Within-Patient Reproducibility of the Aldosterone:Renin Ratio in Primary Aldosteronism

Gian Paolo Rossi, Teresa Maria Seccia, Gaetana Palumbo, Anna Belfiore, Giampaolo Bernini, Graziella Caridi, Giovambattista Desideri, Bruno Fabris, Claudio Ferri, Gilberta Giacchetti, Claudio Letizia, Mauro Maccario, Francesca Mallamaci, Massimo Mannelli, Anna Patalano, Damiano Rizzoni, Ermanno Rossi, Achille Cesare Pessina, Franco Mantero; for the Primary Aldosteronism in the Prevalence in Hypertension (PAPY) Study Investigators

Abstract—The plasma aldosterone concentration:renin ratio (ARR) is widely used for the screening of primary aldosteronism, but its reproducibility is unknown. We, therefore, investigated the within-patient reproducibility of the ARR in a prospective multicenter study of consecutive hypertensive patients referred to specialized centers for hypertension in Italy. After the patients were carefully prepared from the pharmacological standpoint, the ARR was determined at baseline in 1136 patients and repeated after, on average, 4 weeks in the patients who had initially an ARR ≥40 and in 1 of every 4 of those with an ARR <40. The reproducibility of the ARR was assessed with Passing and Bablok and Deming regression, coefficient of reproducibility, and Bland-Altman and Mountain plots. Within-patient ARR comparison was available in 268 patients, of whom 49 had an aldosterone-producing adenoma, on the basis of the “4-corner criteria.” The ARR showed a highly significant within-patient correlation (r=0.69; P<0.0001) and reproducibility. Bland-Altman plot showed no proportional, magnitude-related, or absolute systematic error between the ARR; moreover, only 7% of the values, for example, slightly more than what could be expected by chance, fell out of the 95% CI for the between-test difference. The accuracy of each ARR for pinpointing aldosterone-producing adenoma patients was ≈80%. Thus, although it was performed under different conditions in a multicenter study, the ARR showed a good within-patient reproducibility. Hence, contrary to previously claimed poor reproducibility of the ARR, these data support its use for the screening of primary aldosteronism. (Hypertension. 2010;55:00-00.)

Key Words: secondary hypertension ■ aldosteronism ■ renin assay ■ aldosterone ■ diagnosis

Recent compelling evidence indicates that primary aldosteronism (PA) is associated with prominent cardiovascular and renal damage, adverse metabolic consequences, and an excess rate of cardiovascular events. In the Primary Aldosteronism Prevalence in Hypertension (PAPY) Study, PA was diagnosed in 11.2% of 1125 newly diagnosed hypertensive patients referred to hypertension centers; moreover, in 4.8% it was attributed to an aldosterone-producing adenoma (APA). Therefore, it is likely that PA represents the most common form of endocrine arterial hypertension that is surgically curable in a substantial proportion of the cases. Currently the plasma aldosterone concentration (PAC):renin ratio (ARR) is the most popular screening test for identifying PA, and although it has obvious limitations, its widespread adoption can enhance the identification of PA. Nonetheless, the within-patient reproducibility of this test remains unknown.

We report here the results of the study of within-patient comparison of the ARR that was determined at the baseline screening and repeated on average after a month.

Subjects and Methods

All of the patients originally recruited in the PAPY Study and 11 additional patients who were investigated with an identical protocol at the internal medicine department of the General Hospital of Legnano were included in this study. The PAPY Study protocol followed the Statement for Reporting Studies of Diagnostic Accuracy recommendations and the requirements of the Declaration of Helsinki. Briefly, consecutive newly diagnosed hypertensive patients, referred to specialized hypertension centers nationwide in Italy, were recruited after informed consent was obtained. A previous diagnosis of a secondary form of hypertension and the patient’s refusal to participate in the study were the only exclusion criteria. The general flowchart of the study protocol for the purpose of this study is summarized in Figure S1 (please see the online Data Supplement at http://hyper.ahajournals.org). Briefly, the
patients were prepared from the pharmacological standpoint as follows: drugs interfering with the renin-angiotensin-aldosterone system were withdrawn for ≥2 weeks (for angiotensin-converting enzyme inhibitors, angiotensin II type 1 receptor blockers, β-blockers, and diuretics) or 6 weeks (mineralocorticoid receptor antagonists) before performing the screening test and until the saline infusion test, if required by protocol. A long-acting calcium channel blocker (CCB) and/or doxazosin were prescribed whenever necessary to minimize the risks of uncontrolled hypertension and continued up to the saline infusion test. At baseline screening, all of the patients underwent measurement of the 24-hour Na+ urine excretion and, the next morning, while they remained in the sitting position, of plasma renin activity (PRA), PAC, and plasma cortisol concentration (PCC) before and after a captopril challenge with 50 mg of oral captopril.10 In those with an ARR ≥40 and in 1 of every 4 of those with an ARR <40, the PRA, PAC, and PCC measurements were repeated before the saline infusion test, after, on average, 4 weeks, during which the treatment remained unchanged. For calculation of the ARR, the lowest value of the denominator, PRA, was set to 0.2 ng·mL⁻¹·h⁻¹ to avoid overinflating the ARR.

