Original Article

Test characteristics of the aldosterone-to-renin ratio as a screening test for primary aldosteronism

Pieter M. Jansen[a], Bert-Jan H. van den Born[b], Wijnanda J. Frenkel[b], Emile L.E. de Bruijne[c], Jaap Deinum[d], Michiel N. Kerstens[e], Yvo M. Smulders[f], Arend Jan Woittiez[g], Johanna A.M. Wijbenga[h], Robert Zietse[a], A.H. Jan Danser[a], and Anton H. van den Meiracker[a]

Background: The aldosterone-to-renin ratio (ARR) is a widely used screening test for primary aldosteronism. Current guidelines recommend a cut-off value of 91 pmol/mU. Studies on its sensitivity, specificity, reproducibility and the role of medication have been conflicting. We prospectively assessed the test characteristics of the ARR and the effect of combination antihypertensive treatment.

Methods: In 178 patients with persistent hypertension despite the use of at least two antihypertensives, plasma renin and aldosterone were assessed twice within an interval of 4 weeks. All patients underwent an intravenous salt loading test. A posttest plasma aldosterone exceeding 235 pmol/l was considered diagnostic for primary aldosteronism. ARR was repeated after 4 weeks of standardized treatment with a calcium channel blocker and/or α-adrenergic-receptor blocker.

Results: The prevalence of primary aldosteronism was 15.2%. The median ARR was 35.0 (interquartile range 16.2–82.0) in primary aldosteronism versus 7.1 (2.2–17.5) pmol/mU in essential hypertensive patients ($P < 0.001$). Under random medication, the ARR had 22.2% sensitivity and 98.7% specificity. On standardized treatment, the ARR rose from 9.6 (2.5–24.8) to 21.4 (10.8–52.1) ($P < 0.001$). Multivariate regression showed that angiotensin-converting enzyme (ACE)-inhibitors and angiotensin II-receptor blockers were responsible for the lower ARR during random treatment. The area under the receiver operating characteristic curve was, however, similar under random and standardized treatment (84 vs. 86%, respectively, $P = 0.314$). Bland–Altman plots showed an almost five-fold difference in ARR values taken under the same conditions.

Conclusion: ARR sensitivity for primary aldosteronism is low when the recommended cut-off is used. Reproducibility is also poor, stressing the need for alternative screening tests.

Keywords: aldosterone-to-renin ratio, hypertension, primary aldosteronism, reproducibility, sensitivity, specificity

Abbreviations: AB, α-adrenergic receptor blocker; ACEi, angiotensin-converting enzyme inhibitor; APA, aldosterone-producing adenoma; ARB, angiotensin II receptor blocker; ARR, aldosterone-to-renin ratio; AUC, area under the curve; BB, β-adrenergic receptor blocker; CCB, calcium channel blocker; PAC, plasma aldosterone concentration; RAS, renin–angiotensin system; ROC, receiver operating curve

INTRODUCTION

Since the introduction of the aldosterone-to-renin ratio (ARR) [1], the diagnosis of primary aldosteronism has increased dramatically [2]. Primary aldosteronism is now considered to be the most prevalent cause of secondary hypertension with reported prevalences between 3.2 and 20% [3–15]. The Endocrine Society recommends screening of patients with Joint National Commission stage 2, stage 3 or resistant hypertension, hypertension and spontaneous or diuretic-induced hypokalemia, hypertension with an adrenal incidentaloma, or hypertension and a family history of early-onset hypertension, stroke at a young age or a first-degree relative with primary aldosteronism [16]. The ARR is the screening test of choice with a recommended cut-off value of 91 pmol/mU [16]. However, the diagnostic workup for primary aldosteronism remains a challenge and several factors are known to influence the ARR, including posture [17], time of blood sampling [17,18], age [19,20], salt intake [20,21], presence of hypokalemia [22], sex [23] and use of contraceptives [23–25], NSAIDs [26] and certain selective serotonin reuptake inhibitors [27].

Antihypertensive treatment is a major consideration when interpreting the ARR and many antihypertensive

Journal of Hypertension 2013, 31:000–000

[a]Erasmus Medical Centre, Department of Internal Medicine, Rotterdam, [b]Academic Medical Centre, Department of Internal Medicine, Amsterdam, [c]Red Cross Hospital, Department of Internal Medicine, Beverwijk, [d]Radboud University Nijmegen Medical Centre, Department of Internal Medicine, [e]University of Groningen, University Medical Centre Groningen, Department of Endocrinology, Groningen, [f]Vrije Universiteit Medical Centre, Department of Internal Medicine, Amsterdam, [g]Ziekenhuis Groep Twente, Department of Internal Medicine, Almelo and [h]Vlietland Hospital, Department of Internal Medicine, Schiedam, The Netherlands

Correspondence to Anton H. van den Meiracker, MD, PhD, Division of Pharmacology and Vascular Medicine, Department of Internal Medicine, Room D432, Erasmus MC, 's Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands. Tel: +31 10 7034220; e-mail: a.vandenmeiracker@erasmusmc.nl

Received 10 January 2013 **Revised** 9 May 2013 **Accepted** 26 July 2013

J Hypertens 31:000–000

DOI:10.1097/HJH.0b013e3283656b54

drugs are known to affect the ARR [28]. It is generally accepted that β-adrenergic receptor blocking agents and centrally acting antihypertensives, related to their renin suppressive effect, can lead to falsely elevated ARR levels [28–31]. In contrast, angiotensin-converting enzyme inhibitors (ACE-Is) and angiotensin II type 1 receptor blockers (ARBs) lead to a decrease in ARR [28,30]. Diuretics, including potassium-sparing, are also thought to lower the ARR [16,31,32], although we recently observed a small and borderline significant increase in ARR in patients with essential hypertension treated with eplerenone [33]. The current guidelines recommend to assess the ARR under an α-adrenergic-receptor blocker, hydralazine and/or a calcium channel blocker (CCB), preferably of the nondihydropyridine subclass, as these drugs have little or no effect on the ARR [16]. The role of dihydropyridine CCBs is unclear. In theory, these agents cause a decrease in ARR via an increase in renin levels through reflex sympathetic mechanisms and a decrease in aldosterone levels through inhibition of calcium-dependent adrenal aldosterone production [28,34]. These effects are probably negligible under stable treatment and, in support of this, no effect of dihydropyridine CCBs on the ARR was found in a cross-sectional study [30]. Despite the known effects of individual antihypertensives on the ARR, their clinical relevance when used in combination treatment is less clear and whether patients on combination treatment should be switched to a standardized regime before assessment of the ARR has been debated [35]. Gallay *et al.* [35] concluded that the ARR remains a valid screening test when antihypertensive medications are continued, but their study design did not allow for a proper assessment of sensitivity and specificity. Another study showed that in patients on combination treatment, it is mainly β-adrenergic receptor blocking agents, ACE-Is and ARBs that affect the ARR [30]. A different cut-off value for the ARR may be needed when taken under antihypertensive treatment [36].

Although the ARR is widely used, studies on its sensitivity and specificity have been inconsistent. Sensitivities from 66 to 100% and specificities from 61 to 100% have been reported [11,37–43]. These wide ranges can be explained by differences in cut-off values, laboratory assays, study population and sampling conditions. Furthermore, many studies have methodological limitations [44]. In addition to a high sensititivity and specificity, a screening test should be reproducible. Unfortunately, there is a wide spontaneous variation in plasma renin concentration (PRC) and plasma aldosterone concentration (PAC), even in patients with primary aldosteronism [45]. This may imply that a single ARR is inadequate to confirm or exclude the diagnosis of primary aldosteronism [45].

Our aim was to assess the test characteristics of the ARR in patients with difficult-to-control hypertension and to evaluate whether combination antihypertensive treatment affects the ARR to a degree that a change in medication is warranted.

MATERIALS AND METHODS

Patients

In this multicentre study, patients aged 18–65 years with an office SBP above 140 mmHg and/or DBP above 90 mmHg despite the use of at least two antihypertensive drugs were invited to participate. Patients were excluded if a known cause of hypertension was present, if they suffered from cardiogenic chest pain or heart failure and in case of a cerebrovascular or cardiovascular event within 6 months before study entry or pregnancy.

Design

β-adrenergic receptor blocking agents and potassium-sparing diuretics were discontinued at least 4 weeks before start of the study protocol. All other antihypertensive agents were continued. When hypokalaemia was present, oral potassium replacement was started. PRC and PAC were assessed twice within an interval of 2–4 weeks. At the second visit, all patients also underwent an intravenous salt loading test (SLT) consisting of an infusion of 2 l of NaCl 0.9% in 4 h. Patients with a postinfusion PAC exceeding 235 pmol/l were considered to have primary aldosteronism [46]. Subsequently, the random antihypertensive medication regime was replaced by a standardized medication regime consisting of a combination of a CCB (in most cases amlodipine 5 or 10 mg q.d.) and doxazosin 4 or 8 mg q.d., depending on BP levels. After 4 to 6 weeks on this combination of drugs, PRC and PAC measurements were repeated. Patients with a positive SLT underwent a computed tomography (CT) scan of the adrenals to screen for the presence of an aldosterone-producing adenoma (APA).

