CLINICAL STUDY

Comparison of active renin concentration and plasma renin activity for the diagnosis of primary hyperaldosteronism in patients with an adrenal mass

Nicole Unger¹, Ingo Lopez Schmidt¹, Christian Pitt⁴, Martin K Walz⁴, Thomas Philipp², Klaus Mann^{1,3} and Stephan Petersenn¹

¹ Division of Endocrinology, Medical Center, ² Division of Nephrology and Hypertension, Medical Center, ³ Department of Clinical Chemistry, University of Essen and ⁴ Department of Surgery and Centre of Minimally Invasive Surgery, Kliniken Essen-Mitte, Essen, Germany

(Correspondence should be addressed to S Petersenn, Division of Endocrinology, Medical Center, University of Essen, Hufelandstr. 55, 45122 Essen, Germany; Email: stephan.petersenn@uni-essen.de)

Abstract

Objective: Plasma aldosterone concentration (PAC) to plasma renin activity (PRA) ratio is an established screening test for primary hyperaldosteronism. Due to the increased recognition of adrenal incidentalomas, reliable parameters are required. Determination of active renin concentration (ARC) in contrast to PRA offers advantages with regard to processing and standardization. The present study compared PRA and ARC under random conditions to establish thresholds for the diagnosis of primary hyperaldosteronism.

Design and methods: Fifty patients with various adrenal tumors, including ten patients with aldosterone-secreting adrenal adenomas, as well as ten hypertensive patients and 23 normotensive volunteers were studied. PAC and PRA were measured by radioimmunoassay. ARC was determined by an immunoluminometric assay.

Results: Receiver operating curve (ROC) analysis suggested a PAC to ARC ratio threshold of 90 ((ng/l)/(ng/l)) (sensitivity 100%, specificity 98.6%) and a ratio threshold of 62 by additional consideration of $\text{PAC} \geq 200 \text{ ng/l}$ (sensitivity 100%, specificity 100%) for the diagnosis of aldosterone-secreting adrenal adenomas.

Conclusions: A PAC to ARC ratio of ≥ 62 in patients with PAC levels $\geq 200 \text{ ng/l}$ is a reliable screening method for primary hyperaldosteronism in patients with an aldosterone-producing adenoma under random conditions. Because of its advantages with regard to probe processing and its independence from endogenous angiotensinogen levels, ARC may be preferred to PRA.

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Introduction

Primary hyperaldosteronism is classically characterized by a combination of hypertension, hypokalemia, suppressed renin and an elevated non-suppressible plasma aldosterone concentration. Measurement of these single parameters does not allow a reliable diagnosis. Normokalemia is noted in patients with primary hyperaldosteronism (1–3), as well as suppressed renin in hypertensive patients (4, 5). Due to improved methods in computed tomography (CT) or magnetic resonance imaging (MRI), adrenal incidentalomas are increasingly found. Accurate parameters to distinguish aldosterone-secreting adrenal adenomas from other functioning or non-functioning adrenal masses are therefore required.

The plasma aldosterone concentration (PAC) to plasma renin activity (PRA) ratio was suggested as a screening tool for the diagnosis of primary hyperaldosteronism (1). In contrast to single measurement of

either PAC or PRA, the PAC to PRA ratio seems to be independent of diurnal and day-to-day variations, salt intake and diuretics. It was demonstrated as a reliable and convenient screening tool for ambulatory conditions, independent of body posture (6). However, a recent study revealed a low reproducibility of the ratio in patients with an aldosterone-producing adrenal adenoma under random conditions (7). PRA is determined by radioimmunological measurement of angiotensin-1 generated from angiotensinogen during *in vitro* incubation with plasma renin. However, determination is limited to physiological angiotensinogen levels. Conditions with decreased angiotensinogen levels (e.g. liver cirrhosis, severe cardiac failure) result in the underestimation of renin (8, 9). In contrast, estrogen treatment increases angiotensinogen levels, resulting in an overestimation of renin (10, 11). The lack of standardization is another methodical disadvantage. Processing of plasma samples, especially in regard to incubation time, pH and dilution varies among different

Table 1 Clinical characterization of the population studied. Values are means $\pm$ s.d.