Additional Tests
The further diagnostic workup was based on the results of ARR baseline and/or after the captopril and/or the diagnostic discriminant function test.11 The patients positive at such tests were submitted to an imaging test for identification of adenocortical nodules and also to adrenal vein sampling (AVS)12 or to dexamethasone-suppressed adrenocortical scintigraphy to identify a lateralized aldosterone excess production. AVS was used to diagnose lateralization of hyperaldosteronism only if bilaterally selective; ACTH stimulation was not systematically used during AVS because, although it improves assessment of selectivity of catheterization, it does not enhance the diagnostic accuracy.13,14

Biochemical Measurements
Serum creatinine, serum and urine Na⁺ and K⁺ levels, PRA, PAC, and PCC, and estimated glomerular filtration rate were measured as described.4 Normal ranges, intra-assay and interassay coefficients of variation, and antibody cross-reactivity for the hormonal measurements have already been reported.4

Diagnostic Criteria
Identification of APA required all of the following criteria: (1) evidence of PA at the screening test as defined above; (2) lateralization of aldosterone secretion at AVS or at NPSA adrenocortical scintigraphy; (3) evidence of adenoma at pathology; and (4) demonstration of normokalemia and cure or improvement of hypertension at follow-up after adrenalectomy by reported criteria.4 Patients with biochemical evidence for PA but without conclusive evidence for a lateralized aldosterone excess were presumed to have idiopathic hyperaldosteronism (IHA).

Statistical Analysis
Skewed variables as PRA, PAC, and PCC were analyzed after achievement of a normal distribution by log transformation. One-way ANOVA followed by the Bonferroni posthoc test was used to compare quantitative variables across groups. Distribution of categorical variables was investigated by χ² analysis. To assess the reproducibility of the ARR, several techniques were used, including correlation and regression analyses and Bland-Altman and Mountain plots.15,16 For regression, the Passing and Bablok procedure was preferred to the ordinary linear regression method, because it makes no assumptions regarding the distribution of the samples and the measurement errors.17 In this analysis, a test for linearity is used to evaluate how well a linear model fits the data; moreover, the result does not depend on the assignment of the variables to the x and y axes. To confirm results, Deming regression was also used, because it takes measurement errors for both methods into account.18 Other than furnishing summary statistics for each ARR, both of these regression techniques use a test for linearity to evaluate how well a linear model fits the data and furnish a scatter plot with regression line and estimate intercept A and slope B along with their 95% CI. The CI is used to challenge the null hypothesis that A=0 and B=1. For the intercept A, the null hypothesis is accepted if the CI for A contains the value 0; rejection of this hypothesis leads to the conclusion that the 2 methods differ at least by a constant amount. For the slope B, the null hypothesis is accepted if the CI for B contains 1; rejection of this hypothesis implies that there is a proportional difference between the methods. To identify proportional error, magnitude-dependent bias, and systematic error, the Bland-Altman and Mountain plots were used.

Finally, to assess and compare the accuracy between ARR for identifying APA we also used receiver operator characteristics (ROC) curves10 with the MedCalc software (version 8.1.1.0, MedCalc Software). For all of the other analyses, the SPSS for Windows (version 17.0, SPSS Inc) was used; significance was set at P<0.05.

Results

Baseline Characteristics
There were 268 patients with complete ARR data on the 2 occasions and with a conclusive diagnosis available for this study. The baseline features of the patients, divided by diagnosis (Table 1), did not differ for demographic, clinical, and hormonal data from the total PAPY Study cohort (all P values not significant).

