Variability of aldosterone, renin and the aldosterone-to-renin ratio in hypertensive patients without primary aldosteronism

Original Article

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Objectives: The aldosterone-to-renin ratio (ARR) is commonly used in the screening of primary aldosteronism. However, limited information is available with regard to the intra-patient variability in this ratio. Our objective is to determine whether ARR measurements are reliably consistent over both the short- and long-term.

Methods: We assessed the short-term variability of the aldosterone-to-renin ratio in 116 unmedicated, essential hypertensive participants who had two blood samples taken in the morning of the same day for measurement of aldosterone and active plasma renin concentration. Long-term variability was studied in 22 unmedicated, essential hypertensive participants who had two blood samples taken approximately 1 year apart. All samples were taken under highly standardized conditions.

Results: Our data show that renin, aldosterone and the aldosterone-to-renin ratio show marked variations, both when measured on the same day and when assessed at a longer interval. The ARR becomes increasingly variable as its mean value increases. Its degree of variability is similar in both the short-term and the long-term.

Conclusions: Based on our findings, we conclude that the aldosterone-to-renin has acceptable short-term variability in the lower ranges, but increasingly dubious reliability as aldosterone-to-renin values rise. Thus, in a clinical context, great caution should be taken in interpreting point-measurements of moderate to high aldosterone-to-renin ratio values.

Keywords: aldosterone, ratio, renin, screening, variability

Abbreviations: APRC, active plasma renin concentration; ARR, aldosterone-to-renin ratio; PA, primary aldosteronism; PAC, plasma aldosterone concentration; RAAS, renin–angiotensin–aldosterone system

INTRODUCTION

Current guidelines and position papers recommend the usage of the aldosterone-to-renin ratio (ARR) in the screening of primary aldosteronism (PA) [1,2]. Although this test has proven to be a useful adjunct in the workup of patients suspected of having primary aldosteronism (PA), there is still some concern regarding the intra-patient reproducibility of the ARR. Given that in clinical practice elevated versus nonelevated ARR values are usually determined by a strict cut-off value, it is of great importance to know whether intra-patient variability is sufficiently high that the same patient may easily fall on either side of said cut-off at any given measurement moment. This is certainly relevant as a low value would preclude further testing while a high value would lead to, often costly, additional diagnostic investigations. Being aware of the variability of the ARR test result, could help the clinician to decide if in a specific case a repeat test would be necessary before dismissing or not a presumptive diagnosis of PA.

Inasmuch as studies on this topic are available, they have produced conflicting results. However, much of this research has pointed to alarming rates of variability in renin, aldosterone and ARR measurements, with spot-measurements coming under increasing scrutiny [3–8]. Part of the discrepancies between these studies may be due to insufficient standardization and the fact that many patients continued to be treated with (supposedly neutral) antihypertensive agents. Moreover, reproducibility has been tested for the medium-term (approximately 4 weeks) only while there is no information on shorter-term or long-term variability of the ARR. Therefore, we aimed to study these latter two aspects in more detail. We chose to only study those patients in whom PA had been ruled out, given that the majority of those screened can be expected not to have PA in clinical practice. We hypothesized that the ARR would be stable within the same day but very different when long-term reproducibility is assessed.

METHODS

Study population

A total of 116 patients who had been referred to our Hypertension Clinic for evaluation of their hypertension

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participated in the short-term variability study and 22 did so in the long-term variability study. The latter group consisted primarily of patients who despite having been prescribed antihypertensive drugs, did not respond well to the treatment or had chosen to discontinue their medication. Nevertheless, they remained under our supervision and as part of our clinical routine were re-investigated approximately 1 year later. All participants gave informed consent to partake in this study, which was approved by the Institutional Review Board.

