The aldosterone–renin ratio based on the plasma renin activity and the direct renin assay for diagnosing aldosterone-producing adenoma

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Background **The screening for primary aldosteronism is based on the aldosterone–renin ratio calculated with the plasma renin activity (PRA) value as denominator. A direct measurement of active renin (DRA) is being used as an alternative to PRA, but its diagnostic performance remains unclear.**

Method **We, therefore compared, head-to-head, the aldosterone–renin ratio based on PRA with that based on DRA, at baseline and after captopril administration, for identifying aldosterone-producing adenoma (APA) in 251 patients of the Primary Aldosteronism Prevalence in hYpertension Study (PAPY). The area under the receiver operator characteristics curves was used for estimating the accuracy of the aldosterone–renin ratio based on either renin assay for identifying APA and for the comparison between tests.**

Results **The rate of primary aldosteronism was 13.2%; 6.4% of the patients had an APA and 6.8% idiopathic hyperaldosteronism; 218 (86.8%) had primary hypertension. The area under the receiver operator characteristics curve for identifying APA was higher than 0.50 for the aldosterone–renin ratio based on both renin values (0.870 ± 0.058 for DRA and 0.973 ± 0.028 for PRA) ($P < 0.0001$ for both) and did not differ significantly between the aldosterone–renin ratios calculated with either renin assay. For the aldosterone–renin ratio based on DRA, the optimal cutoff value for identifying APA was 27.3 ng/mIU, remarkably similar to that previously determined for the aldosterone–renin ratio based on PRA.**

Conclusion **Thus, the aldosterone–renin ratio based on DRA is a valuable alternative to that based on PRA for detecting APA.** *J Hypertens* **28:1892–1899 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

Journal of Hypertension 2010, **28**:1892–1899

Keywords: aldosterone, aldosteronism, renin assay, secondary hypertension

Abbreviations: APA, aldosterone-producing adenoma; ARR, plasma aldosterone-to-renin ratio; ARR-D, ARR based on the DRA; ARR-P, ARR based on the PRA; AUC, area under the curve; AVS, adrenal venous sampling; CCB, calcium channel blocker; CI, confidence interval; DRA, active renin; GFR, glomerular filtration rate; IHA, idiopathic hyperaldosteronism; PAC, plasma aldosterone concentration; PAPY, Primary Aldosteronism Prevalence in hYpertension; PCC, plasma cortisol concentrations; PRA, plasma renin activity; ROC, receiver operator characteristics

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Received 27 November 2009 **Revised** 10 April 2010
Accepted 26 April 2010

Introduction

In the largest prospective Primary Aldosteronism Prevalence in hYpertension (PAPY) study, which surveyed newly diagnosed hypertensive patients referred to hypertension centers, primary aldosteronism was shown to have a prevalence of 11.2%, which was accounted for by an aldosterone-producing adenoma (APA) in almost half of the patients [1–3]. Further evidences [4,5] overall suggest that we might be facing an unrecognized epidemic of this curable form of arterial hypertension [6,7], which is underdiagnosed. This is worrying, as an early diagnosis and specific treatment can prevent the excess cardiovascular disease (for review see [8]) and event rate associated with primary aldosteronism [9]. These considerations underline the crucial importance of developing accurate and feasible strategies for timely diagnosing this condition.

The plasma aldosterone-to-renin ratio (ARR) is currently the most popular screening test for identifying primary aldosteronism [10,11]. In a within-patient comparison study [12] we recently showed that, when properly performed under standardized condition, the ARR is reproducible, thus providing support to its clinical use. Nonetheless, this ratio has been validated mainly using the plasma renin activity (PRA) value as denominator [2,3]. However, with this assay, the low, or very low, renin values that are commonly seen in primary aldosteronism patients can be measured accurately only by repeating the assay using long incubation times whenever low renin values are found, which is hardly feasible in routine laboratory practice [13–16].

* Listed in the 'Acknowledgements' section.

0263-6352 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI:10.1097/HJH.0b013e32833d2192

The newer direct measurement of active renin (DRA) has the advantages of being simpler and less labor-consuming and time-consuming than the PRA assay. Moreover, it is unaffected by the availability of angiotensinogen [17], and it allows a direct comparison of results across laboratories with use of renin calibrators. Hence, many centers have replaced the PRA with the DRA, notwithstanding the fact that its use in the ARR calculation has been validated only at one referral center [18]. Thus, the advantages, if any, of the ARR based on the DRA (ARR-D) over that based on the PRA (ARR-P) remain unclear. Moreover, although the optimal cutoff values of the ARR-P have been defined [2], whereas those of ARR-D remain unknown. The PAPY study Steering Committee, therefore, planned to prospectively perform a within-patient comparison of the ARR-D and the ARR-P for diagnosing APA, the primary aldosteronism subtype that can be conclusively diagnosed. We herein report on the results of this comparison.

