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ORIGINAL ARTICLE

A reverse postural test as a screening tool for aldosterone-producing adenoma: a pilot study

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Abstract The aldosterone-to-renin ratio (ARR) is an accepted screening tool for primary hyperaldosteronism (PA). An ambulatory case finding test to separate surgically remediable aldosterone-producing adenoma (APA) from other forms of PA, however, is currently not available. The aim of this study was to evaluate a reverse postural test (RPT) as a novel tool for identifying APA. We investigated 6 healthy controls, 19 primary hypertensive patients, and a prospective cohort of 32 patients clinically suspicious for primary hyperaldosteronism. We diagnosed seven patients with surgically proven APA, and three patients with idiopathic hyperaldosteronism. Serum aldosterone was measured after 30-min of moderate exercise (Aldo-1) and after a subsequent 2-h supine resting period (Aldo-2) with calculation of the ratio Aldo-2/Aldo-1. Aldosterone significantly decreased after supine resting in both healthy controls and primary hypertensives, but not in patients with APA. Receiver-operating-curve analysis revealed that the RPT was suitable for the screening of APA. A combination of the ratio Aldo-2/Aldo-1 >0.59 and Aldo-2 >160 pg/ml correctly identified all the patients with APA, with no false positives. Although the high sensitivity of the RPT here observed needs to be confirmed in larger studies, the high positive predictive value of RPT could be useful for the identification of APA in outpatients.

Keywords Primary hyperaldosteronism · Aldosterone-producing adenoma · Idiopathic hyperaldosteronism · Hypertension · Aldosterone · Reverse postural test

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Introduction

Primary hyperaldosteronism (PA) is recognized as an important potentially curable form of secondary hypertension [1]. Screening for PA in hypertensive patients with resistance to drug therapy or unexplained hypokalaemia is widely accepted, because early diagnosis with specific treatment reduces morbidity. Measurement of the aldosterone-to-renin ratio (ARR) is the most common screening test for PA [2]. However, an established and simple-to-perform ambulatory case finding test for the differentiation of PA subtypes such as aldosterone-producing adenoma (APA) or idiopathic hyperaldosteronism (IHA) is currently not available. Such a test identifying patients with APA in a screening test would be of considerable interest because APA should be cured by surgical treatment, whereas surgery is contraindicated in patients with other forms of PA like IHA, who can be effectively treated by drugs such as aldosterone antagonists.

Postural stimulation testing is usually performed in inpatients after an overnight sleep and can be helpful to

discriminate between patients with APA and IHA, after the presence of PA has been confirmed. This is because sensitivity to angiotensin II and there by aldosterone response to erect position in patients with IHA is exaggerated [3, 4], whereas more than 60% of subjects with APA secrete excess aldosterone independent of angiotensin II and thus can show unchanged or even decreased plasma aldosterone during the test. Thus, although currently not widely used, measuring aldosterone levels during the postural stimulation test (with the transition from the supine to the erect position) is useful particularly if the test shows lack of responsiveness, consistent with angiotensin II-unresponsive APA [3–5].

However, we are not aware of any studies that reported whether aldosterone responses after the transition from the erect to the supine position are different in patients with the most common sub-types of PA and primary hypertension. Thus, the aim of the present study was to investigate whether the measurement of serum aldosterone in a reverse postural test (RPT) could be used in hypertensive patients to screen for PA or subtypes like IHA or APA in outpatients.

Results

RPT in healthy and in hypertensive controls

During RPT with the transition from the erect to the supine position, serum aldosterone levels significantly decreased in the six healthy controls (247 ± 77 pg/ml (Aldo-1) vs. 42 ± 6 pg/ml (Aldo-2), $P = 0.035$) (Fig. 1a), with a mean Aldo-2/Aldo-1 ratio of 0.29 ± 0.09. Three subjects had a relatively high Aldo-1 (Fig. 1a) with an elevated PRA most likely reflecting the post-exercise situation. There was also a significant decrease in serum aldosterone during RPT in primary hypertensive patients that were untreated (Fig. 1b) or under mono therapy with various antihypertensive drugs (90 ± 14 vs. 36 ± 5 pg/ml, $P = 0.0001$) (Fig. 1c), with a mean Aldo-2/Aldo-1 ratio of 0.44 ± 0.05. The Aldo-2/Aldo-1 ratio was similar in all the hypertensive patients irrespective of whether they were treated with beta-blockers (0.34 ± 0.05), ACE-inhibitors (0.35 ± 0.04) or calcium antagonists (0.43 ± 0.04).