Following the present study, secondary causes of hypertension were excluded in all participants after appropriate diagnostic procedures. These included the measurements of active plasma renin concentration (APRC), plasma aldosterone concentration (PAC), calculation of the ARR and additional tests such as saline loading if indicated. Antihypertensive medication, if any, had been discontinued for three weeks prior to the investigations and all patients had been put on a 55 mmol/day sodium intake (equivalent to 3.2 g NaCl/day) for 1 week. We confirmed adherence to the diet by measuring sodium output in 24-h urine collections.

Procedures

All investigations took place in a metabolic ward to which patients had been admitted prior to the investigations. On the day of testing, after an overnight fast and complete bed rest for 10 h, participants stayed in the laboratory from 0800 h until noon. After we had inserted an indwelling needle into the right antecubital vein, patients were allowed to rest for another 45 min. Thereafter, we took the first blood sample for determination of plasma renin and aldosterone concentrations (APRC-1 and PAC-1). Two hours later and without any interfering procedures in the meantime, we took the second sample (APRC-2 and PAC-2). Blood was collected in chilled tubes and spun immediately under cooled conditions and the plasma stored at −80°C until assay.

Throughout the entire period, blood pressure was measured at 5-min intervals with an automatic, oscillometric device (Dinamap, Tampa, Florida, USA), while patients remained in the supine position until the end of the study.

Active plasma renin concentration was measured by a direct immunoradiometric assay that detects active renin. Its characteristics are: sensitivity 2.5 mIU/l, intra-assay variability 2.6% and inter-assay variability 4.3%. Plasma aldosterone concentration was measured by solid-phase radioimmunoassay (antibody-coated tubes) with a sensitivity of 55 pmol/l, an intra-assay variability of 4.3%, and an inter-assay variability of 6.7%. ARR-1 and ARR-2 were calculated from the respective PAC and APRC results.

Statistical methods

We first applied paired *t*-tests to compare mean values of PAC-1 and PAC-2, APRC-1 and APRC-2, and ARR-1 and ARR-2. In addition, Pearson correlation analyses were performed on these respective datasets. Using the averages of the aldosterone, renin and ARR values as well as the differences between these values, we constructed Bland−Altman plots for the entire population as well as for males and females separately. This was done for both the quantitative differences in values and for the percentage differences from the mean. Finally, we constructed a moving average curve for the ARR using 10-patient samples. This was achieved by arranging patients in ascending order of their mean ARR and to calculate the moving average for this variable. Subsequently, we plotted the respective averages of the highest and the lowest values for each average ARR result against that average.

Finally, we generated a histogram of the difference between ARR-1 and ARR-2 (ARR-1 minus ARR-2) to visualize any potential systematic effects.

For all analyses, a *P*-value of <0.05 was considered statistically significant. We used IBM SPSS Statistics Version 26 for Windows (IBM, Armonk, New Year, USA) to do the overall analyses and Prism9 to construct the graphs.

RESULTS

Demographic characteristics of all participants are summarized in Table 1.

Short-term variability

APRC-1 and 2, PAC-1 and 2 and ARR-1 and 2 all showed strong and significant positive Pearson correlations ($r = 0.85$, $P < 0.001$; $r = 0.70$, $P < 0.001$ and $r = 0.88$, $P < 0.001$, respectively). The average values of the first and second measurements of APRC (28 vs. 27 mIU/l) did not differ significantly. However, significant differences were noted for PAC (477 vs. 436 pmol/l, $P = 0.032$) as well as for the ARR (23 vs. 21, $P = 0.018$). Differences between the first and the second measurements of APRC, PAC and ARR values all showed normal distributions with some skewing and a large splay from negative to positive values (Fig. 1, left panel). Interindividual differences in measurements showed a similar pattern in men and women (Fig. 2).