Patients and methods

The PAPY study protocol followed the Statement for reporting Studies of Diagnostic Accuracy [19] recommendations and the requirements of the Declaration of Helsinki [2]. The PAPY study protocol has already been reported [2,20], and will be briefly recapitulated. We recruited consecutive newly diagnosed hypertensive patients referred to specialized hypertension centers nationwide in Italy, after an informed consent was obtained [2]. A prior diagnosis of a secondary form of hypertension and the patient's refusal to participate in the study were the only exclusion criteria.

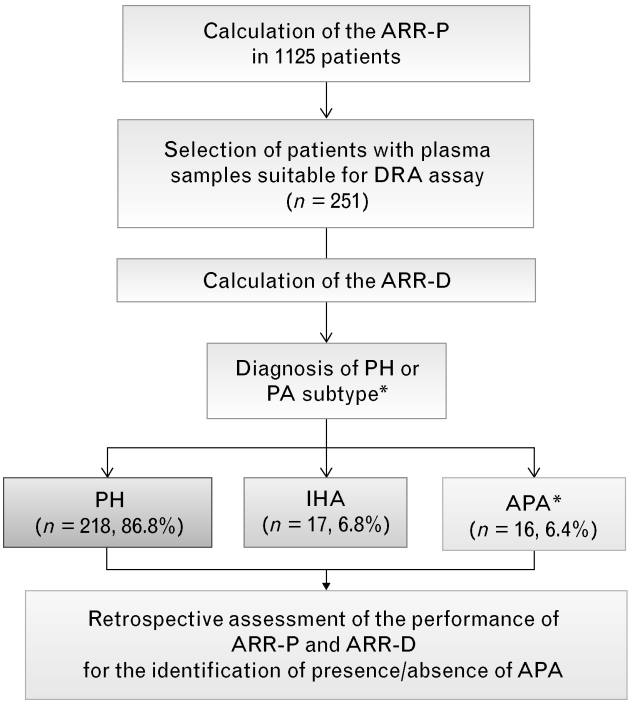
The flowchart for the current study is shown in Fig. 1. The patients were prepared from the pharmacologic standpoint as follows: a long-acting calcium channel blocker (CCB), which entailed nifedipine GastroIntestinal Therapeutic System or amlodipine in 95% of the patients, alone or combined with doxazosin, was prescribed whenever necessary for minimizing the risks of uncontrolled hypertension [21]. All patients underwent measurement of the 24-h Na^+ urine excretion, and were challenged with captopril (50 mg orally) in the sitting position [21].

Before and after captopril, PRA, plasma aldosterone concentration (PAC), and plasma cortisol concentration (PCC) were measured as previously detailed [21].

Further tests

The patients positive at the ARR baseline, after the captopril, at the logistic discriminant function test, or all [2,11] were submitted to an imaging test for identification of adrenocortical nodule and, regardless of its results, to adrenal venous sampling (AVS) or NP59 scintigraphy [22] to identify a lateralized aldosterone excess production. AVS was deemed to provide a lateralization diagnosis only if bilaterally selective [22]; adrenocorticotropic hormone stimulation was not used for the diagnosis because, even though it improves assessment of selectivity of catheterization, it does not enhance the diagnostic accuracy [23].

Fig. 1



Flowchart of the study design. From the 1125 eligible patients recruited in the PAPY study, we selected a subset of 251, who had both a conclusive diagnosis regarding the presence/absence of PA and the PA subtype and plasma samples suitable for the DRA assay. APA, aldosterone-producing adenoma; ARR, plasma aldosterone-to-renin ratio; ARR-D, ARR based on the DRA; ARR-P, ARR based on the PRA; DRA, active renin; IHA, idiopathic hyperaldosteronism; PA, primary aldosteronism; PAPY, Primary Aldosteronism Prevalence in hYpertension; PH, primary hypertension; PRA, plasma renin activity. *Diagnosed by the 'four-corner criteria' [3].

Biochemical measurements

Serum creatinine, serum and urine Na^+ and K^+ levels, PRA, PAC, PCC, and estimated glomerular filtration rate (GFR) were measured as described [24]. For the head-to-head comparison of the PRA, plasma were collected on iced water, centrifuged, then immediately frozen and stored at $-20^{\circ}C$ until assayed. For the DRA, plasma was obtained at room temperature and then immediately frozen at $-20^{\circ}C$. The PRA was determined at each participating center with the same commercial kit as described, usually within a week from sampling [2]. The measurement of DRA was centralized at a core laboratory at the University of Padua, using Liaison Direct Renin kit (DiaSorin, Saluggia, Italy), and was performed on average after 3 years from blood sampling. Samples received from participating centers that were not perfectly frozen were excluded from the analysis.