The study was approved by the Institutional Review Board and Ethical Committee of the Erasmus MC in Rotterdam and has been registered at www.clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT00407784). All patients gave written informed consent to participate.

Clinical and biochemical measurements

At baseline, clinical data, height and weight, and number and types of antihypertensive drugs were recorded. Patients were screened for the presence of left ventricular hypertrophy (LVH) using a standard 12-lead ECG on the basis of the Sokolow–Lyon criteria [47]. Blood pressure (BP) was measured at all visits as three subsequent measurements after at least 5 min in a sitting position with a validated automatic BP measurement device. The mean value of the last two readings was used for the analysis.

At all visits, serum sodium, potassium, urea and creatinine levels were measured using locally available routine laboratory techniques. In addition, 24-h urinary samples were collected for determination of sodium, potassium and creatinine excretion. PRC and PAC were determined by collecting blood samples in EDTA plasma by venipuncture between 0800 and 1000 h after 10 min in sitting position. The samples were centrifuged at room temperature for 10 min at 3000*g*. The plasma was then collected and stored at −20°C until analysis. PAC was measured with a radioimmunoassay (Coat-a-Count; Diagnostics Product Corporation, Los Angeles, California, USA). This assay has a detection limit of 30.5 pmol/l (11 pg/ml) and a coefficient of variance of 8.4%. PRC was assessed using an immunoradiometric assay (Renin III; Cisbio, Gif-sur-Yvette, France) with a detection limit of 2.02 mU/l and a coefficient of variance of 7.2%. All PAC and PRC

measurements were performed at the hypertension research laboratory of the Erasmus MC.

Statistical analysis

Values are expressed as mean ± SD, or as median and interquartile range (IQR) when not normally distributed. Categorical values are reported as percentages.

Medication use was quantified by adding up the total number of different antihypertensive drugs, as well as by assessing the defined daily dose (DDD) per drug and for total drug use according to the WHO Anatomical Therapeutic Chemical (ATC) index [48]. Antihypertensive agents were grouped in the following categories: diuretics, renin-angiotensin-system (RAS) blockers (i.e. ACE-Is, ARBs and renin inhibitors), CCBs, β-adrenergic receptor blocking agents, adrenergic-receptor blockers and other antihypertensive drugs.

Differences between two groups were tested with the Student's *t*-test or the Wilcoxon-signed rank test when not normally distributed. Sensitivity and 1−specificity for different cut-off values of the ARR were plotted in receiver operating characteristic (ROC) curves. Areas under the curve (AUC) were compared using a nonparametric approach [49]. The optimal cut-off point was assessed using Youden's J statistic and by identifying the cut-off value at which 95% sensitivity was reached.

To evaluate the reproducibility of the ARR, the values of the first two visits were subjected to natural logarithmic transformation (LnARR1 and LnARR2). The differences between LnARR1 and LnARR2 were plotted against the mean of these values in Bland–Altman plots. The 95% limits of agreement of these differences were calculated and expressed as the ratio between ARR1 and ARR2 by reversing the Ln-transformation.

The effect of individual medication groups on the ARR was assessed in a multivariate linear regression model with the relative change in ARR after medication change from random medication to standard medication as a dependent variable after natural logarithmic transformation [$(\mathrm{Ln}(\mathrm{ARR_{SM}}/\mathrm{ARR_{RM}})$]. The main classes of antihypertensive medication, the presence or absence of primary aldosteronism, age and sex were included as covariates.

Analyses were performed in SPSS Statistics 20 for MacOS X (IBM, Armonk, New York, USA) and Graphpad Prism 5.02 for Windows (GraphPad Software Inc., La Jolla, California, USA). ROC curve analyses were performed in MedCalc 12.3.0.0 for Windows (MedCalc Software, Mariakerke, Belgium).

RESULTS

Patients

A total number of 186 patients were included in the study. Six patients were excluded from the analysis because their β-adrenergic receptor blocking agent or potassium-sparing diuretic was not stopped after inclusion. Two patients were lost to follow-up before an SLT could be performed. In total, data of 178 patients from 13 hospitals were available for analysis. Of these patients, 27 had a positive SLT (PA) and 151 had a negative SLT (essential hypertension). This resulted in a 15.2% prevalence of primary aldosteronism in our population. Thirteen of the 27 patients with primary aldosteronism had evidence of an adrenal adenoma on the CT scan.

Table 1 shows the baseline characteristics of patients with essential hypertension and primary aldosteronism. SBP and DBP were similar in both patient groups, as was the median number of antihypertensive drugs. Medication use expressed in DDD tended to be lower in patients with primary aldosteronism ($P=0.084$). The most frequently used treatment combinations were diuretics, RAS blockers and CCBs (40%), diuretics and RAS blockers (14%), RAS blockers and CCBs (13%), and diuretics, RAS blockers, CCBs and adrenergic-receptor blockers (12%).

There were no differences in age, sex, BMI and percentage of diabetic individuals between essential hypertensive and primary aldosteronism groups. Also, the proportion of patients with previous cardiovascular complications was similar, but the albumin-to-creatinine ratio was higher in patients with primary aldosteronism (2.09 vs. 1.19 mg/mmol, $P=0.035$). Patients with primary aldosteronism had a higher serum Na^+ and lower serum K^+ than patients with essential hypertension. A higher proportion of patients with primary aldosteronism was hypokalemic or on potassium replacement. As expected, PRC was lower, and the PAC and ARR were higher in patients with primary aldosteronism than in those with essential hypertension.

Sensitivity and specificity of the aldosterone-to-renin ratio

Median PRC was 27.2 (IQR 13.6–87.5) mU/l in essential hypertension and 16.4 (8.6–28.6) mU/l in primary aldosteronism ($P=0.003$). Median PAC was 227 (158–380) pmol/l in essential hypertension compared with 565 (371–725) pmol/l in primary aldosteronism ($P<0.0001$). As a consequence, the ARR was higher in primary aldosteronism than in essential hypertensive patients (35.0 versus 7.1 pmol/mU, $P<0.001$; Fig. 1). Patients with primary aldosteronism with an APA on CT scan tended to have a lower PRC and higher PAC than patients without an APA, whereas the ARR was significantly higher in patients with an APA (median ARR 53.4 versus 22.4 pmol/mU, $P=0.024$; Fig. 2).

When a cut-off value of 91 pmol/mU was used, only six patients with primary aldosteronism demonstrated an elevated ARR resulting in a sensitivity of 22.2%. Two essential hypertensive patients had a positive ARR resulting in a specificity of 98.7% (Fig. 1). The positive predictive value (PPV) of the ARR at this cut-off was 75.0% and the negative predictive value (NPV) was 84.8% in our population. The positive likelihood ratio was 17.1 and the negative likelihood ratio was 0.79. Figure 3 shows the ROC curve for the ARR. The area under the curve (AUC) was 0.85 (SE 0.04, $P<0.0001$). The optimal cut-off point according to Youden's J statistic was an ARR of 24.0 [95% confidence interval (CI) 12.4–34.9] pmol/mU, resulting in a sensitivity of 70.4% and a specificity of 83.4% (Youden's J index 0.54 (95% CI 0.36–0.64). However, for a screening test, a high sensitivity is the most important feature. To achieve 95% sensitivity, the cut-off value of the ARR should be further

TABLE 1. Baseline characteristics of patients with essential hypertension and primary aldosteronism