Bland−Altman analysis of the APRC data revealed a trend of moderate proportional bias with limits of agreement between 19 and −17 mIU/l. Also, in the Bland–

TABLE 1. Demographic characteristics of the two study populations

	Short-term population ($n = 116$)	Long-term population ($n = 22$)
Age (years)	44 + 12	43 + 10
Sex (M/F)	83/33	14/8
Height (males) (cm)	178 + 6	173 + 6
Height (females) (cm)	164 + 5	164 + 4
Weight (males) (kg)	83 + 8	77 + 6
Weight (females) (kg)	71 + 11	75 + 11
BMI (males) (kg/m^2)	26 + 3	26 + 2
BMI (females) (kg/m^2)	27 + 5	28 + 4
Systolic blood pressure (mmHg)	150 + 25	159 + 26
Diastolic blood pressure (mmHg)	96 + 15	99 + 11
Mean arterial pressure	114 + 17	120 + 14
Heart rate (BPM)	80 + 13	71 + 13
Plasma sodium (mmol/l)	140 + 2	142 + 2
Plasma potassium (mmol/l)	4.0 + 0.3	4.3 + 0.1
Plasma creatinine (μmol/l)	93 + 16	93 + 16
Urinary sodium (mmol/24 h)	47 + 23	36 + 18
Urinary potassium (mmol/24 h)	72 + 22	73 + 1
Urinary creatinine (mmol/24 h)	14 + 3	14 + 3

All data expressed as means and standard deviations.