Normal ranges, intra-assay, and inter-assay coefficient of variation and antibody cross-reactivity for the hormonal measurements have already been reported [2]. For the DRA, the intra-assay and inter-assay coefficient of variations, according to the manufacturer (DiaSorin miniCD, assay manual, version May 2007), were 2.4 and 8.4%, respectively, in the range from 4 to 282 mIU/l. Antibody cross-reactivity for β2-microglobulin, cathepsin D, trypsin, and plasmin were 7.0, 6.9, 4.2, and 0.8%, respectively. According to the manufacturer's specification, the normal range of DRA values, measured in a multiethnic cohort of 89 healthy normotensive individuals, was 2.8–39.9 mIU/l (to convert mUI to ng divide by 1.66) and 4.4–46.1 mIU/l (5th–95th percentile) in the supine and upright position, respectively.

Calculation of the plasma aldosterone-to-renin ratio (ARR) based on the active renin (ARR-D) and based on the plasma renin activity (ARR-P) and diagnostic criteria

The ARR was first calculated using PAC (in ng/dl) as numerator, and all DRA (in mIU/dl) or PRA (in ng/ml per h) values. It was, thereafter, recalculated after setting the lowest possible value of the denominator to 0.6 mIU/dl (corresponding to the 25th percentile) and 0.2 ng/ml per h for the DRA and PRA, respectively, to avoid overinflating the ARR due to inaccurate DRA or PRA measurement in the low range of plasma renin.

APA was diagnosed according to the 'four-corner approach', which includes the following criteria: biochemical evidence of primary aldosteronism; lateralized aldosterone excess at adrenal vein sampling or, if unavailable, at adrenocortical mineralocorticoid scintigraphy; identification of APA at surgery, pathology, or both; demonstration of correction of the hyperaldosteronism and cure, or marked improvement of the hypertension after adrenalectomy [3]. Patients with biochemical evidence of primary aldosteronism, but without a lateralized aldosterone excess, were presumed to have idiopathic hyperaldosteronism (IHA).

Statistical analysis

DRA, PRA, PAC, and ARR values were skewed and, therefore, were analyzed after achievement of a normal distribution by log transformation. One-way analysis of variance followed by Bonferroni's post-hoc test was used to compare quantitative variables across groups. Categorical variables distribution was investigated by χ^2 analysis. To assess the within-patient relationship between DRA and PRA, we used Bland–Altman plots and correlation analysis [25]. The slopes of the regression line obtained at baseline and after captopril administration were compared by the method developed at University of California, Los Angeles (Academic Technology Service, Statistical Consulting Group, http://www.ats.ucla.edu/stat/SPSS/faq/compreg2.htm).

We used the Bland–Altman plot to detect systematic error, proportional error, or a magnitude-dependent bias. We also used the receiver operator characteristics (ROC) curves to assess the accuracy of the ARR-D and the ARR-P for identifying APA [21], and to compare it between the ARR-D and the ARR-P (MedCalc software, version 8.1.1.0 2006; MedCalc Software, Mariakerke, Belgium). The Youden index (J), a main summary statistic of the ROC curve defined as $J = \max(c)\,[\text{sensitivity}(c) + \text{specificity}(c) - 1]$, was employed to determine s the optimal cutoff (c^*), defined as the value that corresponds to the highest average of sensitivity and specificity and, therefore, optimizes the ARR's discriminating ability [26,27]. Finally, the positive and negative predictive values of ARR values were also calculated to obtain information on the performance of the tests. Significance was set at a P value below 0.05; SPSS for Mac (version 18.0; SPSS, Inc., Chicago, Illinois, USA) was used for all but the ROC curve analyses.

Results

Baseline characteristics

This sample of 251 patients of the PAPY study, in which DRA results and a conclusive diagnosis were both available, comprised 16 (6.4%) patients with APA, 17 (6.8%) with IHA, and 218 (86.8%) with primary hypertension. The prevalence rates of primary aldosteronism and its subtypes, as well as the demographic, clinical, and hormonal data of the patients with APA and IHA, were similar to those of the whole PAPY study cohort (all $P = \text{NS}$). Of the 33 patients with primary aldosteronism, 69% and 31%, respectively, underwent AVS and NP59 scintigraphy to ascertain the primary aldosteronism subtype.

At the screening test, 25.1% of the patients were untreated, 44.7% were on a CCB, 1.5% on doxazosin, and 28.6% on a combination of a CCB and doxazosin. Overall, the primary aldosteronism required more often a combination of CCB and doxazosin (40% vs. 27%) than the primary hypertension patients to achieve blood pressure (BP) control. Table 1 shows the anthropometric and biochemical features and the hormonal data of the three diagnosis subgroups. While differing for serum Na^+ and K^+ levels, the groups were similar for age, BMI, BP, serum creatinine, estimated GFR, and urine Na^+ and K^+ excretion.

