A new highly sensitive and specific overnight combined screening and diagnostic test for primary aldosteronism

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Abstract

Context: Primary aldosteronism (PA) is the most common cause of endocrine hypertension that is diagnosed following a two-step process: an initial screening test, based on the serum aldosterone-to-renin ratio (ARR), followed by a relatively laborious and time-consuming confirmatory test to document autonomous aldosterone (ALD) secretion.

Objective: The aim of this study is to develop a simple overnight test for the early and definite diagnosis of PA.

Patients and methods: Totally, 148 hypertensive patients underwent a fludrocortisone–dexamethasone suppression test (FDST) and the new overnight diagnostic test (DCVT) using pharmaceutical RAAS (renin–angiotensin–aldosterone system) blockade with dexamethasone, captopril and valsartan.

Results: Of the 148 patients, 45 were diagnosed as having PA and they all normalized their elevated blood pressure (BP) after administration of spironolactone or eplerenone. The remaining 103 patients were considered as having essential hypertension and served as controls. Using ROC analysis, the estimated sensitivity and specificity were 91 and 100%, respectively, for the post-FDST ARR, whereas 98% and 89% and 100% and 82% for the post-DCVT ARR and post-DCVT ALD, respectively, with selected cutoffs of 0.32 ng/dL/μU/mL and 3 ng/dL respectively. However, considering these cutoffs simultaneously, the estimated sensitivity and specificity were 98 and 100% respectively. Applying these cutoffs, the diagnosis of PA was confirmed in 44 (98%) of the 45 patients who were considered to have the disease.

Conclusions: In this study, a highly sensitive and specific, low-cost, rapid, safe, and easy-to-perform diagnostic test (DCVT) for PA is described, which could be utilized on an outpatient basis potentially substituting conventional laborious testing.

European Journal of
Endocrinology
(2016) **175**, 21–28

Introduction

Primary aldosteronism (PA) is the most common cause of endocrine hypertension secondary to autonomous or inappropriately elevated aldosterone production from the adrenals. The prevalence of PA varies considerably among hypertensive patients in different studies, depending on patient selection, diagnostic methodology employed, and severity of arterial hypertension (1, 2, 3, 4).

The current standard practice for the diagnosis of PA is to initially apply a screening test using the calculation of basal aldosterone-to-renin ratio (ARR) followed by a confirmatory test to document autonomous aldosterone secretion, such as the oral sodium loading test, saline infusion test (SIT), fludrocortisone suppression test (FST), or captopril challenge test. Using current methodology,

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the estimated prevalence of PA has been found to range from 4.6 to 16.6% (1, 3, 5, 6, 7). However, all previous studies have solely evaluated the renin stimulatory effect on aldosterone secretion without taking into consideration the adrenocorticotrophin (ACTH) effect. Following the recently introduced modification of FST, by adding dexamethasone (FDST) to suppress both renin and ACTH effect on aldosterone secretion, the prevalence of PA was found to be as high as 31% (8, 9).

Making the diagnosis of PA is highly relevant as the clinical signs and symptoms of PA and routine biochemistry are not specific, whereas even mild undiagnosed PA may lead to renal impairment, atrial fibrillation, stroke, and/or myocardial infarction (10, 11, 12, 13). Considering the high prevalence of hypertension in the general population and the high prevalence of PA among hypertensive individuals, reliable, easy-to-perform, and cost-effective diagnostic tests are required for early diagnosis of PA (14).

Making an early diagnosis entails the application of specific treatment by either surgical removal of the hyper-functioning adrenal lesion(s) or administration of targeted medical treatment with mineralocorticoid receptor antagonists (MRAs). However, currently available diagnostic tests for PA are complex, time-consuming, relatively expensive, and therefore cannot be applied widely in primary care units. In order to overcome these limitations, we opted to develop a new rapid overnight diagnostic test for PA aiming at suppressing with appropriate targeted drugs the renin–angiotensin–aldosterone system (RAAS), such as captopril and valsartan, and concomitantly suppress ACTH secretion with the administration of dexamethasone. The diagnostic accuracy of the new test will be evaluated using the well-validated FDST (8, 9).