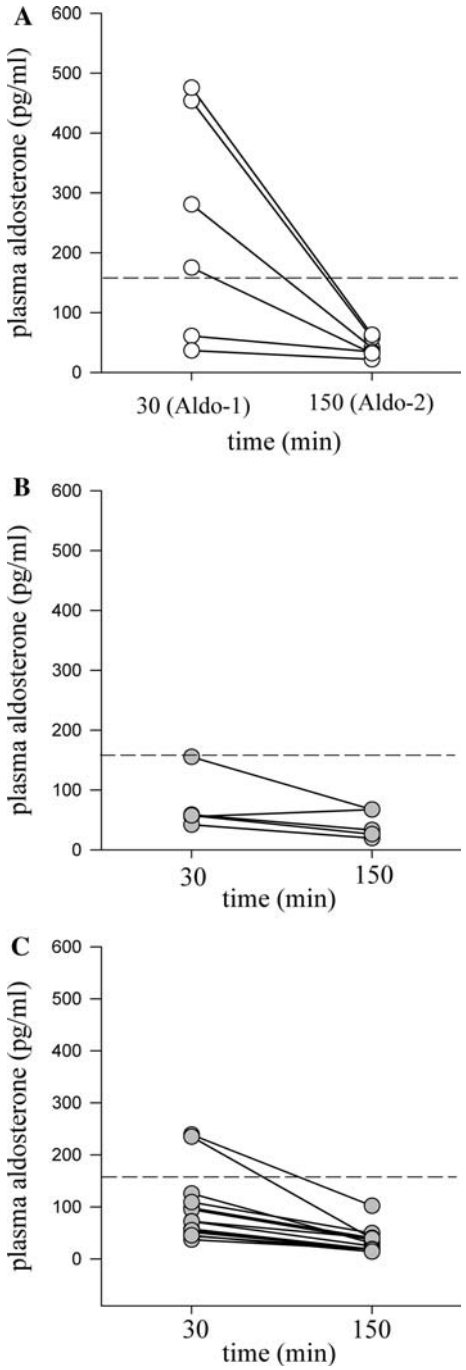

Fig. 1 Serum aldosterone during the RPT in 25 healthy and hypertensive controls. **a** Healthy normotensive controls ($n = 6$), **b** primary hypertensive patients with mild primary hypertension, untreated ($n = 5$), and **c** primary hypertensive patients with antihypertensive mono therapy with beta-blockers, ACE-inhibitors, or calcium antagonists ($n = 14$). *Dotted line*: cut-off (160 pg/ml) for serum aldosterone at 150 min (Aldo2)

RPT in a prospective cohort of patients suspicious for primary hyperaldosteronism

Among the 32 hypertensive patients that were referred because of suspicion for PA, seven patients with APA and three patients with IHA were finally identified (Table 1). All the seven patients with suspected APA were surgically treated and the diagnosis of an APA was proven by the combination of cured hyperaldosteronism after surgery and histological proof of adenoma in all the seven patients. Before surgery, there was no significant decrease of serum aldosterone during the RPT in the patients suspicious for

Table 1 Characteristics of the patients prospectively screened for PA ($n = 32$)

	APA	IHA	Primary hypertension
n	7	3	22
Age (years)	46.3 ± 5.3	63.7 ± 1.8	53.6 ± 2.4
Sex (m/f)	2/5	1/2	6/16
Systolic blood pressure (mmHg)	176 ± 11	150 ± 12	150 ± 5
Diastolic blood pressure (mmHg)	97 ± 5	102 ± 9	90 ± 3
Serum potassium (mmol/l)	3.3 ± 0.2	3.9 ± 0.6	4.2 ± 0.1
Serum aldosterone (pg/ml)	378 ± 50	252 ± 40	211 ± 57
Plasma renin activity (ng/ml/h)	0.14 ± 0.00	0.26 ± 0.12	1.4 ± 0.3
ARR (pg/ml per ng/ml h)	2699 ± 356	1233 ± 285	374 ± 93
Urine aldosterone (μg/24 h)	36.4 ± 4.1	22.5 ± 9.4	25.3 ± 3.7