	EH ($n=151$)	PA ($n=27$)	P
Age (years)	49.9±9.7	47.6±9.4	0.256
Males (%)	53.0	55.6	0.805
BMI (kg/m^2)	28.7 (26.3–31.7)	29.0 (24.7–32.0)	0.826
Whites (%)	67.5	70.4	0.772
Smokers (%)	30.2	23.1	0.461
History of CVD (%)	10.7	3.8	0.274
DM (%)	11.9	7.4	0.494
LVH (%)	18.2	28.0	0.260
ACR (mg/mmol)	1.2 (0.5–2.9)	2.1 (1.1–5.7)	0.035
Duration of HT (months)	261 (94–699)	261 (87–524)	0.567
SBP (mmHg)	155.8±21.4	159.0±15.9	0.452
DBP (mmHg)	94.4±12.2	97.4±11.4	0.241
Pulse (beats/min)	72.9±11.7	68.2±10.9	0.054
Na^+ (mmol/l)	141.9±2.3	142.9±2.2	0.030
K^+ (mmol/l)	3.9±0.5	3.5±0.5	<0.001
Serum creatinine (μmol/l)	80.0±19.1	82.6±22.6	0.526
Na^+ excretion (mmol/24 h)	184 (131–231)	182 (147–219)	0.947
Hypokalemia (%)	15.9	48.1	<0.001
K-suppletion (%)	2.7	23.1	<0.001
TTKG	6.1 (4.8–7.7)	7.3 (5.6–9.5)	0.032
PRC (mU/l)	27.2 (13.6–87.5)	16.4 (8.6–28.6)	0.003
PAC (pmol/l)	227 (158–380)	565 (382–806)	<0.001
ARR (mmol/mU)	7.1 (2.2–17.5)	35.0 (16.2–82.0)	<0.001
No. of antihypertensives	3 (3–4)	3 (2–3)	0.028
DDD	4.5 (3.3–6.0)	3.5 (3.0–6.0)	0.084
Diuretics (%)	80.8	66.7	0.099
RAS blockers (%)	88.7	77.8	0.118
CCB (%)	74.8	77.8	0.744
BB (%)	43.7	29.6	0.172
Alpha-adrenergic blockers (%)	16.6	25.9	0.243
Other α-HT (%)	3.3	0.0	0.338

Data presented are mean±SD or median (interquartile range). α-HT, antihypertensives; ACR, albumin-creatinine ratio; ARR, aldosterone-to-renin ratio; BB, beta-adrenergic blocker; CCB, calcium channel blocker; CVD, cardiovascular disease; DDD, defined daily dose; DM, diabetes mellitus; HT, hypertension; K^+, serum potassium; LVH, left ventricular hypertrophy; Na^+, serum sodium; PAC, plasma aldosterone concentration; PRC, plasma renin concentration; RAS, renin–angiotensin system; TTKG, transtubular potassium gradient.

lowered to 5.6 pmol/mU at the cost of a significant decrease in specificity to 46.9% (95% CI 29.9–70.8).

Due to the study design, patients could be on any type of antihypertensive medication, with the exception of β-adrenergic receptor blocking agents and potassium-sparing diuretics, during the SLT and this may have affected the outcome of the confirmation test. Nine patients (four with essential hypertension and five with primary aldosteronism) were on 'ARR-neutral' medication when the SLT was performed. Their clinical and biochemical profile is shown in Table 2. Of the five patients with primary aldosteronism, only one had an ARR above 91 pmol/mU. All three patients with an APA had ARR levels below this cut-off value.

The effect of medication on the aldosterone-to-renin ratio

After the diagnostic phase, which included assessment of the ARR and performance of the SLT, the random antihypertensive medication was changed to a standardized medication regime consisting of doxazosine and a CCB, in most cases amlodipine. After 4–6 weeks on this antihypertensive regime, the ARR was repeated. Of the total population of 178 patients, this phase could be completed in 145 patients (126 patients with essential hypertension and 19 with primary aldosteronism). The main reasons for not completing this phase were that the BP was or became too high to safely change the medication and the occurrence of side effects.

After changing from random antihypertensive medication to standardized medication, a significant drop in PRC was seen for the group as a whole from 20.9 (11.7–72.1) to 12.4 (7.5–25.0) mU/l ($P<0.0001$), as well as for essential hypertensive and primary aldosteronism patients separately [from 22.8 (12.2–88.5) to 13.7 (8.2–25.6) mU/l ($P<0.0001$) for essential hypertension and from 13.6 (8.6–38.7) to 6.2 (3.3–15.6) ($P=0.0002$) for primary aldosteronism; Fig. 4]. PAC rose significantly in essential hypertensive [from 217 (157–350) to 328 (215–497) pmol/l, $P<0.0001$] but remained unchanged in primary aldosteronism patients [645 (382–898) pmol/l on random antihypertensive medication and 621 (460–892) pmol/l on standardized medication, $P=0.260$]. The ARR was higher on standardized medication than random antihypertensive medication in both essential hypernsive [increase from 7.8 (2.3–19.2) to 18.5 (10.0–43.6) pmol/mU; $P<0.0001$] and in primary aldosteronism patients [increase from 37.5 (16.2–82.0) to 97.0 (42.6–242.0) pmol/mU; $P<0.0001$] (Fig. 4). The influence of the individual medication classes on the change in ARR was tested in a multivariate regression model with $\mathrm{Ln}(ARR_{SM}/ARR_{RM})$ as the dependent variable. Of the main medication groups, only RAS-blockers significantly affected the change in ARR after switching to standardized medication (Table 3). Age had a negative association with the rise in ARR in our

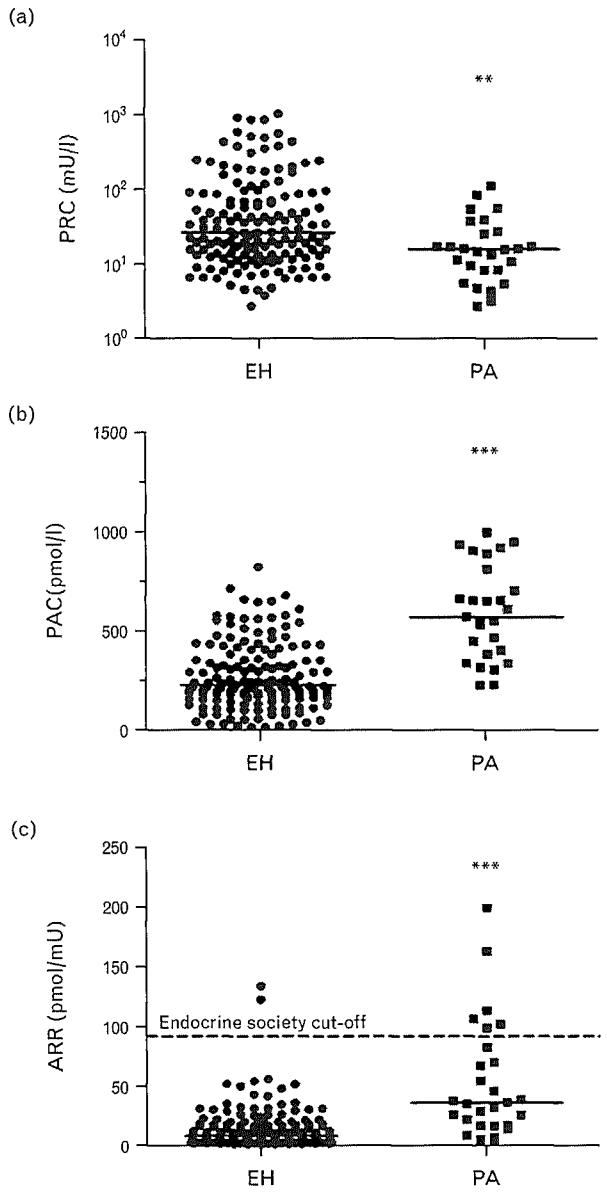

FIGURE 1 Plasma renin concentration (a), plasma aldosterone concentration (b) and aldosterone-to-renin ratio (c) in patients with essential hypertension and primary aldosteronism using random medication. The dashed line in (c) represents the Endocrine Society cut-off value of 91 pmol/mU. $^{**}P<0.01$, $^{***}P<0.001$.

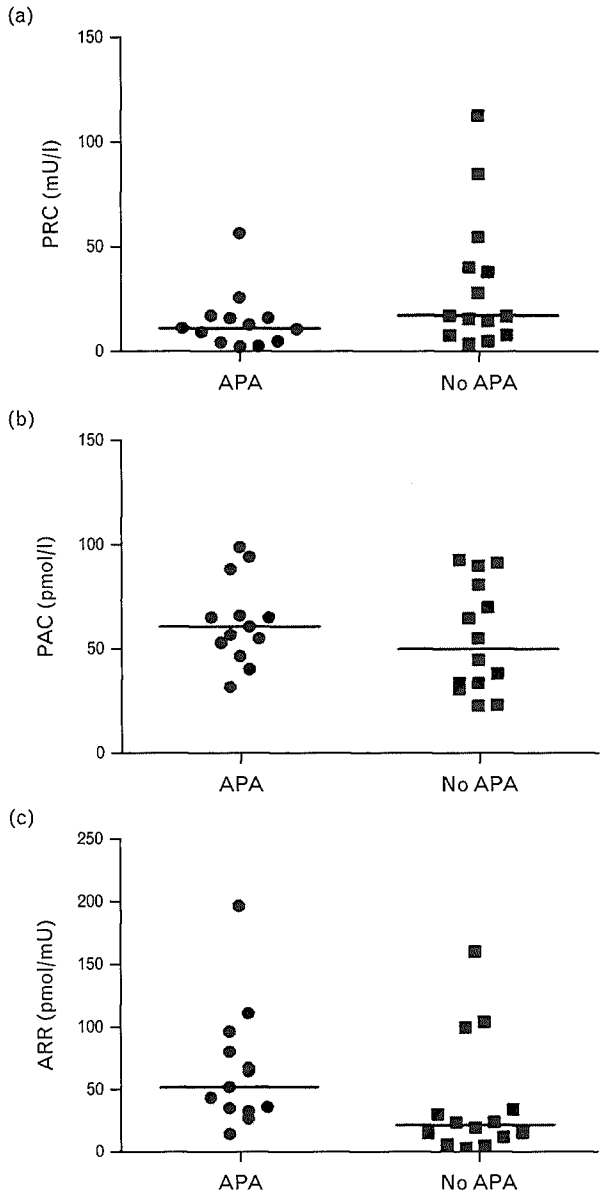

FIGURE 2 Plasma renin concentration (a), plasma aldosterone concentration (b) and aldosterone-to-renin ratio in primary aldosterone patients with (APA) or without (no APA) an aldosterone-producing adenoma on computed tomography scanning.