Diagnosis	No. of patients	Age (years)	Sex (male/female)	Tumor size (cm)
Aldosterone-secreting adenoma	10	51.4 $\pm$ 13.3	3/7	1.8 $\pm$ 0.6
Cortisol-secreting adenoma	7	43.7 $\pm$ 12.5	0/7	3.3 $\pm$ 0.8
Pheochromocytoma	12	49.3 $\pm$ 16.5	6/6	3.8 $\pm$ 2.5
Non-functioning adrenal mass (NFM)	21	51.9 $\pm$ 12.6	10/11	3.9 $\pm$ 2.6
Essential hypertension	10	41.6 $\pm$ 11.4	6/4	na
Healthy volunteers	23	34.2 $\pm$ 12.2	10/13	na
Total	83	44.6 $\pm$ 14.6	35/48	na

na, not available.

laboratories. In patients with suppressed renin, the ratio is clearly dependent on the lower detection limit. An assay sensitivity of 0.5 ng/ml per hour for a PRA assay compared with 0.1 ng/ml per hour for another assay results in an unequivocal lower PAC to PRA ratio.

To avoid these disadvantages, generation of monoclonal antibodies against active renin allowed direct measurement of active renin concentration (ARC) independent from angiotensinogen levels. Standardization of ARC measurement by calibration against a WHO standard enables improved comparability among different laboratories. The present study compared PRA and ARC under random conditions to investigate the use of ARC for the diagnosis of primary hyperaldosteronism in patients with aldosterone-producing adenomas.

Materials and methods

Subjects

Fifty patients with a current adrenal mass were grouped into four categories: ten aldosterone-secreting adrenal adenomas; seven cortisol-secreting adrenal adenomas (one subclinical and six overt Cushing's syndromes), 12 pheochromocytomas; and 21 non-functioning adrenal masses (17 non-functioning adenomas, two cysts, one metastasis of bronchial carcinoma, one myelolipoma). The diagnoses were made preoperatively by appropriate biochemical parameters in combination with evidence of an adrenal mass on CT or MRI. Primary hyperaldosteronism due to an adrenal adenoma was diagnosed by a plasma aldosterone to renin activity ratio ≥ 300 (ng/l to ng/ml per h) and PAC ≥ 200 ng/l (12) and in part confirmed by increased urinary aldosterone-18-glucuronide excretion, saline infusion test, adrenal vein sampling and/or postural stimulation test. Four patients showed a normokaliemic hyperaldosteronism. In these ten patients, CT or MRI revealed a unilateral adrenal mass. Clinical follow-up was carried out in five patients demonstrating a normalized PAC to PRA ratio postoperatively. Because of the limitations of the screening parameters, patients with mild hyperaldosteronism might have been undiagnosed.

Except for five non-functioning adenomas with a size less than 3 cm, all adrenal masses were subsequently

removed. The diagnosis was postoperatively confirmed by histological and immunohistochemical examinations as well as clinical follow-up.

Controls included ten patients with essential hypertension and 23 healthy normotensive volunteers taking an *ad libitum* sodium diet. In patients with essential hypertension, endocrine disorders were excluded by the following tests: primary hyperaldosteronism was excluded by a normal PAC and PAC to PRA ratio; Cushing's syndrome was investigated by midnight plasma cortisol levels and a dexamethasone suppression test; pheochromocytoma was excluded by normal metanephrines and urinary catecholamines. Furthermore, renal artery stenosis was excluded by MRI and/or duplex ultrasound in nine of ten patients. In total, 83 subjects were included in this prospective study (pertinent data are given in Table 1).

At the time of investigation, patients with an aldosterone-secreting adrenal adenoma, patients with pheochromocytoma, and patients with hypertension were on various antihypertensive medications, as well as nine hypertensive patients with non-functioning adrenal adenomas. Three of ten patients with an aldosterone-producing adenoma received spironolactone preoperatively (Table 2).

Blood samples were drawn from patients in an upright body position from a forearm vein between 0800 h and 0900 h. The study protocol was approved by the local ethical committee and informed consent was given by all patients.

Assays

PAC was measured by RIA (Byk & DiaSorin, Dietzenbach, Germany). The normal range in an upright position was 100–350 ng/l. Assay sensitivity was 50 ng/l and assay variability was determined by within-assay coefficients of variation (CV) of 11.2% for 73 ng/l and 8.2% for 308 ng/l and between-assay CV values of 17.7% for 73 ng/l, 10.4% for 208 ng/l and 6.6% for 454 ng/l.