APA aldosterone-producing adenoma; *ARR* aldosterone/renin ratio; *IHA* idiopathic hyperaldosteronism

APA (378 ± 50 pg/ml vs. 364 ± 39 pg/ml, $P = 0.68$, Fig. 2a). The mean Aldo-2/Aldo-1 ratio in patients with APA was 1.02 ± 0.08 (Fig. 2a). After surgery, six out of the seven subjects were available for further testing, and none of them fulfilled any of the criteria for primary hyperaldosteronism anymore (serum aldosterone 30.1 ± 5.8 pg/ml, ARR 61.2 ± 17.1 pg/ml per ng/ml h, systolic blood pressure 132 ± 5 mmHg, diastolic blood pressure 81 ± 4 mmHg, and serum potassium 4.8 ± 0.2 mmol/l), without any antihypertensive medication. After surgery, the Aldo-2/Aldo-1 ratio during the RPT was 0.66 ± 0.08 and serum aldosterone at the Aldo-2 time point was 18.6 ± 2.6 pg/ml.

In the three patients who were diagnosed with IHA, serum aldosterone dropped from 252 ± 40 pg/ml to 68 ± 15 pg/ml during the RPT ($P = 0.017$) (Fig. 2b) with a mean Aldo-2/Aldo-1 ratio of 0.27 ± 0.02. This was similar to the result of the RPT in the patients, in which the presence of PA was highly unlikely ($n = 22$), as indicated either by a normal ARR together with normal electrolytes and normal hormonal values, or a normal confirmatory test (saline infusion test). This group also comprised the nine patients who were referred because of hypertension together with incidentally detected unilateral adrenal mass. In these 22 patients aldosterone significantly decreased from 211 ± 57 to 67 ± 12 pg/ml, $P = 0.007$ (Fig. 2c), with a mean Aldo-2/Aldo-1 ratio of 0.49 ± 0.08. One of the patients with primary hypertension showed a markedly elevated Aldo-1 (1258 pg/ml) but had a high rather than suppressed plasma renin activity (5 ng/ml h) and a normal ARR of 252 pg/ml per ng/ml h. During RPT, a marked decrease of serum aldosterone was observed (Aldo-2/Aldo-1 ratio 0.21).

Serum cortisol during the RPT

Serum cortisol was available from 43 subjects, which significantly decreased during the RPT from 15.0 ± 0.9 to 8.8 ± 0.6 μg/dl ($P < 0.001$) in agreement with the diurnal cortisol rhythm. This decline in cortisol was independent of the fact whether serum aldosterone decreased or not during the RPT. Thus, it can be excluded that stress-induced changes in serum aldosterone have biased the results.

ARR

The ARR was 179 ± 48 pg/ml per ng/ml h in the healthy controls ($n = 6$). In the hypertensive controls ($n = 19$), ARR was 294 ± 95 pg/ml per ng/ml h in untreated patients with mild hypertension ($n = 5$), and 256 ± 39 pg/ml per ng/ml h in the patients under mono therapy with various antihypertensive drugs ($n = 14$). The ARR in these patients was similar irrespective whether patients were treated with beta-blockers (298 ± 32 pg/ml per ng/ml h), ACE-inhibitors (162 ± 96 pg/ml per ng/ml h), or calcium antagonists (291 ± 70 pg/ml per ng/ml h). In patients with APA and IHA, the ARR was 2699 ± 356 and 1233 ± 285 pg/ml per ng/ml h, respectively. This was significantly higher than in the hypertensive patients (374 ± 93 pg/ml per ng/ml h, $n = 22$), and the hypertensive and healthy controls ($P = 0.0001$, respectively).