model. The presence of primary aldosteronism did not affect the change in ARR. Serum potassium had no association with the change in ARR in a univariate analysis (data not shown) and was not included in the model.

Figure 5 shows the ROC curves for these 145 patients during both random antihypertensive medication and standardized medication. The AUC for both curves was of similar magnitude [84.3% (SE 4.5%) for random antihypertensive medication versus 86.3% (SE 4.2%) for standardized medication ($P=0.31$)]. In this subgroup, the optimal ARR cut-off according to the highest Youden's index was 15.2 (95% CI 4.9–20.0) pmol/mU on random antihypertensive medication [Youden's index J 0.54 (95% CI 0.37–0.64); sensitivity 89.5%, specificity 64.3%] and 40.2 (95% CI 16.1–81.6) pmol/mU on standardized medication [Youden's index J 0.63 (95% CI 0.44–0.75); sensitivity 89.5%, specificity 73.8%]. When aiming for a sensitivity of 95%, the cut-off value should be lowered to 5.8 pmol/mU on random antihypertensive medication [associated specificity 45.2% (95% CI 34.9–57.3)] and 16.3 pmol/mU on standardized medication [associated specificity 43.7% (95% CI 32.4–57.9)].

Reproducibility

PRC, PAC and the ARR were assessed on two subsequent visits with an interval of 2–4 weeks. The antihypertensive medications and the conditions of sampling were the same on both occasions. A total number of 158 patients

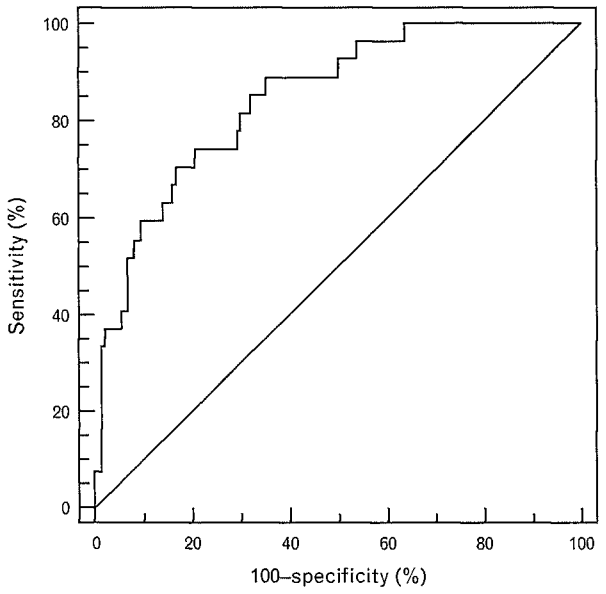

FIGURE 3 Receiver operating characteristic curve for the aldosterone-to-renin ratio under a random antihypertensive medication regime. The area under the curve is 0.85 (SE 0.04, $P < 0.0001$).

were available for this analysis (133 essential hypertensive and 25 primary aldosteronism patients). Six patients (five primary aldosteronism and 1 essential hypertension) had an ARR above 91 pmol/mU at the first visit and eight patients (seven primary aldosteronism and 1 essential hypertension) at the second. Nine patients (seven primary aldosteronism and two essential hypertension) had at least one elevated ARR. Figure 6 shows Bland–Altman plots for ln(PRC), ln(PAC) and ln(ARR). The 95% limits of agreement are shown in the figures. The lower limit was −1.55 (95% CI −1.57 to −1.53) corresponding to an ARR1/ARR2 ratio of 0.21 (0.21–0.22). The upper limit was 1.44 (95% CI 1.42–1.47) corresponding to an ARR1/ARR2 ratio of 4.24 (4.15–4.33). This indicates that the difference between the two readings can range up to almost five-fold.

DISCUSSION

The ARR is the standard screening test for primary aldosteronism. However, data on its sensitivity, specificity and reproducibility have been conflicting. Whether the antihypertensive treatment should be standardized before performing the ARR has remained a subject of debate. The recent Endocrine Society guideline on the diagnosis and treatment of primary aldosteronism recommends a cut-off value for the ARR of 91 pmol/mU [16]. The results shown in this study indicate that the sensitivity of the ARR under a random medication regime, but with the exclusion of β-adrenergic receptor blocking agents and/or potassium-sparing diuretics, is low when this cut-off value is used. Lowering the cut-off value improves sensitivity, but at a considerable loss of specificity.

The sensitivity and specificity of the ARR have been studied by others [11,15,37,38,40–43,50,51] yielding markedly variable findings. Sensitivities between 66 and 100% and specificities between 61 and 96% have been

TABLE 2. Characteristics of patients on aldosterone-to-renin ratio neutral medication during the intravenous salt loading test

Age (years)	Sex	APA	Antihypertensives	SBP (mmHg)	DBP (mmHg)	Na^+ (mmol/l)	K^+ (mmol/l)	Creatinine (μmol/l)	Na^+-excretion (mmol/24 h)	PRC (mU/l)	PAC (pmol/l)	ARR (pmol/mU)
Patients with essential hypertension												
61	F	N/A	Nifedipine 30 mg; Doxazosine 4 mg	146	87	142	3.8	59	186	9.1	219	24.0
52	F	N/A	Nifedipine 60 mg twice daily; Doxazosine 8 mg twice daily	133	87	144	3.0	52	196	11.7	643	54.9
64	M	N/A	Nifedipine 30 mg; Doxazosine 4 mg twice daily	161	95	145	2.9	82	171	9.3	471	50.6
65	M	N/A	Barnidipine 10 mg	157	116	139	3.9	84	N/D	8.9	194	21.8
Patients with primary aldosteronism												
39	M	No	Nifedipine 30 mg twice daily; Doxazosine 4 mg	179	102	144	3.7	96	221	28.6	698	24.4
41	F	Yes	Nifedipine 60 mg	134	88	144	3.3	61	140	16.7	565	33.8
40	F	Yes	Nifedipine 60 mg; Doxazosine 8 mg	137	86	144	3.4	56	183	17.8	648	36.4
57	M	No	Amlodipine 10 mg; Doxazosine 8 mg	174	76	142	3.4	96	200	8.6	911	106.0
48	M	Yes	Amlodipine 10 mg	144	97	142	3.2	72	181	9.8	648	66.1

APA, aldosterone-producing adenoma on computed tomography scan; ARR, aldosterone-to-renin ratio; K^+, serum potassium; N/A, not applicable; N/D, not done; Na^+, serum sodium; PAC, plasma aldosterone concentration; PRC, plasma renin concentration.