PRA was also determined by RIA (Byk & DiaSorin). The normal range in an upright position was 1.8–6.3 ng/ml per hour. Assay sensitivity was 0.1 ng/ml per hour. Within-assay CV values were 7.5% for 0.17 ng/ml per hour, 5.4% for 8.8 ng/ml per hour

Table 2 Medication in patients with hypertension.

Medication	Aldosterone-secreting adenoma	Cortisol-secreting adenoma	Pheochromocytoma	NFM	Essential hypertension
α blockers	2	—	6	1	1
β blocker	6	4	1	3	7
Ca-channel blocker	4	2	2	5	7
ACE inhibitors	4	1	2	4	3
AT1-receptor antagonists	—	2	1	2	1
Diuretics	2	1	1	2	1
Clonidine	1	—	—	1	1
Spironolactone	3	—	—	—	—

ACE, angiotensin converting enzyme; AT1, angiotensin-A.

and 9.9% for 13.5 ng/ml per hour. Between-assay CV values were 7.7% for 2.6 ng/ml per hour, 8.1% for 8.6 ng/ml per hour and 11.5% for 13.0 ng/ml per hour.

ARC was determined in EDTA-treated plasma by a two-site chemiluminescent enzyme immunometric assay with a functional sensitivity of 1 ng/l (Nichols Institute Diagnostics, Bad Nauheim, Germany). The normal range in an upright position was 2.1–26 ng/l by calibration against a WHO standard. Between-assay CV values were 10–14% for 3.9 ng/l, 3.4–4% for 20 ng/l and 3% for 125 ng/l. Within-assay CV values were 7.2% for 4.9 ng/l, 4.0% for 10 ng/l, 3.8% for 49 ng/l and 3.7% for 124 ng/l.

The ratio of PAC to PRA and PAC to ARC was calculated for all subjects. In samples with suppressed PRA and ARC the lower detection limit of the respective assay was used for calculation.

Statistical evaluation

Results are expressed as means $\pm$ S.E.M. GraphPad Prism 3.0 (GraphPad Software Inc., San Diego, CA, USA) was used for statistical analyses. A Spearman's rank correlation analysis was used to determine the relationship between variables. The Kruskal–Wallis test, followed by Dunn's multiple comparison test, was performed where appropriate. Receiver operating curve (ROC) analyses were obtained using MedCalc 6.16 software (MedCalc Software, Mariakerke, Belgium).

Results

PAC to PRA ratio

PAC to PRA ratio was 3570 ± 262 ((ng/l)/(ng/ml per h)) (means $\pm$ S.D.) in patients with primary hyperaldosteronism and 271 ± 32 ((ng/l)/(ng/ml per h)) in the remaining 73 subjects. Ratios for each subgroup are shown in Table 3. The ratio was significantly higher in patients with primary hyperaldosteronism than in pheochromocytomas, cortisol-secreting adenomas, non-functioning adrenal masses, hypertensive patients and healthy volunteers, as analyzed by Kruskal–Wallis and Dunn's multiple comparison test. ROC

analysis suggested a ratio threshold value of 680 (sensitivity 100%, specificity 91.8%, area under the curve (AUC) = 0.992) for the diagnosis of primary hyperaldosteronism. All patients with primary hyperaldosteronism fulfilled this condition, while 66 of the remaining 73 subjects were below this ratio. However, seven subjects (10.6%) were falsely classified as having primary hyperaldosteronism. With the additional consideration of PAC ≥ 200 ng/l for the diagnosis of primary hyperaldosteronism, ROC analysis suggested a threshold ratio of 330 (sensitivity 100%, specificity 100%, AUC = 1.0), as expected by the criteria used for the diagnosis of hyperaldosteronism. Under this condition, none of the control subjects were positively screened for primary hyperaldosteronism (Fig. 1).

Correlation of PRA to ARC

Spearman's test demonstrated a significant correlation for the PAC to PRA and PAC to ARC ratio with $r = 0.91$ ($P < 0.001$) (data not shown).