RPT and ARR for the screening of patients with PA and APA

Since the RPT appeared to be a potential screening test for patients with PA and more specifically for patients harboring an APA, we tested the ability of the Aldo-2/Aldo-1 ratio to identify patients with PA or APA. Receiver operating characteristic (ROC) curves were used to analyze the characteristics of the Aldo-2/Aldo-1 ratio and its suitability as a potential screening test. The specificity was calculated at the 100% sensitivity level, which is preferable for a screening tool. Furthermore, the Aldo-2/Aldo-1 ratio was compared to the ARR as an established screening procedure. Both the ARR (ROC curve: AUC 0.97 ± 0.02, $P = 0.0001$) and the Aldo-2/Aldo-1 ratio (AUC 0.74 ± 0.10, $P = 0.016$) were significantly different from the theoretical area of 0.5 and

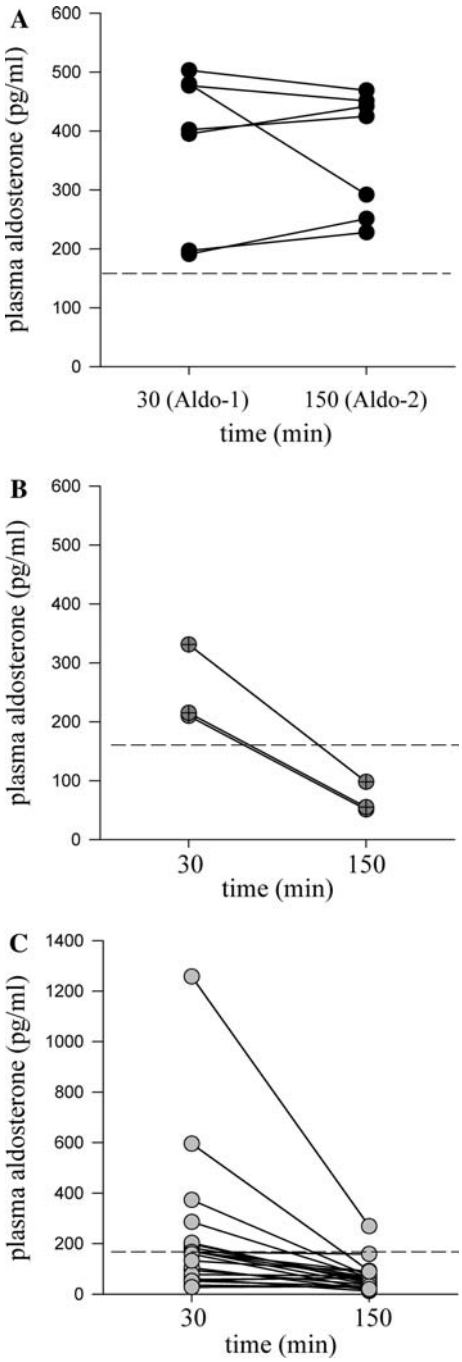

Fig. 2 Serum aldosterone during the RPT in a cohort of 32 hypertensive patients suspicious for primary hyperaldosteronism. **a** Patients with histologically confirmed APA ($n = 7$), **b** patients with IHA ($n = 3$), and **c** patients with primary hypertension ($n = 22$). *Dotted line*: cut-off (160 pg/ml) for serum aldosterone at 150 min (Aldo-2)

thus suitable for the detection of PA (Fig. 3a). The AUC of the ROC curve for ARR, however, was significantly larger than the one for the Aldo-2/Aldo-1 ratio, which indicates superiority of the ARR for the detection of PA ($P = 0.027$) (Fig. 3a). An ARR of >425 pg/ml per ng/ml h, which is comparable to the previously reported cut-offs for the ARR (300–400 pg/ml per ng/ml h) [6, 7], identified all the subjects with PA with a specificity of 82% (Fig. 3a). Although the Aldo-2/Aldo-1 ratio during the RPT of >0.23 identified all the patients with PA, the specificity was only 41%. This is mainly explained by the fact that the Aldo-2/Aldo-1 ratio appeared to systematically miss all the patients with IHA who showed very similar decreases of serum aldosterone in comparison with primary hypertensive patients and healthy controls (ANOVA $P = 0.92$; Figs. 1 and 2).