No differences in age, body mass index, diastolic blood pressure (BP), serum creatinine, estimated glomerular filtration rate, and urine Na⁺ and K⁺ excretions were found between PA and primary hypertension (PH) patients. The APA patients had higher systolic BP and lower serum K⁺ levels than the PH patients. The 74 patients presumed to have IHA did not differ from the APA patients for baseline diastolic BP but had higher serum K⁺ than the APA patients.

At the time of the determinations of either ARR, 40.1% of the patients were untreated, 34.7% were on a CCB, and 25.2% were on a CCB and doxazosin. Significantly more APA than IHA or PH patients required the combination treatment to achieve BP control.

Hormonal Data at the First and Second ARR
On the occasion of both the first and the second hormonal measurements, both the APA and the IHA patients had elevated PAC, suppressed PRA, raised ARR, and lower serum K⁺ than the PH patients. Of note, from the first to the second assay, there was a decrease of PAC and the ARR, which reached significance in the IHA and the PH groups but not the APA group. This decrease was not accompanied by significant changes of PRA in any of the PA groups, although it was associated with a significant, albeit clinically negligible (0.06 ng·L⁻¹·h⁻¹), increase in the PH group. In all of the groups, the decrease of PAC and ARR was paralleled by a significant fall of PCC.

Reproducibility of the ARR
When examining the relationship between first (ARR-1) and second measured (ARR-2) ARRs, we found a high within-patient correlation (R=0.69 [95% CI: 0.63 to 0.74]; P<0.0001) and reproducibility (coefficient of determination: 0.47). As both measurement values showed a skewed distribution, we applied the Passing and Bablok regression method. Figure 1 shows the scatter plot and regression line with 95% CI.
The ROC curves for ARR-1 and ARR-2 (Figure 2) were remarkably similar and their areas under the ROC curve (AUCs), which were both \((P<0.001)\) >0.50, did not differ significantly, indicating that the accuracy of the 2 ARRs was similar. Consistent with this finding, no single APA patient who was pinpointed with the first ARR was missed with the second and vice versa.

**Discussion**

We may be facing an epidemic of PA, and several studies suggest that we should screen all hypertensive patients for PA to prevent cardiovascular and metabolic complications.\(^6,8,19\) To this end, the availability of a screening test capable of providing an accurate identification of PA patients in the huge population of hypertensive patients would be essential. This test should be endowed with a high sensitivity, in order not to miss cases of APA, coupled with a decent specificity, to minimize false-positive results. Moreover, it should ideally provide reproducible results to give reliable information for confirming or excluding the diagnosis. Currently, the ARR is the most popular screening test for PA, although it represents in essence a “crude” bivariate analysis, which can furnish practically identical results starting from very different absolute PAC and PRA values.\(^11,20–22\) Although some empirical solutions to this potential pitfall have been proposed,\(^6\) the crucial issue of the reproducibility of the ARR has never been systematically addressed.

In the PAPY Study, the ARR had to be measured twice in a substantial proportion of the patients: those who tested positive by predefined criteria at the first ARR, and 1 of every 4 consecutive patients who tested negative. This design allowed a within-patient comparison of the 2 ARRs with exclusion of any bias associated with interindividual variability. Moreover, as antihypertensive drugs profoundly affect the ARR,\(^23\) all of the patients had to be carefully prepared pharmacologically to eliminate untoward influences of the antihypertensive treatment on the first measurement of the hormones and were thereafter maintained on the same treatment up to the second ARR.