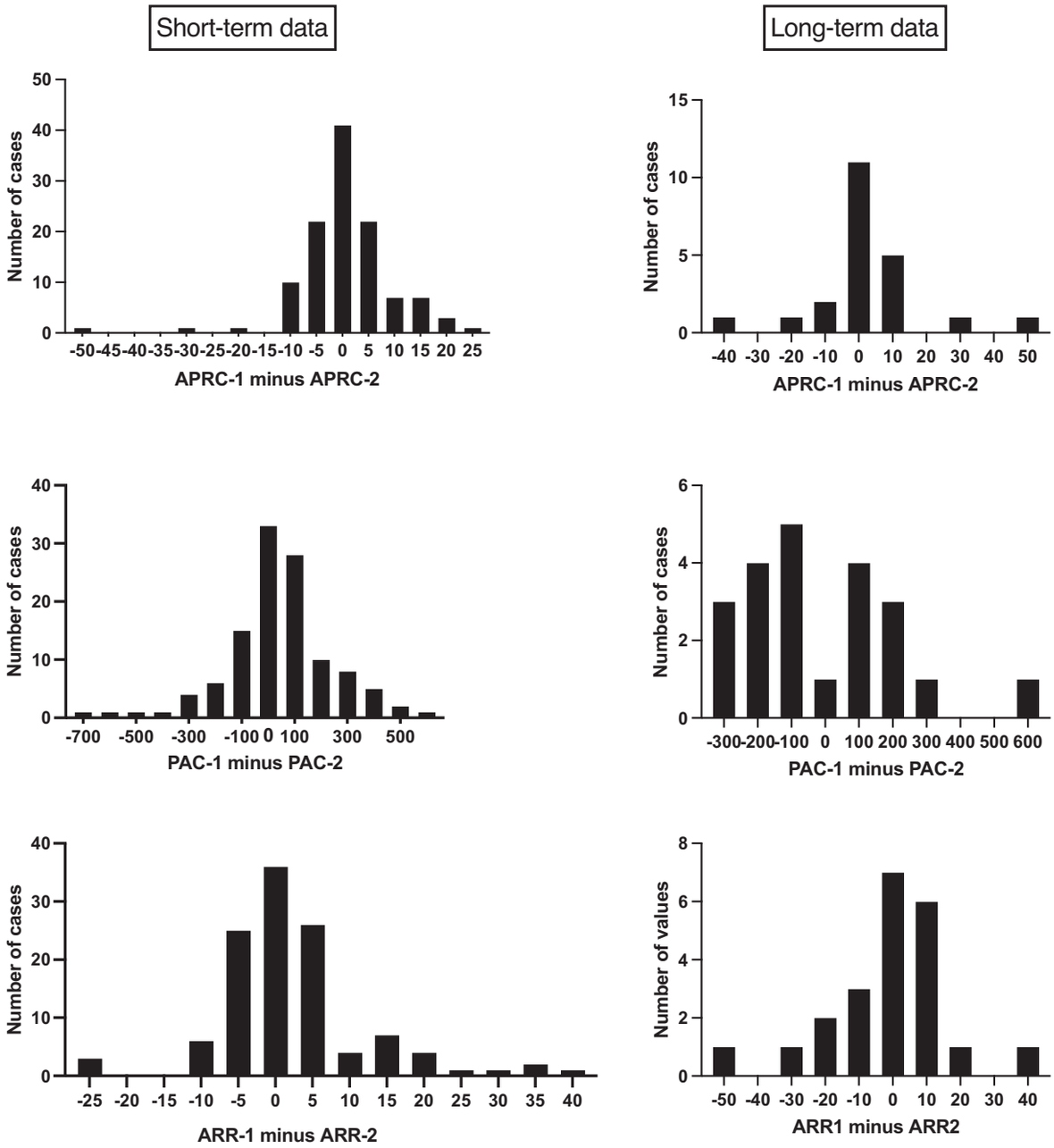

FIGURE 1 Frequency distributions of the differences in APRC, PAC and the ARR in the short-term population (left panel) and long-term population (right panel). ARPC, active plasma renin concentration; ARR, aldosterone-to-renin ratio; PAC, plasma aldosterone concentration.

Altman plot for the PAC, a small-to-moderate proportional bias was observed, the limits of agreement being 440 and −358 pmol/l. Finally, Bland–Altman analysis of the ARR showed a moderate-to-large proportional bias with increasing variance as the intra-patient mean ARR value increased. Although the average bias for the difference was relatively small (1.5), the limits of agreement were wide with an upper value of 22 and a lower value of −18. This proportional bias was seen in both men and women (data not shown). However, when we created a Bland-Altman plot expressing the differences in ARR as the percentage deviation from the mean, the proportional bias was no longer present, but the upper and lower limits of agreement were substantial: 91% and −80% (Fig. 3). Due to similar levels of intra-participant variability in both ARPC and PAC values, it is not clear that either component is the primary driver in ARR variability. Additionally, the large degree of intra-patient variability far exceeds the inter-assay variability of 4.3%.

In the moving average, a general trend of increasing absolute variability was noted as mean ARR values increased, with a small narrowing of variability when the