Patients and methods

We evaluated 148 hypertensive patients, aged 31–71 years, who were investigated for hypertension in our department from 2011 to 2015. Subjects already receiving antihypertensive treatment were considered to be hypertensives and had the date of the diagnosis of hypertension and prescribed medications recorded. Home blood pressure (BP) monitoring was used for assessing the BP status of the participants. Participants were asked to record their BP twice daily (morning and evening) for 7 days. For each BP recording, subjects were instructed to take two consecutive measurements at least 1 min apart while seated. After discarding the measurements

of the first day, we calculated the average value of the remaining measurements to establish the diagnosis of hypertension (systolic BP (SBP) >140 and/or diastolic BP (DBP) >90 mmHg) (15). Exclusion criteria were the presence of cardiovascular, renal or hepatic disease; past or present malignancies; and/or rheumatologic diseases. All participants were euthyroid at their initial visit and remained so during the follow-up period. Postmenopausal women did not receive any hormonal replacement therapy. The reporting of the study conformed to the Strengthening the Reporting of Observational Studies in Epidemiology statement and guidelines. The study protocol was approved by the institution's ethics committee, and informed consent was obtained from all the study participants. At baseline, all participants underwent recording of their medical history and a routine physical examination, including documentation of anthropometric characteristics (weight (kg), height (cm), waist circumference (cm) and body mass index (BMI) (kg/m²)) by the same physician (V Tsiavos).

All hypertensive patients receiving antihypertensive therapy known to affect the renin–aldosterone axis were converted to calcium channel blockers at least 3 weeks before any adrenal hormonal measurement was performed. The newly diagnosed hypertensive patients were also treated with calcium channel blockers. Blood sampling for routine biochemistry and a full blood count were performed, and all participants underwent a FDST as described previously (8). Briefly, fludrocortisone acetate 0.1 mg/6 h per os (PO) and 4 g sodium chloride with meals three times daily were given for 4 days. In addition, 2 mg dexamethasone PO was given at 24:00 h of the fourth day, at least 2 h after the last meal. A blood sample was taken the morning (08:30–9:00 h) of the next day for cortisol (F), aldosterone (ALD), renin (REN), and ACTH measurements, as well as for the calculation of the ALD:REN ratio (ARR). Supplementation with potassium gluconate (4.68 g/8 h) was given to all participants during the FDST to maintain serum K⁺ levels within the normal range (3.5–5.5 mEq/L). In addition, all participants also underwent the overnight diagnostic test (DCVT). The two tests were performed at least 7–13 days apart each other. On the day of the test, the patients did not take their treatment with calcium channel blockers. The rationale of the test was to eliminate all factors known to exert a stimulatory effect on aldosterone secretion acting at various points of aldosterone production. Thus, captopril was administered to inhibit ACE activity, valsartan to counteract the remaining angiotensin activity at the receptor level, and dexamethasone to suppress the ACTH

effect. The ACE inhibitor captopril was preferred because its rapid action within 60–90 min (16) and valsartan because of its high affinity to the AT-I receptor (17). Therefore, all participants received 2 mg dexamethasone, 50 mg captopril, and 320 mg valsartan at midnight, at least 2 h after the last meal the day before the early morning sampling for hormones' measurements to avoid the well-known effect of food on these drugs adsorption. Next morning, due to its short half-life, an extra dose of 50 mg captopril was given 1 h before blood sampling, which was performed between 08:30 and 09:00 h for measurement of circulating cortisol, ALD, REN, ACTH, and potassium levels as well as for the calculation of the ARR. All blood samples were drawn with the participants remaining seated in a non-stressful environment for at least 30 min. In order to evaluate adrenal morphology, computerized tomography (CT) scan of the adrenal glands with 2 mm sections using the Philips Brilliance 16 Spiral scanner was performed in all participants. Adrenal adenomas were defined as well-circumscribed adrenal lesions greater than 10 mm with a non-contrast CT attenuation coefficient of less than 10 Hounsfield units.

Hormonal measurements

Hormonal measurements for ALD, REN, F, and ACTH were performed as described previously (8).