**Table 1. Anthropometric and Biochemical Characteristics of the Patients With PH and PA Caused by an APA and IHA**

<table>
<thead>
<tr>
<th>Variable</th>
<th>APA (n=49)</th>
<th>P (APA vs IHA)</th>
<th>IHA (n=74)</th>
<th>P (IHA vs PH)</th>
<th>PH (n=205)</th>
<th>P (PH vs APA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>49.4±1.8</td>
<td>NS</td>
<td>48.0±1.3</td>
<td>NS</td>
<td>45.5±0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, male/female, n (%)</td>
<td>29/20 (8.8/6.1)</td>
<td>NS</td>
<td>46/28 (14.0/8.5)</td>
<td>NS</td>
<td>110/95 (33.5/29.0)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.4±0.5</td>
<td>NS</td>
<td>26.9±0.5</td>
<td>NS</td>
<td>27.3±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>155±3</td>
<td>NS</td>
<td>153±1</td>
<td>0.02</td>
<td>146±1</td>
<td>0.004</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>96±1</td>
<td>NS</td>
<td>100±1</td>
<td>0.03</td>
<td>96±1</td>
<td>NS</td>
</tr>
<tr>
<td>Mean BP, mm Hg</td>
<td>116±2</td>
<td>NS</td>
<td>117±1</td>
<td>0.02</td>
<td>112±1</td>
<td>NS</td>
</tr>
<tr>
<td>s-Creatinine, mmol/L</td>
<td>82±2</td>
<td>NS</td>
<td>80±2</td>
<td>NS</td>
<td>79±1</td>
<td>NS</td>
</tr>
<tr>
<td>GFR, mL/min</td>
<td>86±2</td>
<td>NS</td>
<td>90±2</td>
<td>NS</td>
<td>91±1</td>
<td>NS</td>
</tr>
<tr>
<td>s-Na⁺, mmol/L</td>
<td>142±1</td>
<td>NA</td>
<td>141±1</td>
<td>NS</td>
<td>141±1</td>
<td>NA</td>
</tr>
<tr>
<td>s-K⁺, mmol/L</td>
<td>3.48±0.07</td>
<td>0.0001</td>
<td>3.90±0.05</td>
<td>0.004</td>
<td>4.08±0.02</td>
<td>0.0001</td>
</tr>
<tr>
<td>Na⁺ uV, mmol/24 h</td>
<td>136±11</td>
<td>NS</td>
<td>137±7</td>
<td>NS</td>
<td>147±5</td>
<td>NS</td>
</tr>
<tr>
<td>K⁺ uV, mmol/24 h</td>
<td>62±5</td>
<td>NS</td>
<td>59±3</td>
<td>NS</td>
<td>59±2</td>
<td>NS</td>
</tr>
<tr>
<td>APA diameter, mm</td>
<td>9±1</td>
<td>NS</td>
<td>NA</td>
<td>...</td>
<td>NA</td>
<td>...</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No drugs, %</td>
<td>21</td>
<td>0.02</td>
<td>35</td>
<td>0.02</td>
<td>47</td>
<td>0.02</td>
</tr>
<tr>
<td>CCB</td>
<td>35</td>
<td>NS</td>
<td>36</td>
<td>NS</td>
<td>34</td>
<td>NS</td>
</tr>
<tr>
<td>CCB+α-blockers</td>
<td>43</td>
<td>0.02</td>
<td>29</td>
<td>NS</td>
<td>19</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data are mean±SD unless otherwise specified. BMI, body mass index; Na⁺ uV, sodium urinary excretion; K⁺ uV, potassium urinary excretion; NS, not significant; NA, not applicable. For treatment, the percentage values with respect to the total patient sample are reported in parentheses.
Reproducibility of the ARR

The tight direct relationship between the 2 ARRs despite the fact that they were performed 4 weeks apart suggests that the ARR furnishes consistent results (Figure 1A) when performed under standardized conditions in the same patient.

The between-test, within-patient pairwise comparison showed a fall of PAC that translated into a decrease of the ARR in the IHA and PH but not in the APA group (Table 2). Because this decrease occurred despite no change in drug treatment and unchanged PRA, but was closely mirrored by a decrease of PCC in all of the groups, it was most likely related to the attenuation of the stress reaction to blood testing occurring from the first to the second blood drawing.

Importantly, the lack of PAC and the ARR decrease in the APA patients not only accords well with the view that aldosterone secretion is autonomous in this condition, but also suggest that a raised ARR on 2 separate occasions is a hallmark of APA. Hence, it would indicate the need to proceed to performing AVS without undertaking an exclusion test.