Relationship between the active renin and the plasma renin activity

The PRA and DRA values showed the expected differences across groups: the DRA values showed lower values in the APA group as compared with both the IHA and the primary hypertension group. Because of the spread of the data, the difference between the latter two groups did not reach statistical significance. The ARR-P and the ARR-D were higher in the patients with APA than in those with primary hypertension, whereas the IHA had intermediate values (Table 1).

Table 1 Anthropometric and biochemical features of the patients with primary hypertension and with an APA or IHA

	APA ($n=16$)	*P* (APA vs IHA)	IHA ($n=17$)	*P* (IHA vs. primary hypertension)	Primary hypertension ($n=218$)	*P* (APA vs. primary hypertension)
Age (years)	50 ± 15	NS	49 ± 12	NS	46 ± 11	NS
Sex (male/female) (%)	7/9 (44/56)	NS	8/9 (53/47)	NS	130/88 (59/41)	NS
BMI (kg/m^2)	26 ± 3	NS	26 ± 5	NS	26 ± 4	NS
SBP (mmHg)	152± 12	NS	144 ± 16	NS	148 ± 16	NS
DBP (mmHg)	94 ± 10	NS	97 ± 9	NS	95 ± 9	NS
eGFR (ml/min per 1.73 m^2)	84 ± 7	NS	91 ± 9	NS	91 ± 2	NS
s-Na$^+$ (mEq/l)	142 ± 2.6	NS	141 ± 2.7	NS	140 ± 2.3	NS
s-K$^+$ (mEq/l)	3.4 ± 0.4	NS	3.7 ± 0.4	NS	3.9 ± 0.4	0.0001
Na$^+$ uV (mEq/24 h)	107 ± 41	NS	91 ± 34	0.01	153 ± 75	NS
K$^+$ uV (mEq/24 h)	47 ± 13	NS	51 ± 34	NS	58 ± 23	NS
PAC (ng/dl)	43.2 ± 6.7	NS	30.2 ± 3.5	<0.0001	11.3 ± 0.5	<0.0001
DRA (mIU/dl)	0.77 (0.61–2.37)	0.0001	2.64 (1.55–2.99)	NS	2.03 (1.74–2.32)	0.0001
PRA (ng/ml/h)	0.20 (0.16–0.45)	NS	0.30 (0.24–0.52)	<0.0001	1.23 (1.37–1.83)	<0.0001
ARR-D (ng/mIU)	149.9 ± 61.3	0.0001	40.9 ± 19.6	NS	12.2 ± 1.2	<0.0001
ARR-P (ng/dl / ng/ml per h)	220.9 ± 53.1	NS	121.8 ± 32.1	<0.0001	18.5 ± 3.0	<0.0001

Data are given as mean ± SD or SE; median and interquartile range for DRA and PRA. APA, aldosterone-producing adenoma; ARR, plasma aldosterone-to-renin ratio; ARR-D, ARR based on the DRA; ARR-P, ARR based on the PRA; BP, blood pressure; DRA, direct renin assay; eGFR, estimated glomerular filtration rate; IHA, idiopathic hyperaldosteronism; K$^+$ uV, potassium urinary excretion; Na$^+$uV, sodium urinary excretion; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

The DRA and PRA values showed a significant within-patient correlation [$r=0.26$, 95% confidence interval (CI) 0.14–0.37, $P<0.0001$] in the whole cohort, which was tighter for the postcaptopril (Fig. 2b) than for the baseline values (Fig. 2a), indicating that the between-method concordance is higher when renin secretion is stimulated. Hence, the slope of the regression lines was significantly higher after captopril administration than at baseline [0.652 (95% CI 0.57–0.81) vs. 0.271 (95% CI 0.16–0.41), $P<0.0001$].

The Bland–Altman plot was carried out using *Z* score for DRA and PRA values to avoid creating an artificial proportional error, due to the different units of measure of the two assays. It evidenced a 'funnel effect' that confirmed a magnitude-related (proportional) difference between the methods (Fig. 3a).

To further investigate the relationship between assays, we plotted the baseline and postcaptopril data altogether and fit the data using nonlinear equations. An exponential equation furnished an excellent fit of the data, as evidenced by the very narrow confidence band (Fig. 3b) and by a high goodness of fit index ($R^2=0.53$). The flattening of the curve in the low range of PRA values was associated with a widening of the prediction band (Fig. 3c), indicating that with the DRA assay, the measurement of low plasma renin levels becomes less accurate in the low range.

Diagnostic accuracy of the plasma aldosterone-to-renin ratio based on the plasma renin activity (ARR-P) and the plasma aldosterone-to-renin ratio based on the active renin (ARR-D)

Figure 4 shows the ROC curves for ARR-D and ARR-P for the identification of APA used as reference. The area under the curve (AUC) of both ARRs differed significantly from that under the identity line, indicating that both ARRs provide a gain in diagnostic accuracy for the identification of APA. The difference between the AUC for the ARR-D (0.870 ± 0.058) and the ARR-P (0.973 ± 0.028) was borderline significantly ($P=0.051$).