Statistical analysis

Statistical analysis was performed using the SPSS software package (SPSS, version 17.0). A Student's *t*-test and the nonparametric Mann–Whitney test were used to compare the continuous variables with and without

normal distribution respectively. The χ^2 test was used for comparison of categorical variables. The mean $\pm$ S.E.M was used to express the results, whereas $P < 0.05$ was considered statistically significant. The 97.5% percentiles were used to define the upper normal cutoffs for aldosterone suppression. Receiver operating characteristic (ROC) curves have been applied to assess the sensitivity and specificity of the post-FDST and DVCT ARR and ALD levels.

Results

All participants' anthropometric characteristics and biochemical profile are presented in Table 1. The diagnosis of PA was based on the normal cutoffs of post-FDST ALD levels and ARR, 3 ng/dL (84 pmol/L) and 0.9 ng/dL/ μ U/mL (26 pmol/IU) respectively, as defined in previous studies (9, 18). Using these normal cutoffs, 41 (28%) patients (148) were diagnosed with PA. However, another four patients were also considered to have PA, although they had a negative FDST, as they presented with uncontrolled hypertension under treatment with two to three antihypertensive drugs, and had spontaneous hypokalemia, suppressed renin levels, and kaliuresis, while all of them normalized their blood pressure after administration of spironolactone or eplerenone. The post-FDST ALD levels and ARR of these four patients ranged from 1.1 to 2.2 ng/dL (30.7–61.6 pmol/L) and 0.3 to 2 ng/dL/ μ U/mL (8–56 pmol/IU) respectively (Supplementary Table 1, see section on supplementary data given at the end of this article). Only these four patients among the tested hypertensives completed the FDST with significant hypokalemia (K^+ levels: 2.5–3.1 mEq/L). Among the remaining 41 PA patients who were positive on FDST,

Table 1 Anthropometric characteristics and biochemical parameters of essential hypertensives (EH) and patients with primary aldosteronism (PA). Data are presented as mean $\pm$ S.E.M.

	All patients	EH	PA	EH vs PA
<i>n</i>	148	103	45	<i>P</i>
Age (years)	53.60 $\pm$ 1.10	52.90 $\pm$ 1.30	55.00 $\pm$ 2.00	NS
SBP (mmHg)	139.30 $\pm$ 1.50	136.60 $\pm$ 1.80	146.00 $\pm$ 2.40	0.007
DBP (mmHg)	85.06 $\pm$ 0.95	83.50 $\pm$ 1.10	88.70 $\pm$ 1.70	0.02
BMI (kg/m ²)	29.25 $\pm$ 0.43	29.50 $\pm$ 0.50	28.30 $\pm$ 0.70	NS
Na ⁺ (mEq/L)	139.5 $\pm$ 0.28	139.30 $\pm$ 0.15	140.80 $\pm$ 0.36	0.01
K ⁺ (mEq/L)	3.95 $\pm$ 0.03	4.04 $\pm$ 0.03	3.82 $\pm$ 0.08	0.001
UrNa ⁺ (mEq/24 h)	139.07 $\pm$ 4.60	140.90 $\pm$ 5.70	137.00 $\pm$ 7.60	NS
UrK ⁺ (mEq/24 h)	65.30 $\pm$ 2.10	58.90 $\pm$ 2.10	82.50 $\pm$ 4.50	<0.0001
UrK ⁺ /Na ⁺	0.52 $\pm$ 0.02	0.41 $\pm$ 0.02	0.65 $\pm$ 0.04	<0.0001

n, number of patients; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; UrNa⁺, UrK⁺, UrK⁺/Na⁺, 24 h urinary Na⁺, K⁺, and K⁺:Na⁺ ratio respectively; NS, not significant.