Figure 1. Reproducibility of the two ARR assays. A, The Passing and Bablok regression equation has values of the intercept A and slope B with 95% CI comprised of 0 and 1 values, respectively, indicating that ARR-1 and ARR-2 do not differ by either a constant amount or a proportional difference. B, The Bland-Altman plot of ARR-1 and ARR-2 shows that (1) the mean value of the difference between ARR-1 and ARR-2 is 0; (2) data are symmetrically distributed around the mean, with a roughly equal number of points above and below the SD; (3) data are mostly dispersed within the 95% limits of agreement for the differences; (4) only 7% of the values fall out the 95% CI (between methods agreement); (5) data are uniformly distributed throughout the plot, with no increase in the degree of differences with increasing mean values and, therefore, with no “funnel” effect. Log(e) natural logarithm to the base “e.” C, The Bland-Altman plot using the average of the ARR-1 and ARR-2 values against the ratio ARR-1/ARR-2, which does not require previous log transformation, confirms a high degree of agreement between the 2 values of ARR-1 and ARR-2 and rules out any systematic bias. D, The Mountain graph, which plots the percentile for each difference between the 2 ARR values against the difference ARR-1–ARR-2 shows that the difference distribution is centered over 0 and has no long tails, thus showing that the 2 ARRs are unbiased with respect to each other, and there are no large differences between the tests. •, patients with PH; closed symbols, patients with PA; squares, APA; triangles, IHA.
A scrutiny of these individual data showed that the PRA was above the normal range in these outliers at the screening test but normalized when measured in the second one. Thus, in a tiny percentage of the patients, notwithstanding a strict adherence to the study protocol, a “carryover” effect of the previous antihypertensive treatment might occur. Therefore, >2 weeks of withdrawal of drugs stimulating renin secretion could be necessary in some patients to achieve unbiased ARR determination. Hence, these results overall provide evidence that the ARR furnishes reproducible results when performed under standardized conditions in adequately prepared patients.

Figure 2. ROC curves for ARR-1 and ARR-2. The AUC for both curves showed a value close to 0.80, indicating that the overall diagnostic accuracy of each ARR for identifying APA is ~80%. The AUC for both ARRs differed significantly (P<0.0001) from the AUC under the diagonal (identity) line. Moreover, at a formal comparison, the 2 AUCs did not differ, indicating that the 2 ARRs had a similar diagnostic accuracy.

Diagnostic Accuracy of the ARR and Clinical Implications

When comparing biochemical tests, the crucial point that separates clinical meaning from “pure” biochemistry is to establish whether the degree of agreement between the ARR is acceptable from the clinical standpoint. The AUC provides an estimate of test accuracy. Hence, to assess the diagnostic performance of the first and the second ARRs, we measured the AUC of both ROC curves, using the diagnosis of APA as a reference, because a conclusive diagnosis is feasible only for this subgroup of PA patients. The findings that both of the AUCs were close to 0.80 indicate that the 2 ARRs have an 80% accuracy. Moreover, the lack of a significant difference between the AUCs of the ARRs indicates a similar accuracy for identifying APA. In fact, all of the APA patients pinpointed with the first ARR were identified with the second and vice versa.

Treatment Effects and Accuracy of the ARR

It has been described that the long-acting CCB amlodipine would decrease PAC and, therefore, the ARR in PA patients, which is difficult to reconcile with the view that aldosterone secretion is mainly regulated via T-type Ca channels, as discussed later. At the first ARR of our patients on a CCB, 95% received nifedipine gastrointestinal therapeutic system or amlodipine. We found no treatment effect on hormone values in the majority of the patients with the aforementioned caveats. The changes occurring from the first to the second ARR were likely attributable, in our view, not to CCB treatment but rather to the waning of either a drug carryover effect and/or of a stress reaction. Because they occurred also in the untreated patients (data not shown), the possibility of a regression toward the mean cannot, however, be dismissed. Thus, these results further support the conclusion that a long-acting CCB and/or doxazosine do not markedly influence the PAC and PRA values, and, thus, the ARR, at least during the 4-week period that elapsed between the first and the second ARR. From the practical standpoint,