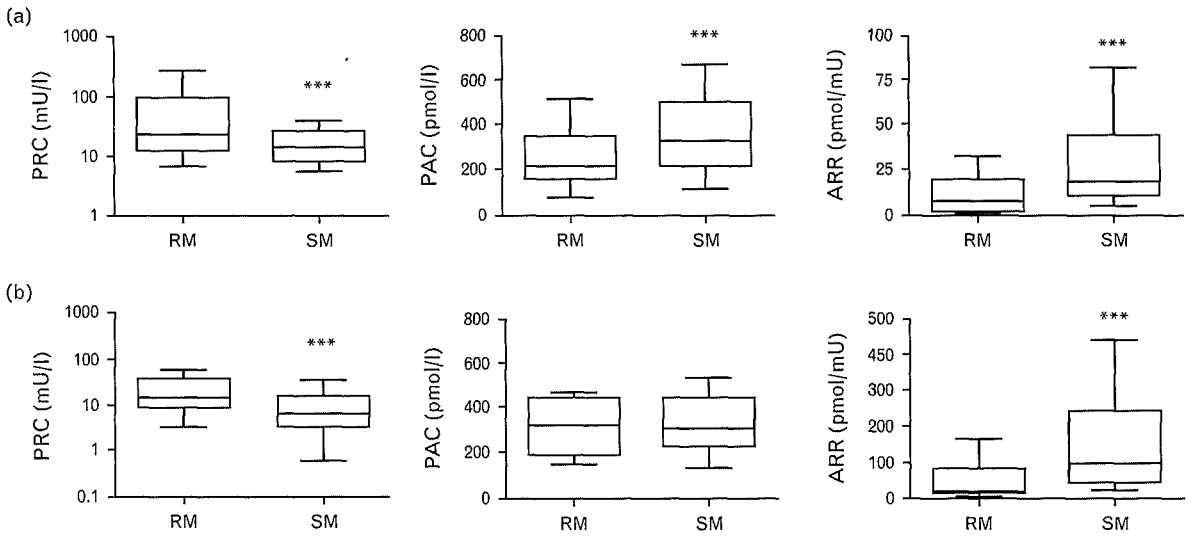

FIGURE 4 *Plasma renin concentration, plasma aldosterone concentration and aldosterone-to-renin ratio on random medication and after switching to a standardized medication regime in patients with essential hypertension (a) and primary aldosteronism (b). The box-and-whisker plots represent the median, IQR and the 10th–90th percentile.* $^{***}P < 0.001$.

reported. Comparing the findings of the various studies is confounded by factors such as differences in cut-off values, laboratory assays (PRA versus PRC), the conditions of testing and the confirmation tests used. Many studies suffer from verification bias, as usually only patients with an ARR above the predefined cut-off value were subjected to confirmatory testing. Such an approach is likely to result in an overestimation of diagnostic performance [44]. The same problem may occur in retrospective studies, in which patients and controls are often selected on the basis of the same diagnostic test that is the subject of evaluation. In our study, both the screening and the confirmation tests were performed prospectively in all participating patients allowing an unbiased evaluation of the diagnostic performance of the ARR.

The use of antihypertensive medication is one of the most important factors to take into account when interpreting the ARR. The effects of individual antihypertensive drugs on renin and aldosterone levels have been discussed by others [28–30,32], but the relevance of combination treatment has been debated [35]. We show that combination treatment significantly affects the ARR and the ARR is lower during random combination antihypertensive treatment than during standardized treatment with a CCB and α-adrenergic receptor blocker. A multivariate analysis pointed out that the use of RAS blockers is responsible for the lower ARR in nonstandardized combination treatment. This is not an unexpected finding, as RAS blockers increase the PRC to a greater extent than that they lower the PAC, resulting in a higher ARR. Contrary to the decrease in PRC, PAC did not rise in primary aldosteronism after the medication change, likely reflecting the autonomy of aldosterone production in these patients. It should be noted that β-adrenergic receptor blocking agents and potassium-sparing diuretics were ceased prior to the start of the study protocol. Therefore, no conclusion can be made regarding the contribution of these agents. However, β-adrenergic receptor blocking agents are known to lower renin levels and to increase the ARR both as monotherapy [28,29] and as part of combination treatment [30]. With this in mind, our results are in agreement with the observations by Seifarth *et al.* [30], showing that in combination treatment, both β-adrenergic receptor blocking agents and ACE-I/ARBs have the largest influence on the ARR. Despite the observed change in ARR in our study, the AUC under the ROC curve remained unchanged, indicating that the test will perform just as well in a multidrug setting, but that a lower cut-off value is required [36]. In theory, this means

TABLE 3. Multivariate regression analysis with the natural logarithm of the relative rise in aldosterone-to-renin ratio after changing from random medication to standardized medication [Ln(ARR_{SM}/ARR_{RM})] as a dependent variable

	β-coefficient	SE	*P*
Age (years)	−0.018	0.009	0.045
Sex (0, F; 1, M)	0.238	0.173	0.171
PA (0, no; 1, yes)	−0.176	0.252	0.486
Diuretics	−0.038	0.201	0.851
RAS blockers	1.053	0.284	<0.001
CCB	0.273	0.243	0.263
Alphablockers	0.113	0.206	0.585

CCB, calcium channel blockers; PA, primary aldosteronism; RAS, renin–angiotensin system; SE, standard error.

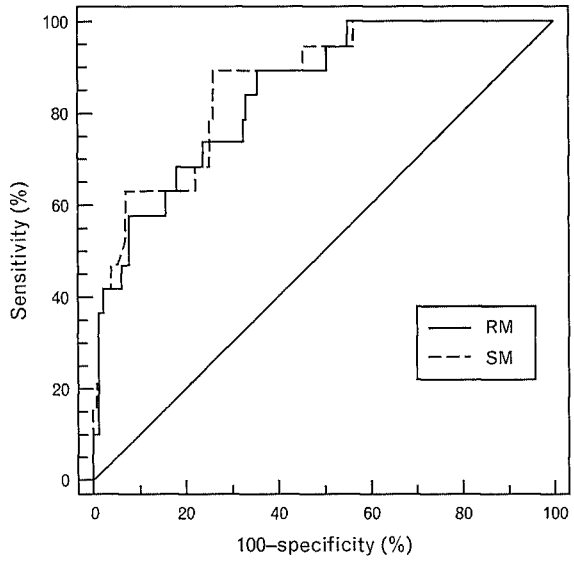

FIGURE 5 Receiver operating characteristic curve for the aldosterone-to-renin ratio taken under a random antihypertensive medication regime and under standardized medication with a calcium channel blocker and doxazosine. The area under the curve (AUC) is 0.84 (SE 0.04) for RM and 0.86 (SE 0.04) for SM ($P=0.31$).

that combination treatment does not need to be stopped. The question, however, remains what cut-off value should be chosen under random antihypertensive treatment. The change in ARR will be hard to predict, due to different combinations of ACE-Is, ARBs and/or beta-adrenergic blockers and variations in the prescribed doses and, for this reason, a clear-cut cut-off cannot be defined. This strongly favours a standardized approach. Many other drugs including NSAIDs [26], certain SSRIs [27] and oral contraceptives [23–25] can influence the ARR. The use of these drugs was not systematically recorded in this study and could have had a small effect on the test results.

For an optimal evaluation of a screening test, the gold standard should be reliable. A limitation in our design was that the SLT was performed during a random medication regime, including diuretics and RAS blockers. The Endocrine Society guideline recommends to perform the SLT under ARR-neutral medication [16]. Although it is reasonable to assume that antihypertensives (diuretics and RAS blockers in particular) have an effect on the outcome of the SLT, this has never been systematically studied. Our protocol reflects clinical reality where it is often more feasible and practical to continue ongoing antihypertensive treatment during confirmation testing. In our study, nine patients (five with primary aldosteronism and four with essential hypertension) were on a CCB and α-adrenergic receptor blocker when the SLT was performed and their clinical profile and ARR values are reported individually. Although the generalizability of these data is limited, they suggest that even under optimal conditions, only a minority of patients with primary aldosteronism have an elevated ARR. Notably, three of these patients had an APA that would have been missed if the ARR cut-off value of 91 pmol/mU had been used.

Another point of debate is the cut-off value for a positive SLT, which was 235 pmol/l in our study [46]. Unfortunately, the optimal SLT cut-off value has never been unequivocally defined. The guideline [16] states that a postinfusion PAC above 277 pmol/l (100 pg/ml) makes the diagnosis of primary aldosteronism very likely and below 139 pmol/l (50 pg/ml) unlikely. A postinfusion PAC between these values is indeterminate. However, the literature on this is limited and local protocols have their own cut-off values [16]. A postinfusion PAC of 139 pmol/l was shown to be the optimal cut-off by some [52,53], with a sensitivity of 88% compared with the fludrocortisone suppression test (FST) [53]. On the contrary, Streeten *et al.* [46] found 235 pmol/l to be the optimal cut-off with 77% sensitivity compared with the FST, although not all patients were subjected to the FST. Our protocol required a single cut-off value and, although arbitrary, in our view, the chosen cut-off provides a fair compromise of all available data and has been used by others [14,46,50]. When a postinfusion PAC cut-off of 277 pmol/l had been used, the prevalence of primary aldosteronism would have been 12.4% in our population. Six primary aldosteronism patients and two essential hypertensive patients would have had an ARR above 91 pmol/mU resulting in a sensitivity of 27.3% and a specificity of 98.7%. Therefore, a more stringent criterion for the SLT would not have altered the outcome of the study significantly.