PAC to ARC ratio

PAC to ARC ratio was 388 ± 292 ((ng/l)/(ng/l)) in patients with primary hyperaldosteronism and 24.1 ± 3.4 in 29 control subjects (Table 3). The ratio was significantly higher in primary hyperaldosteronism than in all other groups. ROC analysis suggested a ratio threshold value of 90 (sensitivity 100%, specificity 98.6%, AUC = 0.996) for the diagnosis of primary hyperaldosteronism. Two control subjects (2.7%) were above that threshold. With additional consideration of PAC > 200 ng/l, ROC analysis revealed a threshold ratio of 62 with a similar sensitivity, but an increased specificity of 100% (AUC = 1). Under this condition, the control subjects remained below the threshold (Fig. 2).

Discussion

This study compared the ARC and PRA for the diagnosis of primary hyperaldosteronism. We did not include

Table 3 PAC, PRA and ARC levels as well as PAC to PRA ratios and PAC to ARC ratios for the six subgroups. Values are means $\pm$ S.D.

Diagnosis	PAC (ng/l)	PRA (ng/ml per h)	ARC (ng/l)	PAC to PRA ratio (ng/l)/(ng/ml per h)	PAC to ARC ratio (ng/l)/(ng/l)
Aldosterone-secreting adenoma	544 $\pm$ 328	0.2 $\pm$ 0.1	1.7 $\pm$ 1.0	3570 $\pm$ 262	388 $\pm$ 292
Cortisol-secreting adenoma	126 $\pm$ 117	1.6 $\pm$ 1.8	14.8 $\pm$ 15.0	163 $\pm$ 211**	16.0 $\pm$ 19.1**
Pheochromocytoma	128 $\pm$ 60	1.5 $\pm$ 1.2	20.0 $\pm$ 15.4	317 $\pm$ 525**	19.0 $\pm$ 24.8**
NFM	159 $\pm$ 170	1.6 $\pm$ 2.9	20.4 $\pm$ 35.3	291 $\pm$ 296**	27.4 $\pm$ 39.2**
Essential hypertension	141 $\pm$ 121	1.2 $\pm$ 1.9	18.5 $\pm$ 36.5	409 $\pm$ 402*	33.2 $\pm$ 30.3*
Healthy volunteers	161 $\pm$ 121	1.1 $\pm$ 1.2	12.7 $\pm$ 10.4	202 $\pm$ 148**	22.2 $\pm$ 22.4**

* $P < 0.05$, ** $P < 0.001$ in relation to aldosterone-producing adenomas.

patients with bilateral hyperplasia with potentially lower levels of aldosterone. Our aim was to establish an accurate screening tool for the diagnosis of adrenal masses with special regard to ambulatory conditions. Blood was obtained from patients in an upright posture after random activity.

Diagnosis of primary hyperaldosteronism was established following the recommendations of a recent NIH consensus statement (12). Without consideration of a threshold for PAC, our ROC analysis suggested a PAC to PRA ratio ≥ 680 with a sensitivity of 100% and a reduced specificity of 91.8%. Various threshold values between 200 and 1000 are mentioned in the literature (1, 2, 12–16). In our study, specificity decreased to 86% for a ratio of ≥ 500 , 70% for a ratio of ≥ 330 and 62% for a ratio of ≥ 200 , while sensitivity remained unchanged.

The influence of antihypertensive medication on the accuracy of PAC to PRA and ARC ratios respectively is controversial. Because of our aim of establishing a screening tool in ambulatory settings we favored the continuation of antihypertensive medication. Gallay *et al.* (2) showed no significant correlation between different values of the PAC to PRA ratio and numbers or types of antihypertensive medications. However, Seifarth *et al.* (17) found some influence of antihypertensive medication on the PAC to ARC ratio. β blockers significantly increased the ratio, while angiotensin converting enzyme (ACE) inhibitors, aldosterone antagonists and AT₁ receptor antagonists significantly decreased the ratio. Two other studies of hypertensive patients on antihypertensive medication revealed a ratio threshold value of 270 (sensitivity 93%) and 1000 (sensitivity 100%) respectively, without consideration of elevated PAC or suppressed PRA (2, 13).

In contrast, several studies discontinued antihypertensive treatment in hypertensive patients revealing a PAC to PRA ratio range of 200 to 500 (1, 14–16). The ratios are nearly similar to those in studies with continued antihypertensive medication.