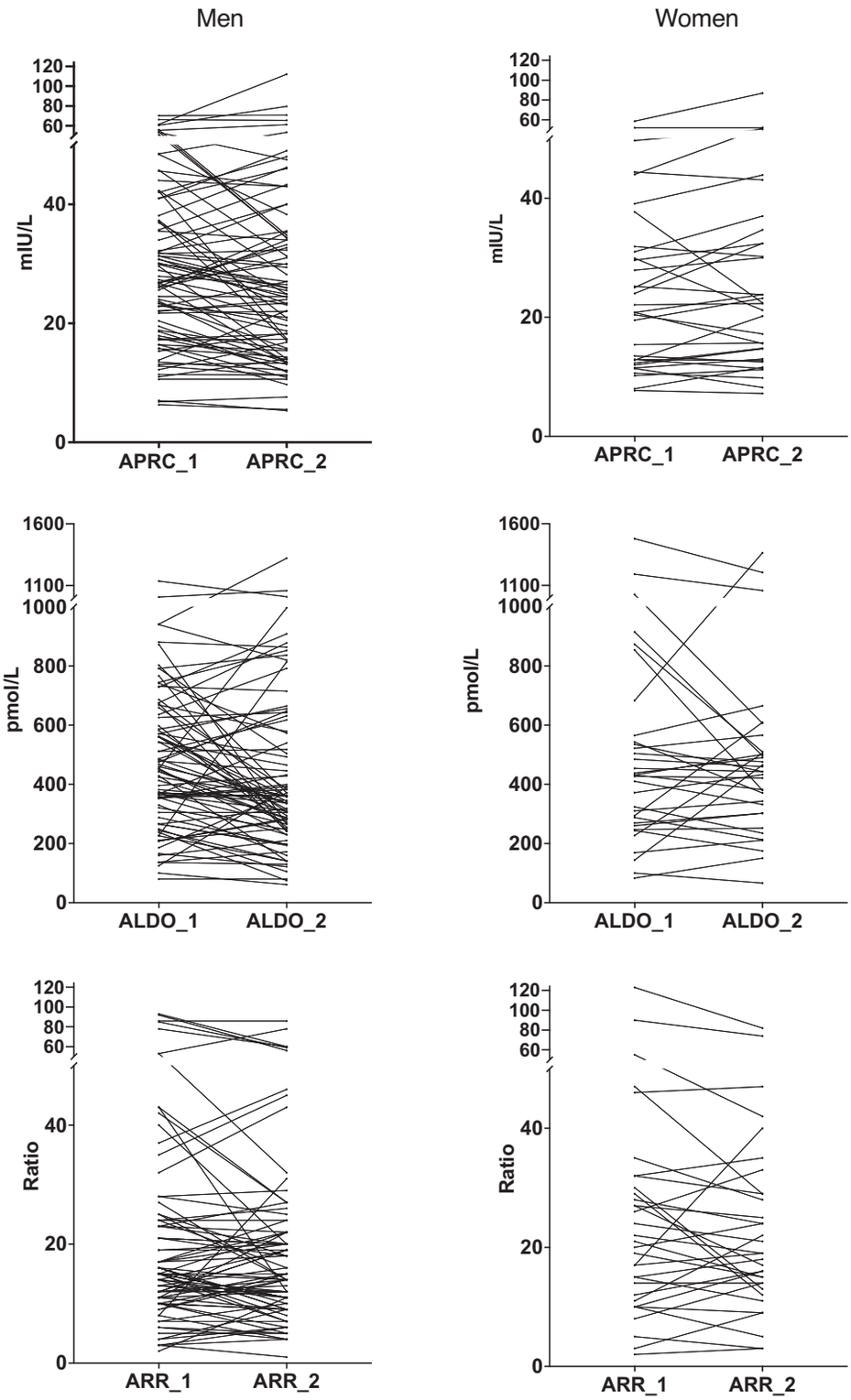

FIGURE 2 Individual differences in APRC, PAC and ARR, broken down by sex. ARPC, active plasma renin concentration; ARR, aldosterone-to-renin ratio; PAC, plasma aldosterone concentration.

ARR was still within the 25–30 range (Fig. 4). However, when expressed as a percentage of the mean value, the difference between the highest and the lowest values remained nearly constant at 29%.

Looking at the upper and lower moving average ARR values, the lower moving average passes the 30 threshold when the upper value is 42, and the upper value passes below the 30 threshold when the lower value is 23. This