Many of our primary aldosteronism patients had a normal ARR because their PRC was not or only marginally suppressed. This can be explained in part by medication use, but even under standardized conditions, many of these patients had relatively high PRC levels. Therefore, additional explanations for these higher than expected PRC levels need to be considered. In our study, the ARR was based on PRC and not on PRA. Many of the earlier studies reporting on the ARR have used the PRA. The assessment of the PRA has disadvantages, being time-consuming, dependent on endogenous angiotensinogen levels and difficult to standardize [54]. Use of the PRC allows for standardization of the test procedure and comparison with international reference values. Studies have shown that this can be a reasonable alternative for the PRA [55–58], although the PRC may be less sensitive in the very low range [54,58]. The assay used here has a detection limit of 2.02 mU/l, which should enable detection of suppressed levels of PRC. Cryoactivation of prorenin (allowing its detection in a renin assay) can be a cause for falsely elevated PRC levels [59], particularly at low PRC levels. Incubation of samples at 37°C (such as during a PRA measurement) facilitates the return of prorenin to its closed, inactive conformation so that it can no longer display activity nor will be detected in a renin assay. Theoretically therefore, cryoactivation is expected to affect PRA to a lesser degree than PRC. Nevertheless, in our samples, PRC levels were only rarely close to the detection limit of the assay (Fig. 1A). Moreover, to prevent prorenin cryoactivation, samples were always processed at room temperature and the plasma was rapidly frozen until analysis. Furthermore, all PRC as well as PAC measurements were performed in one laboratory with extensive experience in performing these measurements.

Salt intake is a strong determinant of RAS activity and low salt intake can lead to a marked rise in renin [60]. Although salt intake was not actively controlled for, it was generally

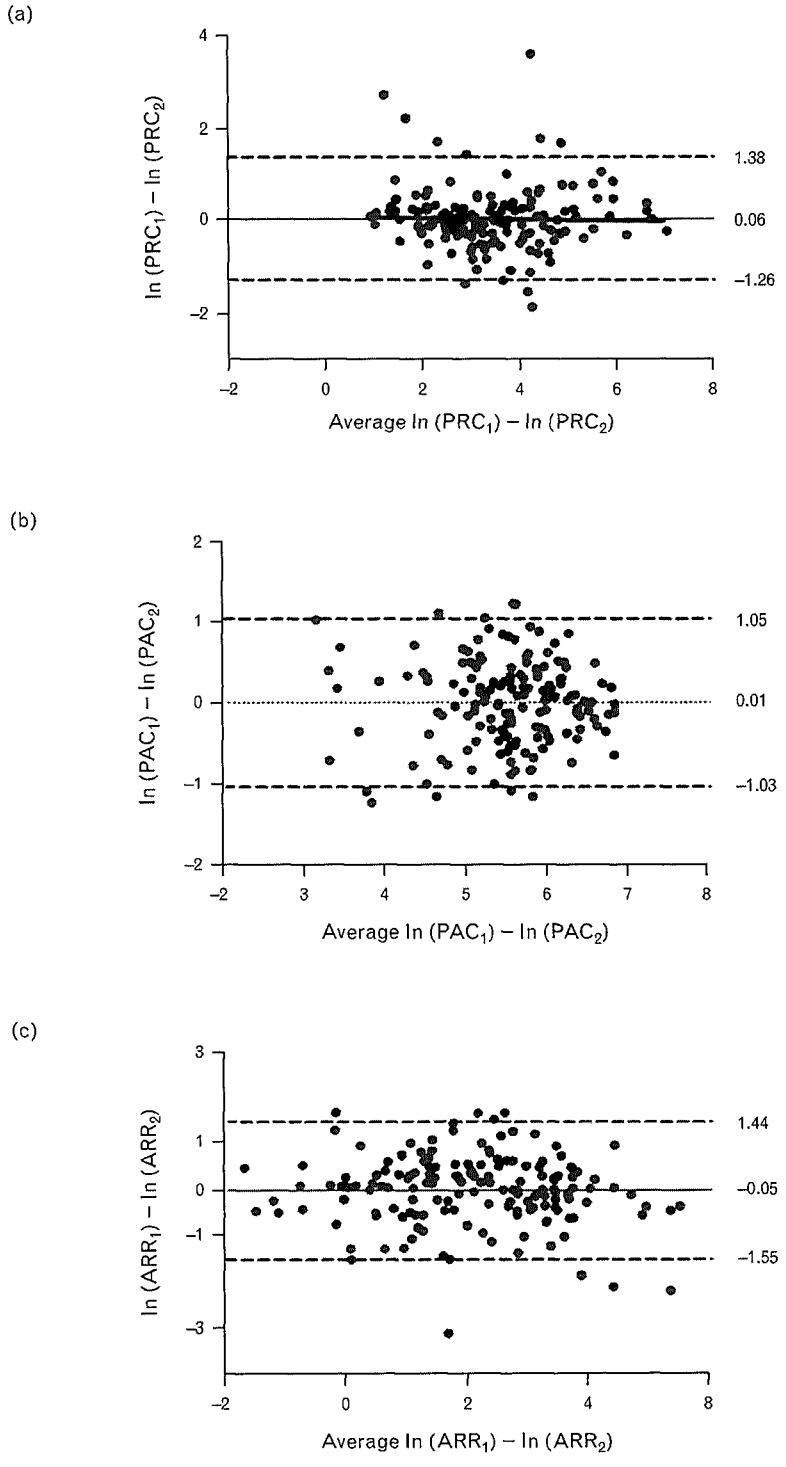

FIGURE 6 *Bland–Altman plots for plasma renin concentration (a), plasma aldosterone concentration (b) and aldosterone-to-renin ratio (c) on 2 subsequent visits after natural logarithmic transformation. The dashed lines represent the 95% limits of agreement.*

high in our population, and therefore, it is highly unlikely that the sometimes high PRC values can be explained by a low salt intake. It has also been suggested that the diagnosis of primary aldosteronism can be masked if hypertensive kidney damage is present, manifested by renal arteriolosclerosis leading to a rise in renin levels [61]. We selected patients with a relatively normal creatinine clearance to exclude the possibility of hypertension caused by renal disease. Nonetheless, subclinical renal damage may have contributed to the relatively high PRC levels in some of our primary aldosteronism patients.

Another factor to take into account is serum potassium levels. Potassium by itself can stimulate aldosterone secretion, whereas hypokalemia can inhibit aldosterone secretion, leading to lower PAC levels [22]. Failure to correct hypokalemia can therefore lead to falsely low ARR levels and an underdiagnosis of primary aldosteronism. Although we aimed to correct hypokalemia as much as possible, this was not successful in all patients. Twelve out of 27 primary aldosteronism patients had a serum potassium level below 3.5 mmol/l and three patients below 3.0 mmol/l at the time of the first ARR measurement. To correct hypokalemia completely in patients with severe primary aldosteronism can be a challenge. As aldosterone production is largely autonomous in primary aldosteronism, it may be questioned whether hypokalemia will affect PAC to an extent that is clinically relevant. Indeed, Tanabe *et al.* [45] observed an inverse relation between PAC and serum potassium concentration in primary aldosteronism, supporting the concept that serum potassium levels are determined by PAC in primary aldosteronism and not the other way around.

The question remains whether an elevated ARR is a good reflection of (relatively) autonomous aldosterone production. Montori *et al.* [32] showed that the ARR is mainly driven by renin and is therefore not a good indicator of inappropriately elevated aldosterone levels in relation to renin. On the contrary, it is debatable whether suppressed renin levels are a prerequisite for the diagnosis of primary aldosteronism. Previous studies, supported by the present findings, have shown that a significant number of primary aldosteronism patients have nonsuppressed renin levels even in the presence of an APA [62,63]. In addition to the various possibilities mentioned above, this may be related to individual differences in sensitivity and thresholds for RAS activation. Furthermore, the clinical picture of primary aldosteronism encompasses a broad spectrum with mild biochemical abnormalities on the low end and a florid phenotype with high aldosterone and markedly suppressed renin levels on the high end. This study confirms that primary aldosteronism patients with an APA on average have lower PRC and higher ARR values than patients without evidence of an APA on the CT-scan.

Another important consideration is test reproducibility. PRC, PAC and ARR are known to be subject to significant diurnal variations [18]. Sampling at a standardized time helps reducing variability in ARR levels [17]. Blood sampling was performed mid-morning in accordance with recommendations [16]. Nonetheless, day-to-day variations can be considerable with negative effects on reproducibility [45,64]. Our data show that ARR levels can display an almost five-fold difference when determined under the same conditions. The implication of this is that a single negative test cannot sufficiently rule out primary aldosteronism, although it remains to be determined how many tests are needed.

