable alternative for definitive diagnosis of PA where insufficient resources and experience are available to carry out FST, which should still be regarded as the most reliable approach. In our hands, the best cutoff for the PAC post iv SLT is 5 ng/dl, which confirms the findings of two previous studies (9, 10). However, most confirmed APA (95%) had a PAC post iv SLT higher than 7.5 ng/dl, and therefore, this cutoff may be considered to further reduce the costs by reducing the number of CT scans and AVS.

In conclusion, this study demonstrates that the iv SLT is a reasonably reliable alternative to the FST for the confirmation of the diagnosis of primary aldosteronism in patients who are positive after the screening test.

TABLE 3. Blood pressure variation during iv SLT and FST.

	iv SLT (range)	FST (range)	Significance
SBP after − SBP before	9 (0–13)	10 (0–15)	n.s.
DBP after − DBP before	5 (0–10)	5 (0–10)	n.s.
% SBP variation	5.3 (0–9.4)	6.6 (0–12)	n.s.
% DBP variation	4.5 (0–11)	5 (0–12)	n.s.
BP increase > 30/15 mm Hg	13%	16%	n.s.

Data are expressed as median (25th–75th percentiles). SBP, Systolic blood pressure, DBP, diastolic blood pressure; n.s., not significant.

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