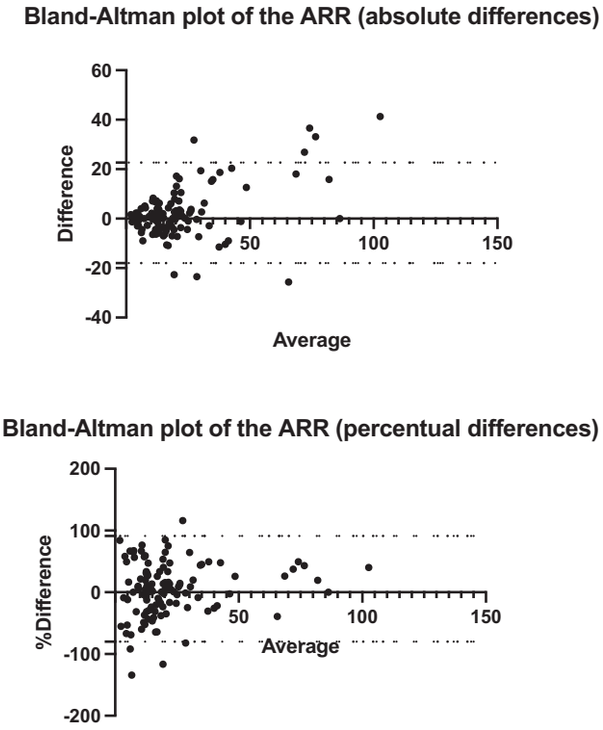

FIGURE 3 Bland–Altman plot for the ARR when differences are expressed as absolute values (upper panel) or as a percentage (lower panel). ARR, aldosterone-to-renin ratio.

indicates a range of values from 23 to 42 (or roughly from 20% below to 30% above the reference value) in which a single ARR measurement does not rule out a change in outcome upon a repeat measurement. Variability in ARR did not correlate with age or the level of blood pressure.

In our population of 116 EH participants, 71% had consistently normal ARR values, 4% had a normal mean ARR with one abnormal measurement, 7% had an abnormal mean with one normal measurement, and 18% had a consistently abnormal ARR (Fig. 5, left panel).

Long-term variability

The time period between the two assessments ranged from 10 to 15 months. Although blood pressure values had varied over the year, there was no significant difference in blood pressure at the first and the second examination. The average values of the first and second measurements of APRC (26 vs. 27 mIU/l) did not significantly differ from each other ($P = 0.94$). However, those of PAC (415 vs. 424 pmol/l) and the ARR (21 vs. 23) did ($P = 0.047$ and $P = 0.003$, respectively).

APRC-1 and -2 did not correlate well with each other ($r = 0.37$, $P = 0.094$), whereas PAC-1 and 2 and ARR-1 and ARR-2 both showed significant, yet weak Pearson correlations ($r = 0.43$, $P = 0.047$ and $r = 0.61$, $P = 0.003$, respectively). By and large, differences between the first and the second measurements of APRC, PAC and ARR values showed normal distributions, again with a large splay from negative to positive values (Fig. 1, right panel). Again, patterns were similar for men and women and there was no correlation between individual differences and either age or blood pressure.

Bland–Altman analysis of the ARR showed a moderate-to-large proportional bias with increasing variance as the intra-patient mean ARR value increased, much like our findings regarding short-term variability. The limits of agreement were very wide with an upper value of 33 and a lower value of −36.

Twelve of the 22 participants had a normal ARR twice, six had a normal mean ARR with one abnormal measurement, one had an abnormal mean with one normal measurement, and three had two abnormal ARR measurements (Fig. 5, right panel).