In conclusion, primary aldosteronism is the most prevalent cause of secondary hypertension, but establishment of this diagnosis can be challenging. The ARR is the standard screening test for primary aldosteronism, but our data show that its sensitivity is poor when the recommended cut-off value is used even under a standardized medication regime. Overall, test performance does not improve under a standardized treatment regime but requires a different cut-off value. Reproducibility is also low, stressing the need for multiple tests to establish the diagnosis. Despite these difficulties in screening, it remains clinically important to identify patients with primary aldosteronism, especially with surgically correctable forms. It is recommended to proceed quickly to a confirmation test when clinical suspicion is high.

ACKNOWLEDGEMENTS

J.H.A. Arnoldus, J.D. Banga, M. van den Berge, R.A. Carels, A. Dees, B.P. Imholz, E. Jäger, M.Y. Gerrits, S. Mohkamsing and B. van der Velde are gratefully acknowledged for their contribution to this study.

P.M.J. is financially supported by the Dutch Kidney Foundation (Grant no. C05-2151).

This study was financially supported by Pfizer, The Netherlands. Pfizer was not involved in the analyses nor in the preparation of the manuscript.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Hiramatsu K, Yamada T, Yukimura Y, Komiya I, Ichikawa K, Ishihara M, *et al.* A screening test to identify aldosterone-producing adenoma by measuring plasma renin activity. Results in hypertensive patients. *Arch Intern Med* 1981; 141:1589–1593.
2. Mulatero P, Stowasser M, Loh KC, Fardella CE, Gordon RD, Mosso L, *et al.* Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. *J Clin Endocrinol Metab* 2004; 89:1045–1050.
3. Williams JS, Williams GH, Raji A, Jeunemaitre X, Brown NJ, Hopkins PN, Conlin PR. Prevalence of primary hyperaldosteronism in mild to moderate hypertension without hypokalaemia. *J Hum Hypertens* 2006; 20:129–136.
4. Loh KC, Koay ES, Khaw MC, Emmanuel SC, Young WF Jr. Prevalence of primary aldosteronism among Asian hypertensive patients in Singapore. *J Clin Endocrinol Metab* 2000; 85:2854–2859.
5. Fogari R, Preti P, Zoppi A, Rinaldi A, Fogari E, Mugellini A. Prevalence of primary aldosteronism among unselected hypertensive patients: a prospective study based on the use of an aldosterone/renin ratio above 25 as a screening test. *Hypertens Res* 2007; 30:111–117.
6. Mosso L, Carvajal C, Gonzalez A, Barraza A, Avila F, Montero J, *et al.* Primary aldosteronism and hypertensive disease. *Hypertension* 2003; 42:161–165.
7. Westerdahl C, Bergenfelz A, Isaksson A, Wihl A, Nerbrand C, Valdemarsson S. High frequency of primary hyperaldosteronism among hypertensive patients from a primary care area in Sweden. *Scand J Prim Healthcare* 2006; 24:154–159.
8. Gordon RD, Stowasser M, Tunny TJ, Klemm SA, Rutherford JC. High incidence of primary aldosteronism in 199 patients referred with hypertension. *Clin Exp Pharmacol Physiol* 1994; 21:315–318.
9. Lim PO, Dow E, Brennan G, Jung RT, MacDonald TM. High prevalence of primary aldosteronism in the Tayside hypertension clinic population. *J Hum Hypertens* 2000; 14:311–315.
10. Fardella CE, Mosso L, Gomez-Sanchez C, Cortes P, Soto J, Gomez L, *et al.* Primary hyperaldosteronism in essential hypertensives: prevalence, biochemical profile, and molecular biology. *J Clin Endocrinol Metab* 2000; 85:1863–1867.
11. Rossi GP, Bernini G, Caliumi C, Desideri G, Fabris B, Ferri C, *et al.* A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. *J Am Coll Cardiol* 2006; 48:2293–2300.

12. Douma S, Petidis K, Doumas M, Papaefthimiou P, Triantafyllou A, Kartali N, *et al.* Prevalence of primary hyperaldosteronism in resistant hypertension: a retrospective observational study. *Lancet* 2008; 371: 1921–1926.
13. Stowasser M, Gordon RD, Gunasekera TG, Cowley DC, Ward G, Archibald C, Smithers BM. High rate of detection of primary aldosteronism, including surgically treatable forms, after 'nonselective' screening of hypertensive patients. *J Hypertens* 2003; 21:2149–2157.
14. Strauch B, Zelinka T, Hampf M, Bernhardt R, Widimsky J Jr. Prevalence of primary hyperaldosteronism in moderate to severe hypertension in the Central Europe region. *J Hum Hypertens* 2003; 17:349–352.
15. Calhoun DA, Nishizaka MK, Zaman MA, Thakkar RB, Weissmann P. Hyperaldosteronism among black and white subjects with resistant hypertension. *Hypertension* 2002; 40:892–896.
16. Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasser M, *et al.* Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2008; 93:3266–3281.
17. Tiu SC, Choi CH, Shek CC, Ng YW, Chan FK, Ng CM, Kong AP. The use of aldosterone-renin ratio as a diagnostic test for primary hyperaldosteronism and its test characteristics under different conditions of blood sampling. *J Clin Endocrinol Metab* 2005; 90:72–78.
18. Lamarre-Cliche M, de Champlain J, Lacourciere Y, Poirier L, Karas M, Larochelle P. Effects of circadian rhythms, posture, and medication on renin-aldosterone interrelations in essential hypertensives. *Am J Hypertens* 2005; 18:56–64.
19. Yin G, Zhang S, Yan L, Wu M, Xu M, Li F, Cheng H. Effect of age on aldosterone/renin ratio (ARR) and comparison of screening accuracy of ARR plus elevated serum aldosterone concentration for primary aldosteronism screening in different age groups. *Endocrine* 2012; 42:182–189.
20. Koch M, Aker S, Haastert B, Rump LC. Clinical relevance of dietary salt intake on aldosterone and the aldosterone-to-renin ratio as screening parameters for primary aldosteronism. *Clin Nephrol* 2010; 74:182–189.
21. Kerstens MN, Kobold AC, Volmer M, Koerts J, Sluiter WJ, Dullaart RP. Reference values for aldosterone-renin ratios in normotensive individuals and effect of changes in dietary sodium consumption. *Clin Chem* 2011; 57:1607–1611.
22. Cain JP, Tuck ML, Williams GH, Dluhy RG, Rosenoff SH. The regulation of aldosterone secretion in primary aldosteronism. *Am J Med* 1972; 53:627–637.
23. Pizzolo F, Raffaelli R, Memmo A, Chiecchi L, Pavan C, Guarini P, *et al.* Effects of female sex hormones and contraceptive pill on the diagnostic work-up for primary aldosteronism. *J Hypertens* 2010; 28:135–142.
24. Pizzolo F, Pavan C, Corrocher R, Olivieri O. Laboratory diagnosis of primary aldosteronism, and drospirenone-ethinylestradiol therapy. *Am J Hypertens* 2007; 20:1334–1337.
25. Ahmed AH, Gordon RD, Taylor PJ, Ward G, Pimenta E, Stowasser M. Effect of contraceptives on aldosterone/renin ratio may vary according to the components of contraceptive, renin assay method, and possibly route of administration. *J Clin Endocrinol Metab* 2011; 96:1797–1804.
26. Mitnick PD, Greenberg A, DeOreo PB, Weiner BM, Coffman TM, Walker BR, *et al.* Effects of two nonsteroidal anti-inflammatory drugs, indomethacin and oxaprozin, on the kidney. *Clin Pharmacol Ther* 1980; 28:680–689.
27. Ahmed AH, Calvird M, Gordon RD, Taylor PJ, Ward G, Pimenta E, *et al.* Effects of two selective serotonin reuptake inhibitor antidepressants, sertraline and escitalopram, on aldosterone/renin ratio in normotensive depressed male patients. *J Clin Endocrinol Metab* 2011; 96:1039–1045.
28. Mulatero P, Rabbia F, Milan A, Paglieri C, Morello F, Chiandussi L, Veglio F. Drug effects on aldosterone/plasma renin activity ratio in primary aldosteronism. *Hypertension* 2002; 40:897–902.
29. Ahmed AH, Gordon RD, Taylor P, Ward G, Pimenta E, Stowasser M. Effect of atenolol on aldosterone/renin ratio calculated by both plasma renin activity and direct Renin concentration in healthy male volunteers. *J Clin Endocrinol Metab* 2010; 95:3201–3206.
30. Seifarth C, Trenkel S, Schobel H, Hahn EG, Hensen J. Influence of antihypertensive medication on aldosterone and renin concentration in the differential diagnosis of essential hypertension and primary aldosteronism. *Clin Endocrinol (Oxf)* 2002; 57:457–465.
31. Stowasser M, Ahmed AH, Pimenta E, Taylor PJ, Gordon RD. Factors affecting the aldosterone/renin ratio. *Horm Metab Res* 2012; 44:170–176.
32. Montori VM, Schwartz GL, Chapman AB, Boerwinkle E, Turner ST. Validity of the aldosterone-renin ratio used to screen for primary aldosteronism. *Mayo Clin Proc* 2001; 76:877–882.
33. Jansen PM, Frenkel WJ, van den Born BJ, de Bruijne EL, Deinum J, Kerstens MN, *et al.* Determinants of blood pressure reduction by eplerenone in uncontrolled hypertension. *J Hypertens* 2013; 31:404–413.
34. Kondo T, Goto R, Sonoda K, Yasuda T, Ono K, Takaki Y, *et al.* Plasma renin activity and aldosterone concentration are not altered by the novel calcium channel antagonist, azelnidipine, in hypertensive patients. *Intern Med* 2010; 49:637–643.
35. Gallay BJ, Ahmad S, Xu L, Toivola B, Davidson RC. Screening for primary aldosteronism without discontinuing hypertensive medications: plasma aldosterone-renin ratio. *Am J Kidney Dis* 2001; 37:699–705.
36. Niizuma S, Nakahama H, Kamide K, Fukuchi K, Iwanaga Y, Nakata H, *et al.* The cutoff value of aldosterone-to-renin ratio for the diagnosis of primary aldosteronism in patients taking antihypertensive medicine. *Clin Exp Hypertens* 2008; 30:640–647.
37. Schwartz GL, Chapman AB, Boerwinkle E, Kisabeth RM, Turner ST. Screening for primary aldosteronism: implications of an increased plasma aldosterone/renin ratio. *Clin Chem* 2002; 48:1919–1923.
38. Bernini G, Moretti A, Orlandini C, Berti P, Miccoli P, Bardini M, *et al.* Plasma and urine aldosterone to plasma renin activity ratio in the diagnosis of primary aldosteronism. *J Hypertens* 2008; 26:981–988.
39. Eng PH, Tan KE, Khoo DH, Tan CE, Lim HS, Lim SC, *et al.* Aldosterone to renin ratios in the evaluation of primary aldosteronism. *Ann Acad Med Singapore* 1997; 26:762–766.
40. Giacchetti G, Ronconi V, Lucarelli G, Boscaro M, Mantero F. Analysis of screening and confirmatory tests in the diagnosis of primary aldosteronism: need for a standardized protocol. *J Hypertens* 2006; 24:737–745.
41. Nishizaka MK, Pratt-Ubunama M, Zaman MA, Cofield S, Calhoun DA. Validity of plasma aldosterone-to-renin activity ratio in African American and white subjects with resistant hypertension. *Am J Hypertens* 2005; 18:805–812.
42. Hirohara D, Nomura K, Okamoto T, Ujihara M, Takano K. Performance of the basal aldosterone to renin ratio and of the renin stimulation test by furosemide and upright posture in screening for aldosterone-producing adenoma in low renin hypertensives. *J Clin Endocrinol Metab* 2001; 86:4292–4298.
43. Schwartz GL, Turner ST. Screening for primary aldosteronism in essential hypertension: diagnostic accuracy of the ratio of plasma aldosterone concentration to plasma renin activity. *Clin Chem* 2005; 51:386–394.
44. Montori VM, Young WF Jr. Use of plasma aldosterone concentration-to-plasma renin activity ratio as a screening test for primary aldosteronism. A systematic review of the literature. *Endocrinol Metab Clin North Am* 2002; 31:619–632; xi.
45. Tanabe A, Naruse M, Takagi S, Tsuchiya K, Imaki T, Takano K. Variability in the renin/aldosterone profile under random and standardized sampling conditions in primary aldosteronism. *J Clin Endocrinol Metab* 2003; 88:2489–2494.
46. Streeten DH, Tomycz N, Anderson GH. Reliability of screening methods for the diagnosis of primary aldosteronism. *Am J Med* 1979; 67:403–413.
47. Sokolow M, Lyon TP. The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. *Am Heart J* 1949; 37:161–186.
48. WHO Collaborating Centre for Drug Statistics Methodology. ATC/DDD Index 2012. http://www.whocc.no/atc_ddd_index/ [accessed 23 August 2013]
49. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; 44:837–845.
50. Seiler L, Rump LC, Schulte-Monting J, Slawik M, Borm K, Pavenstadt H, *et al.* Diagnosis of primary aldosteronism: value of different screening parameters and influence of antihypertensive medication. *Eur J Endocrinol* 2004; 150:329–337.
51. Trenkel S, Seifarth C, Schobel H, Hahn EG, Hensen J. Ratio of serum aldosterone to plasma renin concentration in essential hypertension and primary aldosteronism. *Exp Clin Endocrinol Diabetes* 2002; 110: 80–85.