As the Aldo-2/Aldo-1 ratio primarily detected patients with APA, we tested the Aldo-2/Aldo-1 ratio during RPT and the ARR as a potential screening test to discriminate between patients with primary hypertension and IHA on the one hand, and APA on the other hand. Both the Aldo-2/Aldo-1 ratios (AUC 0.89 ± 0.06, $P = 0.0001$) and the ARR (ROC curve: AUC 0.97 ± 0.03, $P = 0.0001$) were significantly different from the bisecting line, but the AUCs for the two parameters were not significantly different from each other ($P = 0.32$) (Fig. 3b). The ARR with a calculated cut-off of >425 pg/ml per ng/ml h (optimal cut-off to detect patients with PA) was unsuited to discriminate between subjects with APA and IHA, which is not unexpected, since the ARR has been designed as a case finding test for the detection of PA, but not APA. An ARR >1314 pg/ml per ng/ml h, however, had 88% specificity for the detection of APA at the 100% sensitivity level. An Aldo-2/Aldo-1 ratio >0.59 resulted in 72% specificity to detect APA. This discrimination, however, could be further improved by considering a threshold for Aldo2 of >160 pg/ml. This combination that can be easily performed without any further measurements or calculations correctly detected all the surgically confirmed cases of APA without any misclassification.

Discussion

Distinguishing surgically remediable forms such as unilateral APA from other causes of PA is clinically important. A screening test for the detection of APA could be relevant in the diagnostic work-up and management of patients suspicious for PA. At present, the posture test can be used in the differential diagnosis of IHA and APA [3, 4, 8]. This test, however, is not a screening tool and requires subjects resting for several hours in the supine position before the test can be performed.

Physiological control of aldosterone secretion occurs mainly via the renin-angiotensin system (RAS). Orthostasis decreases renal perfusion thereby triggering renin release from juxtaglomerular cells and thus activation of RAS. This regulatory principle is exploited during the classic posture test that is applied to differentiate between APA

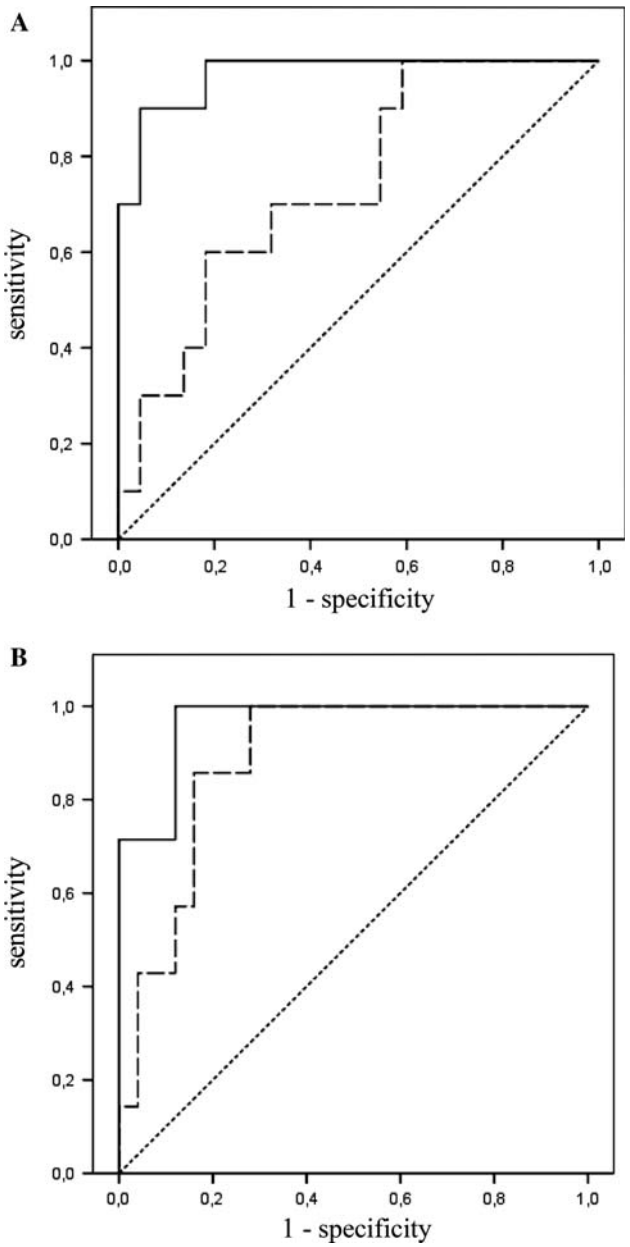

Fig. 3 ROC curves of the ARR and the Aldo-2/Aldo-1 ratio (RPT) for identifying patients with PA (**a**) or APA (**b**). ARR: *solid line*; Aldo-2/Aldo-1 ratio: *dashed line*; bisecting line: *dotted line*