On the basis of these results, the hypothesis was put forward that this difference could depend on the lower precision of the DRA method in the low range of PRA values. This was tested by repeating the analysis after constraining the lowest value of DRA to 0.6 mIU/dl (0.36 ng/dl), which corresponds to the 25th percentile of the DRA values in the overall population, and the PRA to 0.2 ng/ml per h, the value used in clinical practice when calculating ARR-P to avoid overinflating its values due to exceedingly low PRA values [2,3]. With such approach the between-curve difference lost significance, because the AUC of the ARR-D ROC curve slightly increased (0.896) more than that of the ARR-P (0.976) (Fig. 4b).

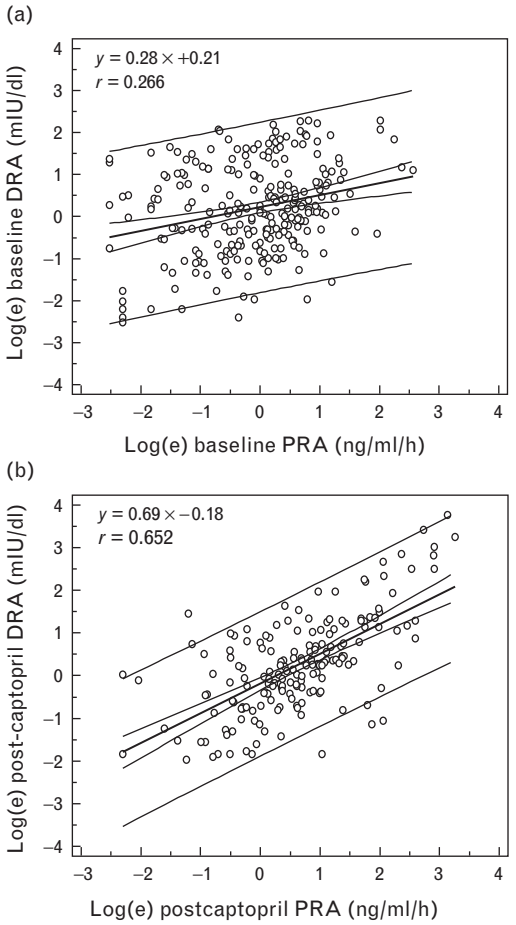
Optimal cutoff of the plasma aldosterone-to-renin ratio based on the active renin (ARR-D) for identification of aldosterone-producing adenoma

The Youden index was used to identify the optimal cutoff for the raw and corrected ARR-D (Table 2) [26,28]. For the former ARR-D, the optimal cutoff was 27.3 ng/mIU (45.3 if calculated as PAC in ng/dl and DRA in ng/dl), a value that is remarkably similar to that (26.85 ng/dl / ng/ml per h) found for ARR-P in the overall PAPY study [2]. For the latter, the optimal cutoff was 19.5. At the observed prevalence of APA (6.4%), the raw ARR-D had a sensitivity of 75%, a specificity of 91.3%, which translated in a moderate (81%) accuracy. For the corrected ARR-D, the sensitivity, specificity and the accuracy were practically identical. At the same prevalence rate of APA, the positive and negative predictive values of the raw and corrected ARR-D were 36.7% and 98.2%, and 39.9% and 98.2%, respectively (Table 2).

Discussion

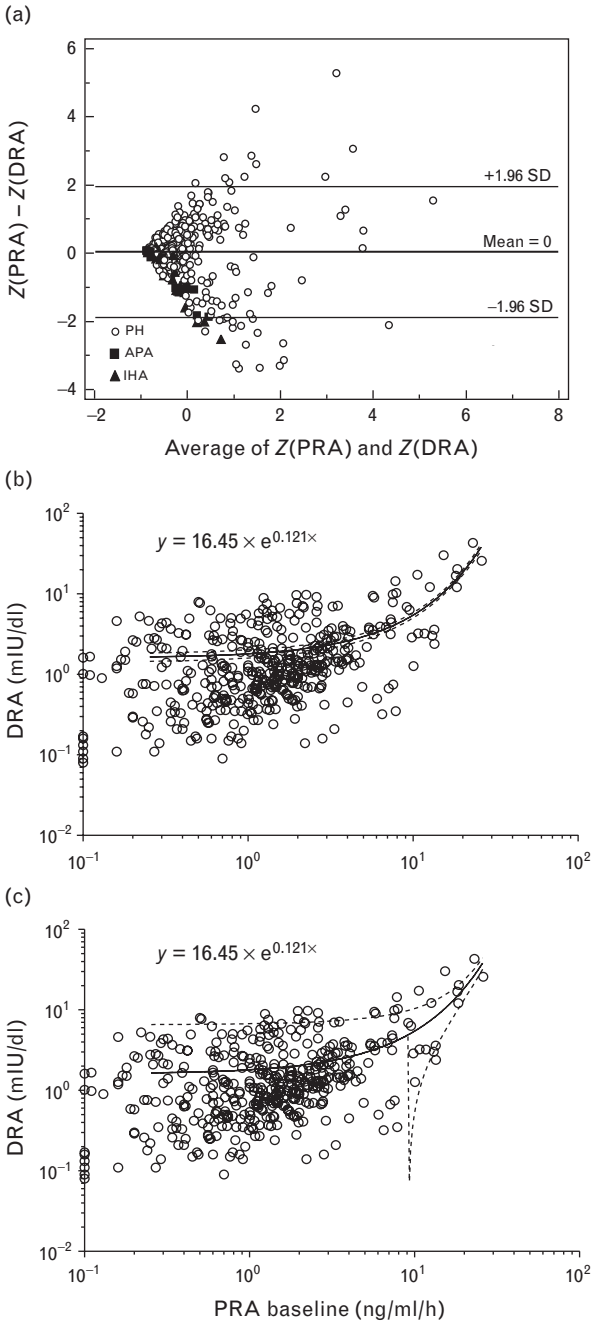
Given the high prevalence of primary aldosteronism and the feasibility of avoiding a life-long medical treatment