52. Holland OB, Brown H, Kuhnert L, Fairchild C, Risk M, Gomez-Sanchez CE. Further evaluation of saline infusion for the diagnosis of primary aldosteronism. *Hypertension* 1984; 6:717–723.
53. Mulatero P, Milan A, Fallo F, Regolisti G, Pizzolo F, Fardella C, *et al.* Comparison of confirmatory tests for the diagnosis of primary aldosteronism. *J Clin Endocrinol Metab* 2006; 91:2618–2623.
54. Sealey JE, Gordon RD, Mantero F. Plasma renin and aldosterone measurements in low renin hypertensive states. *Trends Endocrinol Metab* 2005; 16:86–91.
55. Perschel FH, Schemer R, Seiler L, Reincke M, Deinum J, Maser-Gluth C, *et al.* Rapid screening test for primary hyperaldosteronism: ratio of plasma aldosterone to renin concentration determined by fully automated chemiluminescence immunoassays. *Clin Chem* 2004; 50:1650–1655.
56. Ferrari P, Shaw SG, Nicod J, Saner E, Nussberger J. Active renin versus plasma renin activity to define aldosterone-to-renin ratio for primary aldosteronism. *J Hypertens* 2004; 22:377–381.
57. Unger N, Lopez Schmidt I, Pitt C, Walz MK, Philipp T, Mann K, Petersenn S. Comparison of active renin concentration and plasma renin activity for the diagnosis of primary hyperaldosteronism in patients with an adrenal mass. *Eur J Endocrinol* 2004; 150:517–523.
58. Rossi GP, Barisa M, Belfiore A, Desideri G, Ferri C, Letizia C, *et al.* The aldosterone-renin ratio based on the plasma renin activity and the direct renin assay for diagnosing aldosterone-producing adenoma. *J Hypertens* 2010; 28:1892–1899.
59. Sealey JE, Moon C, Laragh JH, Alderman M. Plasma prorenin: cryoactivation and relationship to renin substrate in normal subjects. *Am J Med* 1976; 61:731–738.
60. Volpe M, Lembo G, Morganti A, Condorelli M, Trimarco B. Contribution of the renin-angiotensin system and of the sympathetic nervous system to blood pressure homeostasis during chronic restriction of sodium intake. *Am J Hypertens* 1988; 1 (4 Pt 1):353–358.
61. Oelkers W, Diederich S, Bahr V. Primary hyperaldosteronism without suppressed renin due to secondary hypertensive kidney damage. *J Clin Endocrinol Metab* 2000; 85:3266–3270.
62. Ignatowska-Switalska H, Chodakowska J, Januszewicz W, Feltynowski T, Adamczyk M, Lewandowski J. Evaluation of plasma aldosterone to plasma renin activity ratio in patients with primary aldosteronism. *J Hum Hypertens* 1997; 11:373–378.
63. Bravo EL, Tarazi RC, Dustan HP, Fouad FM, Textor SC, Gifford RW, Vidt DG. The changing clinical spectrum of primary aldosteronism. *Am J Med* 1983; 74:641–651.
64. Rossi GP, Seccia TM, Palumbo G, Belfiore A, Bernini G, Caridi G, *et al.* Within-patient reproducibility of the aldosterone: renin ratio in primary aldosteronism. *Hypertension* 2010; 55:83–89.

Reviewer's Summary Evaluation

Referee 2

This is a well conducted study aiming at evaluating the performance of the aldosterone-to-renin ratio (ARR) test in the screening of primary aldosteronism. Undoubtedly, this is a hot topic since the best way to screen hypertensive patients for such disease is still a matter of debate. Here, Jansen and colleagues demonstrate that ARR sensitivity is low regardless of the medication regimen, a conclusion that is supported by the prospective design of their work. Unfortunately, though, what cut-off value should be used remains unanswered and the work does not provide an easy alternative to current clinical practice.