and RAS-dependent IHA [3, 4, 8]. In contrast, reversed orthostasis enhances renal perfusion thereby curtailing renin and consecutive aldosterone release from the zona glomerulosa. We hypothesized that this "negative" feedback control of aldosterone secretion might be altered in APA, which in most cases is characterized by autonomous aldosterone production independent of RAS [9]. Thus, a RPT exploiting this negative control mechanism could be a useful "suppression test" in identifying surgically remediable unilateral APA.

In healthy normotensive and in primary hypertensive controls, a marked decline in serum aldosterone was observed during the RPT indicating a rapid negative feedback on circulating aldosterone by changing from the erect to the supine position. Various antihypertensive medications were tested in mono therapy such as ACE-inhibitors, beta-blockers, or calcium antagonists, but none had an obvious effect on the decline of aldosterone during the test expressed as the Aldo-2/Aldo-1 ratio. In a prospective series of patients referred because of suspicion for PA, seven were diagnosed with APA, which was surgically proven. In six out of the seven patients no decrease in aldosterone during RPT was observed. In one patient, serum aldosterone declined during the RPT, which might be explained by some angiotensin II responsiveness of the adenoma [4, 9]. Moreover, in this patient, however, the APA was correctly identified by an elevated Aldo-2/Aldo-1 ratio of 0.61 and an Aldo-2 that was above the cut-off of 160 pg/ml. Consistent with our hypothesis, serum aldosterone decreased during the RPT in patients with IHA in a similar way as in healthy and hypertensive controls.

We tested the RPT for its ability to identify patients with PA, and especially patients with surgically remediable APA. According to ROC analysis, the RPT was clearly inferior to the ARR in the screening for PA. The reason for this appears to be the fact that the RPT systematically missed patients suffering from IHA. The threshold, the sensitivity, and the specificity for ARR determined in our cohorts were similar to those reported by others [6, 7]. The Aldo-2/Aldo-1 ratio (>0.59), however, in combination with a threshold for Aldo-2 (>160 pg/ml) correctly identified all the patients with surgically proven APA. We cannot, however, exclude that we misclassified a patient with early disease and/or with preserved angiotensin II-responsiveness harboring an APA not visible on imaging studies. A recent article demonstrated that in normokalaemic PA, which might reflect early disease, the test procedures established to confirm classical hypokalaemic PA such as the saline infusion test have a low accuracy and fail to reliably identify this variant of PA [10]. Furthermore, we have not routinely performed bilateral adrenal venous sampling (AVS), which recently has been advocated as a gold standard for localizing a unilateral autonomous lesion [5]. Techniques and interpretation, however, vary widely, and concordant criteria for establishing unilateral autonomy by AVS are lacking [3, 4, 11, 12], and the sensitivity of the AVS for diagnosing APA in most reports was less than 100% [13, 14]. Thus, even if the AVS is routinely performed in every patient suspicious for APA, the correct diagnosis could be missed. Nevertheless, the possibility exists that we overlooked a patient with early or normokalaemic APA non-visible on imaging studies. Therefore, the sensitivity of the RPT could be somewhat lower,

i.e., a negative test result may not definitely rule out the presence of APA. It remains to be shown in further prospective studies, whether the negative predictive value of the RPT is indeed as high as suggested by our series.

However, specificity of the RPT and the positive predictive value for the diagnosis of a surgically remediable APA were high, since all the patients with a positive test result had a surgically proven APA, and there were no false positive cases. This means that in our series all the patients with a positive RPT, and a unilateral adenoma visible on imaging studies indeed had a surgically remediable primary aldosteronism. The RPT can be easily included in the screening strategy for PA by combining it with the ARR, which principally detects all the types of PA.