Fig. 2



Scatter plot of the within-patient values of plasma renin measured by the plasma renin activity and active renin method. (a) The coefficient of determination (R^2) for log-transformed DRA and PRA measured at baseline was 0.071 and the coefficient of correlation '*r*' was 0.266 (95% CI 0.147–0.377, $P < 0.0001$). (b) The coefficient of determination (R^2) for log(e)DRA and log(e)PRA postcaptopril challenge was 0.425 and *r* was 0.652 (95% CI 0.558–0.729, $P < 0.0001$). Formal comparison of the slope of the regression lines observed at baseline and after captopril administration revealed that slope of the regression lines was significantly higher after captopril administration than at baseline [0.652 (95% CI 0.57–0.81) vs. 0.271 (95% CI 0.16–0.41), $P < 0.0001$]. These findings indicate that the relationship between the methods was weaker in the low value range. DRA, active renin; PRA, plasma renin activity.

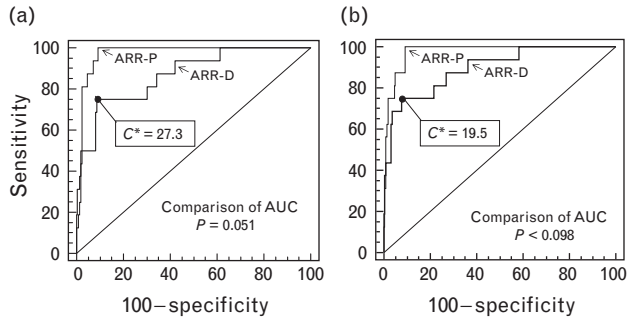
Fig. 3



(a) Bland–Altman plot of the *z* score for the active renin *Z*(DRA) and the plasma renin activity *Z*(PRA). The mean value of the difference between DRA and PRA was zero; overall, the data were symmetrically distributed around the mean, with a roughly equal number of points above and below the SD. Data were mostly dispersed within the 95% limits of agreement for the differences. The value pertaining to primary aldosteronism patients (closed symbols) were scattered at left bottom corner, indicating that with increasing average values of the PRA and DRA, the latter measurement tends to overestimate renin as compared with the former. (b) Distribution of baseline DRA and PRA values with the best fitting curve. The 95% confidence interval, which includes the true regression line with 95% probability, and the 95% prediction interval for the same curve (c) are also plotted. Being taken in the same individual, the baseline and postcaptopril measurement were not statistically independent; therefore, these bands are shown only for visual purposes and not for value prediction. DRA, active renin; PRA, plasma renin activity.

[29], and preventing cardiovascular complications with an early diagnosis, a sensitive, simple, and inexpensive screening test for primary aldosteronism is a hypertensiologist's dream [9,30]. While approaching such features [3,31], the ARR requires an accurate measurement of plasma renin, which is challenging in the primary aldosteronism patients who, by definition, exhibit low, or very low, renin values [3]. Of the available methods for measuring renin, the PRA assay requires two angiotensin I assays before (blank) and after sample incubation under standardized conditions. It also demands precautions during blood sampling, including collection on iced

Fig. 4



Receiver operator characteristics curves for the ARR based on active renin (ARR-D) and plasma renin activity (ARR-P). (a) The AUC for ARR-P was 0.973 (95% CI 0.944–0.990) and did not significantly differ ($P = 0.051$) from the AUC for ARR-D, which was 0.870 (95% CI 0.820–0.910). (b) After constraining the lowest values of DRA or PRA in the ARR denominator to 0.6 mIU/dl or 0.2 ng/ml per h, respectively, both AUC slightly increased (ARR-P AUC to 0.976, 95% CI 0.948–0.992; ARR-D AUC to 0.896, 95% CI 0.850–0.932). No significant differences were found between these AUC after such constrain. ARR, plasma aldosterone-to-renin ratio; ARR-D, ARR based on the DRA; ARR-P, ARR based on the PRA; DRA, active renin; PRA, plasma renin activity.