seven (17%) had also low end-test K^+ levels ranging from 3.1 to 3.4 mEq/L, whereas among the EH patients, only eight (7.8%) had low end-test K^+ levels ranging from 3.2 to 3.4 mEq/L. In order to exclude a false positive test, all these four patients were asked to repeat the test. However, only one from these four patients, with post-FDST ALD levels 1.5 (43 pmol/L), ARR 0.6 ng/dL/ μ U/mL (21 pmol/L) and K^+ 2.5 mEq/L, agreed to repeat the FDST after normalization of serum K^+ levels with IV potassium administration. She was a 63-year-old obese lady (BMI: 35.50) with well-controlled diabetes mellitus on 850 mg metformin daily, arterial hypertension diagnosed at the age of 52 years, low normal serum K^+ levels (usually), spontaneous hypokalemia (occasionally), suppressed renin levels (2 U/L), and kaliuresis (86.2 mEq/24 h), whereas CT examination revealed a right adrenal adenoma (2.5 cm). Following normalization of serum K^+ levels (4.1 mEq/L), the post-FDST ALD levels and ARR were 6 ng/dL (167 pmol/L) and 3 ng/dL/ μ U/mL (85 pmol/IU) respectively, confirming the diagnosis of PA. Therefore, 42 (28.4%) of the participants were considered to have PA, the three PA patients who failed to be diagnosed by FDST were characterized as almost certainly PA and were excluded from the final statistical analysis, whereas the remaining 103 were diagnosed as having essential hypertension (EH) (Fig. 1 and Table 2).

The group of patients with EH (103) were considered as a control for the overnight DCVT, as normotensive volunteers could not be used to define the normal cutoffs of the test. Using the 97.5% percentiles, derived from the EH patients, the normal cutoffs for post-DCVT ALD level and post-DCVT ARR were 5.8 (162 pmol/L) and 0.9 ng/dL/ μ U/mL (25 pmol/IU) respectively. However, further analysis of the results showed that in ten (9.7%) EH patients, the post-DCVT ARR was inappropriately high, ranging from 0.9 to 1.3 ng/dL/ μ U/mL (25–36 pmol/IU), and was regarded as a false positive result, as the post-DCVT ALD levels were low, not compatible with autonomous aldosterone production, and the post-DCVT REN levels suppressed. In addition, in 15 (14.5%) EH patients, the post-DCVT ALD levels were found to be inappropriately elevated ranging from 3 to 12 ng/dL (85–340 pmol/L), false positive results, compatible with autonomous aldosterone secretion, following a rise in REN levels (range 46–322 μ U/mL), usually above the upper normal limit, giving, however, a low post-DVCT ARR.

The performance characteristics of the two tests used were compared by estimating the sensitivity and specificity of the post-FDST and post-DCVT ALD levels and ARR in diagnosing PA using ROC analysis

Figure 1

Post-FDST and post-DVCT aldosterone (ALD) levels and aldosterone:renin ratios (ARR) in essential hypertensives (EH) vs patients with primary aldosteronism (PA). The horizontal lines represent the mean $\pm$ S.E.M.

(Table 3). The estimated sensitivity and specificity for the post-FDST ARR was 100% with a selected cutoff of 0.9 ng/dL/ μ U/mL (26 pmol/IU) and for post-FDST ALD levels 100% and 81.5% with a selected cutoffs of 3.1 ng/dL (85 pmo/L) respectively. Using the post-FDST ARR, no false positive results were observed in this study; thus, the simultaneous consideration of the post-FDST ALD levels was not considered necessary, as no improvement in the diagnostic accuracy of the test was expected. The corresponding sensitivity and specificity for the post-DCVT ARR were 98 and 89%, respectively, and for the post-DCVT ALD 100% and 82% with selected cutoffs of 0.3 ng/dL/ μ U/mL (9 pmol/IU) and 3.1 ng/dL (85 pmol/L) respectively. However, considering these cutoffs for the post-DCVT ALD and post-DCVT ARR simultaneously, the estimated sensitivity and specificity, using the procedure described by Barry and Ebell (19), were 98 and 100% respectively. Applying these cutoffs, the diagnosis of PA was confirmed in 44 (98%) of the 45 patients who were considered as having the disease, including the four patients in whom the FDST failed initially to confirm

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Table 2 Basal and post-FDST/post-DCVT hormonal levels among patients with essential hypertension (EH) and primary aldosteronism (PA). Data are presented as mean $\pm$ S.E.M.