Clearly, larger studies with detailed characterization also of control subjects and studies in unselected populations are needed before the RPT should be introduced in clinical practice. However, if confirmed in larger, independent studies, the RPT could be a simple and valuable additional tool for the detection of patients with APA. Based on our series, in the case of a positive ARR, a positive RPT, and the presence of a unilateral adenoma, the likelihood of a surgically remedial APA appears to be very high.

In summary, the novel RPT procedure here proposed is simple to perform and appears to have noteworthy potential for identifying APA in outpatients. The RPT can be easily combined with the ARR in the screening of PA in a primary care setting and appears useful in the work-up of the hyperaldosteronemic patient.

Materials and methods

Subjects

We studied six healthy controls, five patients with untreated mild hypertension, a group of patients with known primary hypertension, who were under antihypertensive mono therapy with beta-blockers ($n = 5$), calcium antagonists ($n = 5$), or ACE-inhibitors ($n = 4$), and a cohort of hypertensive subjects ($n = 32$) that were referred to the outpatients clinic between April 2005 and April 2006 because of suspicion for primary hyperaldosteronism by clinical criteria. These criteria included the presence of severe hypertension (blood pressure $\geq$160 mmHg systolic and/or $\geq$100 mmHg diastolic, $n = 16$). Five of these patients had treatment-resistant hypertension, defined as blood pressure that remains above goal in spite of the concurrent use of three antihypertensive agents of different classes including a diuretic, which were prescribed at optimal dose amounts. Other criteria for referral in some of the patients were the presence of hypertension of any stage together with spontaneous hypokalaemia or with hypokalaemia provoked by administration of a low-dose diuretic ($n = 6$), or manifestation of hypertension at young age (<30 years, $n = 2$, one of these subjects also having severe hypertension and unprovoked hypokalaemia). Nine of the patients in the cohort of 32 hypertensive subjects presented for further endocrine evaluation because of an incidentally detected adrenal unilateral adrenal mass. Other hormonal causes of secondary hypertension such as Cushing's disease or pheochromocytoma had been ruled out. All the subjects ($n = 57$) were prospectively investigated both using the established ARR and the novel RPT (RPT) here proposed. Diagnostics including drawing of blood samples in the present study were approved by the local ethical committees.

Screening for primary hyperaldosteronism in the cohort of the 32 hypertensive subjects was performed according to a standardized protocol, which included the measurement of blood pressure, electrolytes, serum aldosterone, plasma renin activity, calculation of the ARR and the 24-h urinary aldosterone excretion. RPT and the calculation of the ARR were performed after antihypertensive medication has been paused or switched to calcium antagonist treatment for at least two weeks. None of the patients was taking beta-blockers or aldosterone antagonists at the time of evaluation. Patients showing a combination of low ARR, normal serum aldosterone, normal or elevated plasma renin activity, normal electrolytes, and normal urinary aldosterone excretion were regarded as not having PA. The diagnosis of PA was made in patients with a high ARR, elevated serum aldosterone, and urinary aldosterone excretion, in the presence of low or suppressed plasma renin activity, and hypokalemia. In patients with an elevated ARR a saline infusion test to confirm or rule out PA was performed, if one of the above criteria for PA was not fulfilled. All the patients with an elevated ARR received imaging studies of the adrenals (CT or MRI). Patients with PA and visualization of an unilateral adenoma were considered as having APA, while the imaging-negative patients were tested by a postural test. Seven patients with APA, and three patients with IHA were finally identified. Surgery was performed in the seven subjects that were highly suspicious to have APA, and diagnosis of adrenal adenoma was histologically confirmed in all the seven subjects. In six of the seven patients the ARR and the Aldo-2/Aldo-1 ratio during RPT were re-investigated after surgery. The age of the subjects was comparable between the groups (ANOVA, $P = 0.9$, patients with primary hyperaldosteronism 52.5 ± 4.5 yrs ($n = 10$), patients without primary hyperaldosteronism 53.6 ± 2.4 yrs ($n = 22$), and healthy or hypertensive controls 55.4 ± 3.2 yrs ($n = 25$)), although in our relatively small cohort the age of patients with IHA and APA differed from typical age distribution observed in larger cohorts.