water, immediate centrifugation at 4°C, and freezing of plasma, and plasma storage at −20°C to avoid in-vitro angiotensin I generation. Moreover, detection of a low PRA value mandates repetition of the assay with longer incubation times in order to precisely estimate plasma renin levels in the low or very low range. By contrast, the DRA requires neither low temperatures during blood collection and centrifugation nor repeated angiotensin I assays, thus being simpler and faster. Hence, it is progressively replacing the PRA assay in most laboratories. The widely increasing popularity of the DRA strikingly contrasts with the lack of information on its accuracy for identifying conditions that are, as primary aldosteronism, characterized by low, or very low, plasma renin levels. For example, the recent Endocrine Society Clinical Practice Guideline reported the cutoff values for the ARR based on direct measurement of DRA, but apparently these values were based on consensus of experts rather than being experimentally determined [32].

In the PAPY study, the collection at the initial screening of plasma samples that were suitable for the DRA measurement allowed for a head-to-head comparison of the ARR-P and the ARR-D of renin for identifying patients with a conclusive diagnosis of APA [2,11] in a sizable cohort of referred hypertensive patients.

Relationship between active renin and plasma renin activity

The two methods showed a weak within-patient values correlation under baseline conditions and a strong correlation after captopril administration. As captopril stimulated renin secretion in the majority of the patients in this study who had primary hypertension, the stronger correlation after captopril administration is attributable to the higher precision of both assays, and particularly of the DRA, when plasma renin values are raised. However, with regard to primary aldosteronism, what is important is to examine the relation between assays in the low renin range, wherein the renin values of the primary aldosteronism patients typically sit. In this range, there was a progressively weaker relationship between DRA and PRA with lowering of the renin values, as evidenced by the difference in slope between regression lines (Fig. 2); the widening of the prediction band (Fig. 3c), and the 'funnel effect' at the Bland–Altman plot (Fig. 3a), which testifies a proportional (magnitude-related) error.

The limited ability of the DRA assay to detect the low renin levels in primary aldosteronism patients is intriguing in that, according to the manufacturer, the Liaison Direct Renin kit would have a functional sensitivity of 0.2 mIU/l. Moreover, previous studies carried out with different DRA methods showed a highly significant correlation between PRA and DRA in plasma samples with low renin concentration [33] and a mean value of immunoreactive renin of 1.63 mIU/l (range 0.8–11.7) in 28 patients with APA/IHA, which led to the contention that the ARR-D could be as accurate as ARR-P in identifying primary aldosteronism patients [34].

However, the use of 76 normotensive volunteers as controls, instead of hypertensive patients up to one-third of whom have low renin hypertension, could conceivably have magnified the performance of the test. Preliminary results of other comparative ARR-D/ARR-P studies carried out with the Liaison Direct Renin kit also suggested the possibility of measuring precisely the low renin values

Table 2 Optimal cutoff for the raw and corrected plasma aldosterone-to-renin ratio based on the active renin as identified by the receiver operator characteristics curves, and corresponding sensitivity, specificity, positive, and negative predictive values

	C*	Sensitivity (%)	Specificity (%)	Accuracy (%)	Positive predictive value	Negative predictive value
ARR-D ($ng\,dl^{-1}/mIU\,dl^{-1}$)	27.3	75.0	91.3	81.0	36.7	98.2
Corrected ARR-D ($ng\,dl^{-1}/mIU\,dl^{-1}$)	19.5	75.0	92.2	81.4	39.3	98.2

Corrected ARR were calculated by arbitrarily fixing the renin values in the denominator for DRA to 0.6 mIU/dl. C* is the optimal cutoff of the ARR-D value, which is the value that corresponds to the Youden Index defined as (sensitivity + specificity) − 1. Positive predictive value, calculated as (sensitivity × prevalence)/[sensitivity × prevalence + (1 − specificity) × (1 − prevalence)], estimates the likelihood of APA being present with a positive (ARR-D > the Youden index) test. Negative predictive value, calculated as (specificity × prevalence)/[(1 − sensitivity) × prevalence + specificity) × (1 − prevalence)], estimates the likelihood of APA being absent with a negative (ARR-D < Youden index) test. Accuracy, calculated as (sensitivity x prevalence)/(specificity)(1 − prevalence), estimates the proportion of true results in the population. Both predictive values were calculated at the prevalence of APA of 6.3% that was found in this cohort. For ng/dl/μIU/ml, the C* value must be divided by 10.

of patients with primary aldosteronism, but only eight patients with primary aldosteronism were investigated [35].