Comparing the results of PRA and ARC, we have established a threshold for the PAC to ARC ratio. A threshold value of 90 provided a high sensitivity and specificity for the diagnosis of primary hyperaldosteronism. By considering PAC ≥ 200 ng/l, a ratio threshold of

62 was established with improved specificity. Another study examined the determination of ARC for the diagnosis of primary hyperaldosteronism (18). Trenkel *et al.* (18) studied 17 patients with primary hyperaldosteronism (nine adrenal adenomas, eight idiopathic hyperaldosteronism), 146 hypertensive patients on various medication and 37 normotensive subjects. Primary hyperaldosteronism was diagnosed by a PAC to PRA ratio ≥ 200 and subsequent tests. A PAC to ARC ratio cut-off value of 50 revealed a sensitivity of 89% and a specificity of 96%. In contrast to our study, sensitivity decreased to 84% and specificity increased to 100% by considering a PAC ≥ 200 ng/l as an additional criterion. The high proportion of idiopathic hyperaldosteronism, which is probably susceptible for lower aldosterone levels, might explain the lower threshold. Controls were not diagnosed by determination of PAC to PRA ratio, possibly resulting in an underestimation of primary hyperaldosteronism.

Automated methods for the determination of ARC provide not only standardization but also savings of time. The accuracy of determination has been improved by the development of immunoradiometric and immunoluminometric assays (19). Independence from angiotensinogen levels is another advantage of ARC determination. Plouin *et al.* (9) reported underestimated PRA levels in patients with liver cirrhosis and severe cardiac failure and overestimated PRA levels in women exposed to estrogens due to altered angiotensinogen levels. Valabhji *et al.* (20) described decreased PRA levels in type 1 diabetic patients, while similar concentrations of ARC in type 1 diabetics and volunteers were demonstrated (20). Reduced angiotensinogen levels in diabetic patients may be responsible for an underestimation of PRA (21). Because of the above-mentioned advantages the present study favors ARC over PRA for the diagnosis of primary hyperaldosteronism.

Differentiation of primary hyperaldosteronism from essential hypertension may be difficult. Most studies are therefore based on a population of hypertensive patients. However, increased diagnosis of adrenal masses due to improved imaging requires reliable parameters to separate primary hyperaldosteronism from pheochromocytomas, cortisol-secreting adenomas or incidentalomas. Thus, our study was performed with

Figure 1 (A) Relationship of PAC and PRA in ten patients with primary hyperaldosteronism (squares) and 73 control subjects (triangles). The horizontal line shows the PAC threshold of 200 ng/l. The upper left quadrant demonstrates the subjects positively screened for primary hyperaldosteronism. (B) PAC to PRA ratios in the different subgroups. The right column of each subgroup demonstrates the ratios of subjects with a PAC $\geq$ 200 ng/l (closed symbols). Open symbols demonstrate subjects with a PAC $\leq$ 200 ng/l. + symbols demonstrate patients with aldosterone-producing adenoma. The upper horizontal line shows the ratio threshold of 680 for the diagnosis of primary hyperaldosteronism. The lower horizontal line shows the ratio threshold of 330 in subjects with a PAC $\geq$ 200 ng/l. Pheo, pheochromocytoma; Conn, aldosterone-producing adenoma.

Figure 2 (A) Relationship of PAC and ARC in ten patients with primary hyperaldosteronism (squares) and 73 control subjects (triangles). The horizontal line shows the PAC threshold of 200 ng/l. (B) PAC to ARC ratios in the different subgroups. The right column of each subgroup demonstrates the ratios of subjects with a PAC ≥ 200 ng/l (closed symbols). Open symbols demonstrate subjects with a PAC ≤ 200 ng/l. + symbols demonstrate patients with aldosterone-producing adenoma. The upper horizontal line shows the ratio threshold of 90 for the diagnosis of primary hyperaldosteronism. The lower horizontal line shows the ratio threshold of 62 in subjects with a PAC ≥ 200 ng/l. Pheo, pheochromocytoma; Conn, aldosterone-producing adenoma.

special regard to a population of patients with adrenal masses of various etiologies. We provided a PAC to ARC ratio ≥ 62 in patients with PAC levels ≥ 200 ng/l as a reliable screening method for primary hyperaldosteronism under random conditions.

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