Design of the reverse postural test (RPT)

Subjects presented in the outpatient's clinic between 08:00 and 09:00 a.m. after an overnight fast. The time interval between getting up and presenting in the outpatient's clinic was not exactly defined, but extended 2 h in all the participants. After arrival, blood pressure was measured and subjects were instructed to exercise moderately for 30 min at walking pace. After 30 min of exercise in the metabolic unit subjects were asked to sit down and blood was drawn within the next 5 min, for the measurement of serum aldosterone (Aldo-1), plasma renin activity, serum cortisol, and electrolytes. Thereafter, subjects spent 120 min in supine position, followed by drawing of a second blood sample for the measurement of serum aldosterone (Aldo-2) and cortisol. Serum cortisol was measured to detect potential stress induced effects on serum aldosterone [15]. ARR was calculated from serum aldosterone and plasma renin activity determined at the Aldo-1 time point. In the patients evaluated for PA antihypertensive drugs were paused or switched to calcium antagonists at least two weeks prior to the RPT. The hypertensive control subjects were instructed to take their previously prescribed antihypertensive drugs after the test.

Assays

Serum aldosterone concentrations were measured using a commercially available radioimmunoassay (RIA Aldosterone, Immunotech, Marseille, France). The intra-assay CV is <9.5% and the inter-assay CV is <9.9%. The recovery rate is between 95% and 113%. The cross-reactivity with other steroids such as cortisone, corticosterone, and DHEA-S is extremely low. The normal range of aldosterone levels in serum or plasma samples is given by the manufacturer as follows: supine position: 10–95 pg/ml, upright position: 34–273 pg/ml. Plasma renin activity was measured using a commercially available RIA (RENCTK Angiotensin I Radioimmunoassay, DiaSorin, Saluggia, Italy) with an intra-assay CV of 7.6% and an inter-assay CV of 9.1%. The recovery rate is between 93.6% and 100.9%. Cross reactivity is <0.1% with angiotensin II, and <0.02% with heptapeptide and hexapeptide. The normal range of plasma renin activity is given by the manufacturer as follows—supine position: 0.2–2.8 ng/ml/h, upright position: 1.5–5.7 ng/ml/h. Serum cortisol was measured by a chemoluminescence immunoassay (Bayer Diagnostics, Fernwald, Germany; sensitivity 0.2 µg/dl, intra-assay CV 3.7%, inter-assay CV 4.9%).

Statistical analyses

Data are given as means ± SEM. The ARR was calculated as serum aldosterone-to-plasma renin activity ratio (pg/ml per ng/ml h). Measured values of plasma renin activity below the limit of quantification (LOQ) of the assay (<0.2 ng/ml/h) were set at 0.7 × LOQ [16]. The Aldo2/Aldo-1 ratio was calculated from the aldosterone levels (pg/ml) obtained from the two blood drawings during the RPT. Characteristics of the investigated subjects were compared using ANOVA. The two-tailed paired *t*-test was used to compare parameters during the RPT (time point 30 min (Aldo-1) and 150 min (Aldo-2)). The area under the receiver operating characteristic (ROC) curve (AUC) was used to define variables discriminating between patients with PA, APA, and aldosterone-independent (primary) hypertension. The 95% confidence interval (95% CI) for the ROC-AUC was used to test the hypothesis that the theoretical area is 0.5. When the 95% CI did not include the 0.5 value, the laboratory test was considered to have an ability to distinguish between patients with PA, APA, and primary hypertensive patients. Differences between ROC curves were calculated as described previously [17], using MedCalc Software (MedCalc, Mariakerke, Belgium). The optimal cut-off point for each variable usually is selected by calculating the Youden's index (J = sensitivity + specificity − 1). However, for a screening test, high sensitivity is preferred. Therefore, specificities were calculated at defined sensitivities of 100%. Calculations other than ROC curve analyses were performed using SPSS version 14 (SPSS, Chicago; IL, USA). Statistical significance was defined as $P < 0.05$.

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