It is likely that factors related to the centralization of the DRA assay might account for the seemingly better performance of the ARR-P over the ARR-D in this study. The PRA was measured at experienced laboratories in which long incubation times allowed to accurately measure the low renin values, and thus the ARR-P, in our primary aldosteronism patients. Moreover, notwithstanding the precautions that were taken, the centralization of the DRA measurement, which was meant to limit the interlaboratory variability, might have led to cryoactivation during shipment of the samples, which we could not control for. This might explain why some patients with a conclusive diagnosis of APA did show low renin values by the PRA method but not by the DRA measurement (Fig. 3b and c).

Samples of primary aldosteronism patients could be particularly exposed to this problem because they might have a disproportionate increase of total (cryoactivatable) inactive renin, but preliminary data do not seem to support this possibility [36]. In this regard, the crucial importance of a rigorous control of the preanalytical phase to avoid inadvertent alteration of renin measurement, both with the enzymatic and even more so with the direct assay, has recently been emphasized [32]. According to Campbell *et al.* [32], theoretical considerations could explain why the DRA could be more biased by cryoactivation than the PRA. Hence, some potential mechanisms might explain the high DRA values that were occasionally observed in few APA patients despite low PRA values (Fig. 3a). The concurrence of additional factors cannot, however, be excluded because the lower diagnostic performance of the ARR-D was not simply due to these few disproportionately high DRA values, but more so to the finding of many low DRA values in the primary hypertension population. In fact, the finding of an overlap of DRA values between the IHA and the primary hypertension patients (Table 1) lend further support to the notion of a continuum between these conditions and accounts for the lack of statistically significant differences between these groups.

Diagnostic accuracy of the plasma aldosterone-to-renin ratio based on the active renin (ARR-D) and based on the plasma renin activity (ARR-P) for identifying aldosterone-producing adenoma

The AUC under the ROC curves is an index of the average sensitivity for all values of specificity and *vice versa*, and, therefore, provides an estimate of the diagnostic accuracy. Noteworthy, the AUC was higher than 0.50 for both the ARR-D and the ARR-P, indicating that both are useful for the screening of APA over 'tossing a coin'. Even though a significant correlation between the assays was seen, the raw ARR-D performed slightly, but nonsignificantly, worse than the ARR-P (Fig. 4a), owing to the fact that the DRA became less precise in the low range of renin values [32]. This is indicated by the stronger correlation between DRA and PRA seen after captopril administration and the widening of the prediction band in the low range (Fig. 3b and c). This conclusion is also supported by the observation that when the ROC analysis was repeated after constraining the lowest limit of DRA-measured renin values, the borderline difference between the ARR-D and the ARR-P AUC waned. This arbitrary setting of the minimum DRA concentration at 5 mU/l (3 ng/dl) to avoid overestimating the ARR in cases of very low or undetectable DRA levels is currently done at one referral center that adopted the DRA [18].

Analysis of the Youden index, which captures the performance of a test better than the AUC, showed that the discriminatory accuracy of corrected ARR-D in distinguishing primary hypertension from APA patients was slightly higher than that of uncorrected ARR-D (Table 2).

Strengths and limitations of the study

Strengths of the PAPY study include its prospective design, the use of a conclusive diagnosis of APA [2,11] as referent, and a careful standardization of the conditions for patient's preparation and blood sampling. The recruited patients were newly diagnosed hypertensive and most of them had only mild-to-moderate hypertension; however, they were referred to specialized hypertension centers, which might suggest that the present findings cannot be extrapolated to the general population of hypertensive subjects, and/or to those with long-standing and/or more severe and/or resistant hypertension. The investigation of only a sample of the entire PAPY study cohort might also suggest a selection bias. However, the fact that prevalence of primary aldosteronism and APA (13.2 and 6.4%, respectively) were only slightly higher than those found in the all PAPY study; the different diagnosis subgroups of this study did not differ from their counterpart of the entire PAPY study, collectively would speak against a selection bias and support the generalizability of our findings at least to the population of referred hypertensive patients.

In conclusion, these results allow, in our view, the following conclusions: the precision of the DRA under baseline conditions is lower (in the low range of plasma renin values) than after stimulation by captopril, which suggests that the ARR-D should be ideally used after stimulation of renin secretion. When applied to populations with an enriched prevalence of primary aldosteronism, the ARR-D is useful for detecting APA, but its accuracy can be enhanced by fixing the lowest detectable value of DRA to 0.6 mIU/dl. With this correction, the optimal cutoff of the ARR-D for detecting APA is 19.5 ng/mIU, whereas for the raw ARR-D, it was 27.3 ng/mIU, which is quite similar to the cutoff of 26.85 ng/dl and 26.85 ng/ml per h observed for the ARR-P in the PAPY study [2].

Acknowledgements

This study was supported by research grants from the FOundation for advanced Research In Hypertension and CArdiovascular diseases and the Società Italiana dell'Ipertensione Arteriosa.

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