	EH	PA	P
n	103	45	
Basal ALD (ng/dL)	10.89 $\pm$ 1.31	20.38 $\pm$ 2.43	<0.0001
Basal REN (μ U/mL)	19.01 $\pm$ 1.45	5.32 $\pm$ 0.44	<0.0001
Basal RR (ng/dL/ μ U/mL)	0.62 $\pm$ 0.04	7.51 $\pm$ 1.73	<0.0001
Basal F (μ g/dL)	13.46 $\pm$ 0.52	15.59 $\pm$ 0.91	NS
Basal ACTH (pg/mL)	20.52 $\pm$ 1.90	22.77 $\pm$ 2.90	NS
Urinary F (μ g/24 h)	84.68 $\pm$ 5.53	71.50 $\pm$ 7.20	NS
Post-FDST F (μ g/dL)	1.62 $\pm$ 0.08	2.14 $\pm$ 0.22	NS
Post-FDST ACTH (pg/mL)	6.44 $\pm$ 0.23	5.60 $\pm$ 0.25	NS
Post-FDST ALD (ng/dL)	1.49 $\pm$ 0.07	10.51 $\pm$ 1.42	<0.0001
Post-FDST REN (μ U/mL)	5.30 $\pm$ 0.30	4.46 $\pm$ 0.46	0.01
Post-FDST ARR (ng/dL/ μ U/mL)	0.40 $\pm$ 0.02	3.62 $\pm$ 0.70	<0.0001
Post-FDST K ⁺ (mEq/L)	3.88 $\pm$ 0.04	3.58 $\pm$ 0.07	<0.0001
Post-DCVT F (μ g/dL)	1.86 $\pm$ 0.09	2.32 $\pm$ 0.20	NS
Post-DCVT ACTH (pg/mL)	6.38 $\pm$ 0.24	6.03 $\pm$ 0.23	NS
Post-DCVT ALD (ng/dL)	2.224 $\pm$ 0.25	9.50 $\pm$ 1.33	<0.0001
Post-DCVT REN (μ U/mL)	62.40 $\pm$ 9.20	6.70 $\pm$ 0.70	<0.0001
Post-DCVT ARR (ng/dL/ μ U/mL)	0.11 $\pm$ 0.01	2.14 $\pm$ 0.40	<0.0001
Post-DCVT K ⁺ (mEq/L)	4.42 $\pm$ 0.04	3.86 $\pm$ 0.07	<0.0001

n, number of patients; ALD, aldosterone; REN, renin; ARR, aldosterone:renin ratio; F, cortisol; ACTH, adrenocorticotrophic hormone, FDST, fludrocortisone–dexamethasone–saline test, DCVT, dexamethasone–captopril–valsartan test; NS, not significant.

the diagnosis (Fig. 1). The single PA patient who failed to be diagnosed by DCVT was a 48-year-old lady with a mild form of arterial hypertension diagnosed at the age of 46 years, normal serum K⁺ levels (3.7 mEq/L) without kaliuresis (<60 mEq/24 h), low normal basal renin levels (7 U/L), and significantly increased post-FDST ALD and ARR (11.64 ng/dL (323 pmol/L) and 2.0 ng/dL/ μ U/mL (55.7 pmol/IU) respectively), whereas CT examination revealed a left adrenal adenoma (2.6 cm).

Of particular importance was the effect of FDST and DCVT on serum K⁺ levels. FDST induced a significant decrease in serum K⁺ levels in both EH patients (mean $\pm$ S.E.M., baseline: 4.04 $\pm$ 0.03 mEq/L, post-FDST: 3.88 $\pm$ 0.04 mEq/L, $P=0.0006$) and PA patients (mean $\pm$ S.E.M., baseline: 3.82 $\pm$ 0.08 mEq/L, post-FDST: 3.58 $\pm$ 0.07 mEq/L, $P=0.01$).

By contrast, DCVT induced a significant increase in serum K⁺ levels in EH patients (mean $\pm$ S.E.M., baseline: 4.04 $\pm$ 0.03 mEq/L, post-DCVT: 4.42 $\pm$ 0.04 mEq/L, $P<0.0001$), whereas the test did not affect serum K⁺ levels in patients with PA (mean $\pm$ S.E.M., baseline: 3.82 $\pm$ 0.08 mEq/L, post-DCVT: 3.86 $\pm$ 0.07 mEq/L, $P=0.41$).