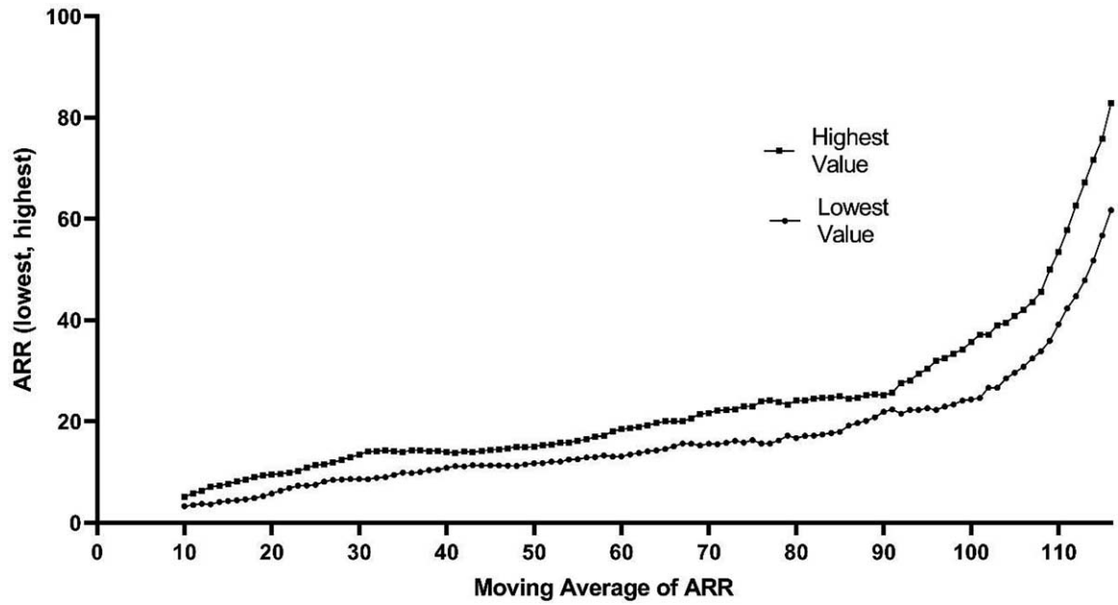

FIGURE 4 Moving average for ARR in short-term population with participants arranged in ascending order of their mean ARR. ARR, aldosterone-to-renin ratio.

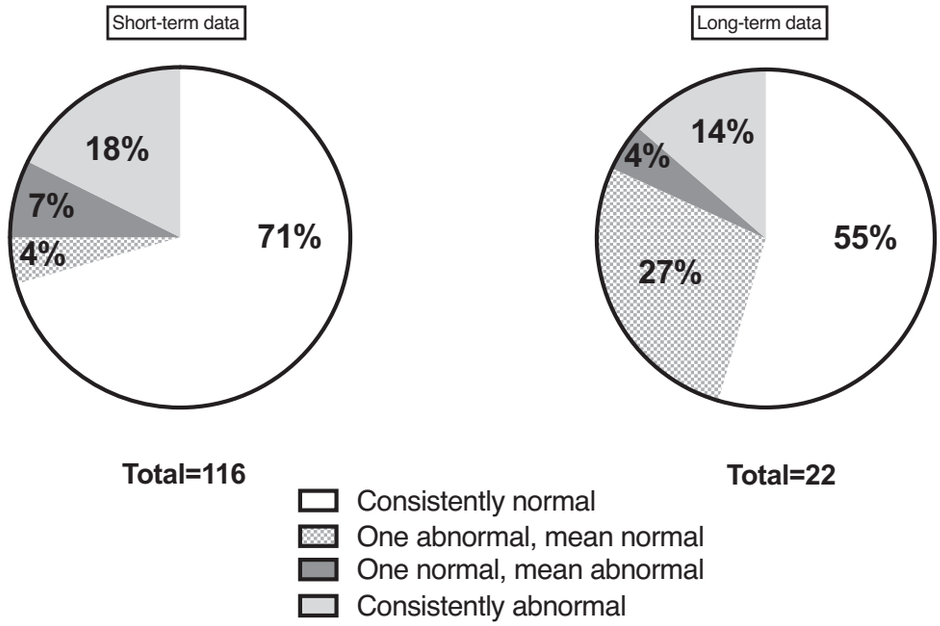

FIGURE 5 Pie charts depicting rates of normal/abnormal values for the ARR in the short-term (left) and long-term (right) patient groups. ARR, aldosterone-to-renin ratio.

DISCUSSION

To the best of our knowledge, this is the first study to investigate reproducibility of the ARR under highly standardized conditions using same-day basal measurements in an otherwise healthy essential hypertensive population. The primary finding of our study was the rather large variability in the ARR as evidenced by the Bland–Altman plots. Even though, on average, the difference between the two measurements, taken on the same day, hardly deviated from zero, both the Bland-Altman and the moving average analysis show that there may be substantial variation between two successive measurements.

As visually illustrated in Fig. 1, no clear systematic pattern emerged, which makes the possibility that our findings are the result of diurnal variations less likely. The lack of significant differences in short-term APRC and PAC values as well as the bidirectional proportional biases in Bland-Altman analyses for APRC and PAC further argues against diurnal effects being responsible for the observed phenomena.