Table 2. Plasma Hormone Concentration and Serum K⁺ Levels Measured on the Occasion of the Baseline Screening (First Assay) and After 4 Weeks (Second Assay) Before the Saline Infusion in the Patients Divided According to Diagnosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>APA (n=49)</th>
<th>APA (First vs Second Assay)</th>
<th>IHA (n=74)</th>
<th>IHA (First vs Second Assay)</th>
<th>PH (n=205)</th>
<th>PH (First vs Second Assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA, ng·L⁻¹·h⁻¹</td>
<td>0.62±0.15</td>
<td>0.57±0.13 NS</td>
<td>0.52±0.07</td>
<td>0.67±0.09 NS</td>
<td>1.27±0.15 **</td>
<td>1.33±0.14††</td>
</tr>
<tr>
<td>PAC, ng·dL⁻¹</td>
<td>31.6±2.9</td>
<td>31.3±3.2* NS</td>
<td>26.3±1.6</td>
<td>23.4±1.6 0.01</td>
<td>18.2±0.8**</td>
<td>15.2±0.8**</td>
</tr>
<tr>
<td>ARR</td>
<td>95.0±9.0</td>
<td>92.5±13.7 NS</td>
<td>79.6±6.6</td>
<td>64.5±6.7 0.0001</td>
<td>36.9±2.6**</td>
<td>31.0±2.2**</td>
</tr>
<tr>
<td>s-K⁺, mmol·L⁻¹</td>
<td>3.51±0.07†</td>
<td>3.57±0.06 NS</td>
<td>3.91±0.05</td>
<td>3.87±0.05 NS</td>
<td>4.00±0.02§</td>
<td>4.02±0.02</td>
</tr>
<tr>
<td>PCC, nmol·L⁻¹</td>
<td>133±6</td>
<td>93±11 0.004</td>
<td>139±6</td>
<td>86±12 0.0001</td>
<td>142±4†</td>
<td>100±5‡</td>
</tr>
</tbody>
</table>

ARR indicates aldosterone (ng·dL⁻¹)/PRA (ng·mL⁻¹·h⁻¹) ratio; NS, not significant.
*P<0.05.
†P<0.01 vs IHA.
‡P<0.05 vs APA.
§P<0.01 vs APA.
¶P<0.001 vs APA.
‖P<0.05 vs IHA.
¶¶P<0.01 vs IHA.
**P<0.001 vs IHA.
this implies that these agents should be used before (and during re-)assessment of the ARR to avoid the risks of uncontrolled hypertension.

**Limitations of the Study**

The patients of the PAPY Study were referred to specialized hypertension centers where PAC and PRA could be measured after strict quality control criteria. Furthermore, the ARR was performed only after the withdrawal of interfering drugs. Thus, it remains to be determined whether our findings could be directly extrapolated to the general population of hypertensives. Data obtained in the Bussolengo Study, which was performed in the setting of general practice, would, however, support the feasibility and usefulness of measuring the ARR at this setting, provided that the patients are adequately prepared from the pharmacological standpoint.

**Conclusions**

In this study, the ARR was found to provide reproducible results and a reasonably accurate identification of the surgically curable forms of PA, like APA, when performed under carefully controlled conditions in patients adequately prepared from the pharmacological standpoint. This test is no more expensive than a lipid profile, but its result can have a much greater impact (than lipid profile) on the lifetime cardiovascular risk of those hypertensives who have a surgically curable subtype of PA. Hence, it should be offered, in our view, to all of the hypertensives who are candidates for adrenalectomy.

**Perspectives**

CCBs, including benidipine, azelnidipine, and efondipine, which block both L- and T-type Ca channels, were reported recently to inhibit angiotensin II– and KCl-induced expression of steroidogenic enzymes, including CYP11B2 (aldosterone synthase), in vitro. They also suppressed steroid biosynthesis in H295R cells, whereas nifedipine, an L-type CCB, was ineffective. Replacement of amlodipine, another T-type CCB, with efondipine was associated with a significant decrease of both PAC and left ventricular mass index in essential hypertensive patients, indicating that the T-type Ca channels mediate aldosterone production and ensuing left ventricular hypertrophy. It was also reported recently that the dihydropyridine CCBs could have a mineralocorticoid receptor antagonist effect, suggesting that they can be particularly effective in PA patients. It can, therefore, be anticipated that CCBs will be widely used in hypertensive patients during the screening for PA; hence, future work should be aimed at assessing the effect on the ARR of CCBs with and without T-type Ca channels and with and without mineralocorticoid receptor blocking properties.