The anthropometric characteristics and biochemical profile of EH and PA patients are presented in Table 1. There were no significant differences regarding age, BMI and urinary Na⁺ concentrations between EH and PA patients, whereas serum K⁺ level was significantly lower and SBP, DBP, serum Na⁺, urinary K⁺, and urinary K⁺:Na⁺ ratio were significantly higher in PA compared with EH patients. Basal and post-FDST hormone levels are presented in Table 2. Basal serum cortisol

Table 3 Normal cutoffs and performance characteristic of the tests, as they defined by ROC analysis.

	Controls (normal cutoffs)	PA patients with values > normal cutoffs	EH patients with values < normal cutoffs	Sensitivity (%)	Specificity (%)
Post-FDST ALD (ng/dL)	3	41/45	103/103	91	100
Post-FDST ARR (ng/dL/ μ U/mL)	0.9	41/45	103/103	91	100
Post-FDST ALD (ng/dL) and post-FDST ARR (ng/dL/ μ U/mL)	3/0.9	41/45	103/103	91	100
Post-DCVT ALD (ng/dL)	3	45/45	86/103	100	83
Post-DCVT ARR (ng/dL/ μ U/mL)	0.3	44/45	93/103	98	90
Post-DCVT ALD (ng/dL) and post-DCVT ARR (ng/dL/ μ U/mL)	3/0.3	44/45	103/103	98	100

ALD, aldosterone; ARR, aldosterone:renin ratio; FDST, fludrocortisone–dexamethasone–saline test; DCVT, dexamethasone–captopril–valsartan test.

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(F), ACTH and 24-h urinary F, and post-FDST/DCVT F and ACTH levels did not differ significantly between EH and PA patients, whereas basal and post-FDST/DCVT ALD, REN, K⁺ levels, and ARR were found to be significantly higher in PA patients (0.01 to <0.0001). CT examination revealed that 19 (42.20%) patients with PA had an adrenal adenoma, a prevalence significantly higher than that observed in patients with EH (22/103, 21.40%) ($P < 0.001$).

Discussion

In this study, an overnight combined screening diagnostic test (DCVT) for PA is described using pharmaceutical RAAS blockade and is compared with the FDST, of high diagnostic accuracy, as it is based on suppressing the two main natural stimuli, renin and ACTH, of aldosterone secretion by sodium chloride loading and concomitant dexamethasone administration. The diagnosis of PA was based on the cutoffs of post-FDST ALD levels and ARR, 3 ng/dL (84 pmol/L) and 0.9 ng/dL/μU/mL (26 pmol/IU) respectively, as they have been defined in normotensive volunteers recruited with strict criteria and extensively validated in previous studies (8, 9, 18). These cutoffs were similar to those derived from the group of hypertensive patients with EH, who were served as controls in this study. Using these cutoffs, 41 of the tested patients (148) diagnosed to have PA. However, FDST failed to reveal autonomous aldosterone secretion in another four patients who were considered to have almost certainly PA. Apparently, this FDST failure is directly related to low potassium levels found at the end test, as the negative FDST in one of these patients became positive after normalization of serum K⁺ levels, obtaining adequate post-FDST ALD level and ARR to confirm the diagnosis of PA.

After the pharmaceutical RAAS blockade, a significant rise in renin levels associated with low or suppressed aldosterone levels giving a low ARR in EH hypertensive patients without autonomous aldosterone secretion was expected. Indeed, using a group of patients as controls, who were considered to have EH, the post-DCVT ARR was found to be low in the majority of cases. In only ten (9.7%) patients with EH, the post-DCVT ARR was inappropriately high and was regarded as a false positive result, as the post-DCVT ALD levels were low, not compatible with autonomous aldosterone production, and the post-DCVT REN levels suppressed. However, in another 15 (14.5%) patients with EH, the post-DCVT ALD levels were found to be falsely elevated (false