Notably, absolute variability seems to increase when the ARR is higher. Not unexpectedly, the variation was even greater when the ARR results one year apart were considered, although this was not much greater than the short-term variability.

Although the majority of our patients showed consistent test results, at least 11% of them appeared to have a discrepant outcome when evaluated a few hours later. This number increases to 31% with a much longer time interval.

Several articles have been published covering the reproducibility of the ARR but there is still no consensus regarding this topic [3–5,9]. While shortcomings with regard to standardization could account for some of the discrepancies, in most studies standardization was not so inferior to fully explain the divergent results. Differences in statistical analysis could also play a role. Although some authors concluded that the ARR showed good reproducibility only on the basis of a statistically significant Pearson's correlation analysis of the data, others used Bland-Altman plots or a Passing–Bablok regression, which are better approaches in this regard [5,10–13].

A recent study by Yozamp *et al.* [6], in which the variability of PAC and ARR measurements was investigated in a confirmed PA population with measurements separated by several days, dovetails nicely with our own study. In that study, the same pattern of proportional bias was noted in the PAC and ARR values in this PA population as in our own essential hypertension population. This suggests that our findings are applicable to all those who are suspected of PA and, together with the paper of Yozamp *et al.*, strongly indicates a pressing need for spot ARR measurements not to be taken at face value.

It is our opinion that the variability noted in our study is unlikely to be driven by an underlying physiological/pathological process that can be readily standardized away. Rather, we feel that it is more likely that this variability is part of normal homeostatic fluctuations in the renin–angiotensin–aldosterone system (RAAS). As investigated by Vieweg, Siragy and their colleagues it would appear that renin and aldosterone is secreted in a burst-like manner, which would naturally result in fairly large fluctuations in values when taken in spot measurements [14,15]. It is, in addition, logical from our point of view that individuals with physiologically higher mean values of various RAAS components would display greater absolute, but similar relative, fluctuations in these values when compared with individuals with lower mean RAAS values.

The implication of our results is that when the laboratory returns an entirely unelevated or low ARR result, the clinician can safely refrain from additional investigations. Likewise, when the ARR is markedly elevated, one can proceed immediately to confirmation tests. However, in clinical

practice one often is confronted with patients who exhibit an ARR that is only marginally elevated or slightly below the upper limit of unelevated values. In those cases, a repeat test seems appropriate before deciding about the further work-up.

The main weakness of our study was that our patients had blood samples taken from the supine position, while current guidelines advise that patients be in the seated position for blood samples when screening for PA. However, we deliberately wanted to avoid posture-related variations in renin and aldosterone levels. Previous research has indicated that the ARR is less variable when measured in the supine position [16], thus, the difference between successive measurements of the ARR may have even been greater when measured in the seated position. Additionally, research regarding posture and systematically higher or lower ARR values have been inconclusive [16], thus, we felt comfortable maintaining the widely used value of 30 as our cut-off. Our population was additionally placed on a fixed low sodium diet, as opposed to the recommended ad libitum diet according to current guidelines [1]. Our reasons for this however, were twofold. Firstly, sodium intake is a potential confounder in renin and aldosterone production, thus a fixed sodium intake allowed us to scrutinize our results with greater certainty. Our choice for a low sodium diet was largely based on the fact that many (presumed) essential hypertensives are placed on sodium restricted diets as part of their treatment, thus low sodium diets are not at all an uncommon occurrence in hypertensive populations being screened for PA.

Our data add to the mounting body of evidence that in search of a secondary cause of hypertension, the intrapatient reproducibility of the ARR is not sufficiently high to rely on single spot measurements as an effective screening technique. Although this may have implications for guidelines on the detection of patients with primary hyperaldosteronism, there is also a need for prospective studies to explore whether dual measurements have better predictive power for a final diagnosis.

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Conflicts of interest

There are no conflicts of interest.

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