**Appendix**

The list of participating centers and PAPY study investigators are as follows: Padova, Italy, DMCS Internal Medicine 4, G.P.R., Andrea Semplicini, and A.C.P.; Padova, Italy, Endocrinology, F.M., Decio Armanini, Giuseppe Opocher, and Mee-Yung Mattarello; Ancona, Italy, Endocrinology, G.G., Vanessa Ronconi, and Marco Boscaro; Reggio Emilia, Italy, Azienda Ospedaliera ASMN di Reggio Emilia Internal Medicine, E.R.; Pisa, Italy, Internal Medicine, G.B. and Angelica Moretti; L’Aquila, Italy, Department of Internal Medicine and Public Health, C.F. and G.D.; Palermo, Italy, Internal Medicine, Giuseppe Andronico and Giovanni Cerassola; Brescia, Italy, Internal Medicine, D.R., Enzo Porteri, and Enrico Agabiti-Rosei; Legnano, Italy, Internal Medicine, G.P., Carlo Costantini, and Maria Teresa Lavazza; Roma, Italy, Internal Medicine, C.L. and Chiara Caliumi; Trieste, Italy, Internal Medicine, B.F.; Firenze, Italy, Endocrinology, M.Man. and Gabriele Parenti; Torino, Italy, Endocrinology, M.Mac. and Ezio Ghigo; Reggio Calabria, Italy, Nephrology, F.M., G.C., and Carmine Zoccoli; Bari, Italy, Internal Medicine, A.B.

**Sources of Funding**

This study was supported by research grants from the Foundation for Advanced Research in Hypertension and Cardiovascular Diseases, the Società Italiana dell’Ipertensione Arteriosa, and Progetti di Ricerca di Interesse Nazionale 2007PRELCC_002. Moreover, the study was supported by the research grants 60% to A.C.P., G.P.R., T.M.S. and Progetto di Ateneo to T.M.S.

**Disclosures**

None.

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for the Primary Aldosteronism in the Prevalence in hYpertension (PAPY) Study Investigators

Hypertension. published online November 23, 2009;

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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http://hyper.ahajournals.org/content/early/2009/11/23/HYPERTENSIONAHA.109.139832.citation

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Within-patient Reproducibility of The Aldosterone/Renin Ratio (ARR) in Primary Aldosteronism

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Running title: Aldosterone-renin ratio for primary aldosteronism.

*listed in the Appendix.

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The flow-chart illustrates the study design: after enrolment, 55 patients were discarded from further analysis because of incomplete data, protocol violations or unwillingness to undergo further testing. The remaining 1131 patients underwent measurement of the PRA and PAC for ARR calculation. Those with an ARR $\geq 40$ baseline, and/or $\geq 30$ after captopril, and/or a logistic discriminant function (LDF) score $\geq 50\%$, and one every 4 consecutive patients not fulfilling this criteria were submitted to the second ARR determination before the saline infusion test. In the patients who underwent both ARR the conclusive diagnosis of APA was then used for the purpose of assessing the diagnostic performance of both tests.
The Bland and Altman method is a quantitative comparison method based on the concept that the agreement between two methods of clinical measurements can be quantified using the differences between observations made with the two methods on the same subjects.\(^1\) The method uses a graphical approach which entails first the plot of the absolute difference between each pair of measurements (test 1 - test 2) from the two different methods on the y-axis against their corresponding averages on the x-axis, and then the calculation of the mean and the standard deviation of the difference. By visually inspecting the data, an agreement between the methods can be gathered from the graph if 1) data have a uniform distribution throughout the plot, with no increase in the degree of differences with increasing mean values; 2) data are symmetrical about the mean, and the mean is roughly equal to the median, 3) the mean is approximately zero; 4) data are mostly dispersed within the 95% limits of agreement for the differences, and about 4.3% of the values fall out the 95% agreement interval. When these conditions are satisfied, the 2 methods agree well and no bias exists (Panel A).\(^2\) Cases for which such conditions are not satisfied are exemplified in panels B to D. If the difference between the two measurements increases along their magnitude, a proportional error of the measurements affects reproducibility (Panel B); if the variation of at least one method strongly depends on the magnitude of measurements, e.g. if a ‘funnel effect’ is apparent,
also a bias impacts the agreement between the two methods (Panel C); if the mean value of the differences is far from the zero, an absolute systematic error prevents reproducibility of the methods (Panel D).

Horizontal lines are drawn at the mean difference, and at the limits of agreement, which are defined as the mean difference plus and minus 1.96 times the standard deviation of the differences.

References