positive results). This was an unexpected finding as such high aldosterone levels following complete blockage of renin–Ang-II system are not expected. A possible explanation for this finding could be an undefined so far rennin-dependent but Ang-II-independent mechanism or due to Ang-II escape from pharmacological blockage (20). Therefore, in 25 (24%) patients with EH, either the post-DCVT ARR or the post-DCVT ALD levels were found to be falsely elevated (false positive results), affecting the specificity of the test when each of them was used separately. In order to exclude the diagnosis of PA from EH patients with false positive results, the simultaneous consideration of both indices of autonomous aldosterone secretion, post-DVCT ARR 0.3 ng/dL/μU/mL (9 pmol/IU) and post-DVCT ALD 3 ng/dL (85 pmol/L), was adopted. Using these normal cutoffs, the diagnosis of PA was confirmed in 44 of 45 (98%) patients with PA, including the four patients in whom the FDST failed to confirm the diagnosis, as the performance characteristics of the DCVT improved significantly with an estimated specificity 100% and a sensitivity 98%.

It is generally accepted that currently available diagnostic tests for PA, which are based on saline loading for RAAS suppression, are complex, time-consuming, and relatively expensive (21). Therefore, they cannot be widely applied to hypertensive patients in primary health care units. In addition, the oral saline loading test for 4 days, which is employed by either the FDST or the classical FST, often induces a significant decrease in serum K⁺ levels at the end of the test despite the simultaneous oral supplementation with potassium gluconate or potassium chloride. In that case, the test has to be repeated after normalization of serum K⁺ levels with IV potassium administration, further increasing the cost and patient's inconvenience. By contrast, the use of pharmaceutical blockage, as described in the DCVT, is not expected to induce any further decrease in serum K⁺ levels. This was exactly the case in this study where DCVT induced a significant increase in serum K⁺ levels in EH patients leaving unaffected serum K⁺ levels in PA patients.

Although it is well known that any decrease in serum K⁺ levels, even within the normal range, is a strong inhibitor of aldosterone secretion (22, 23), this effect on the diagnostic accuracy of saline loading tests has never been validated. A possible reason for this could be the lack of alternative to the saline loading tests with high diagnostic accuracy to compare with. In this study, we found that significant hypokalemia may cause FDST failure to diagnose PA in a significant number of patients

(9%), who were considered having the disease. These findings underline the necessity of doing the FDST, as well as any other saline loading test, with serum K⁺ levels within the normal range.

The standard diagnostic procedure for PA that has been adopted by almost all and is recommended by the recently published guideline of Endocrine Society (24) has been to initially utilize the basal ARR as a screening test for case detection in high-risk groups of hypertensive patients, and then establish the diagnosis of PA using a confirmatory test. The FDST has been recognized as a new useful confirmatory test of PA (24), as it is sensitive, safe to be performed on an outpatient basis, and has been validated in a relatively large number of patients (9). In this respect, the number of hypertensives with PA who are diagnosed by FDST is expected to represent only a subgroup of those identified by the basal ARR. However, in recent studies (9), we have shown that after performing the FDST in all hypertensives, rather than in those only identified by the basal ARR, the number of hypertensives who had PA based on an elevated basal ARR was significantly lower than that identified by the FDST (43/327 vs 94/327). This suggests that basal ARR as a screening test may diagnose only the more severe cases leaving undiagnosed the milder forms of PA. We consider that the diagnostic limitations that are related to the two-step procedure (screening+confirmatory tests) can be overcome using as a screening diagnostic test, the DCVT, which, as shown in this study, is a simple and very-low-cost test, with similar sensitivity and specificity compared with FDST.

In this study, a low-cost, rapid, safe, and easy-to-perform overnight screening diagnostic test for PA is described. It is based on pharmaceutical RAAS blockade and is characterized by higher diagnostic accuracy compared with a saline loading test (FDST). Therefore, the DCVT can be widely used for the diagnosis of PA on outpatient basis in primary health care units.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-16-0003>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

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Clinical Study	V Tsiavos and others	Overnight diagnostic test for primary aldosteronism	175:1	28
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Received 2 January 2016

Revised version received 6 April 2016

Accepted 12 April 2016