Prospective validation of an automated chemiluminescence-based assay of renin and aldosterone for the work-up of arterial hypertension

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Abstract

Background: The availability of simple and accurate assays of plasma active renin (DRC) and aldosterone concentration (PAC) can improve the detection of secondary forms of arterial hypertension. Thus, we investigated the performance of an automated chemiluminescent assay for DRC and PAC in referred hypertensive patients.

Methods: We prospectively recruited 260 consecutive hypertensive patients referred to an ESH Center for Hypertension. After exclusion of six protocol violations, 254 patients were analyzed: 67.3% had primary hypertension, 17.3% an aldosterone producing adenoma (APA), 11.4% idiopathic hyperaldosteronism (IHA), 2.4% renovascular hypertension (RVH), 0.8% familial hyperaldosteronism type 1 (FH-1), 0.4% apparent mineralocorticoid excess (AME), 0.4% a renin-producing tumor, and 3.9% were adrenalectomized APA patients. Bland-Altman plots and Deming regression were used to analyze results. The diagnostic accuracy (area under the curve, AUC of the ROC) of the DRC-based aldosterone-renin ratio (ARR_{CL}) was compared with that of the PRA-based ARR (ARR_{RIA}) using as reference the conclusive diagnosis of APA.

Results: At Bland-Altman plot, the DRC and PAC assay showed no bias as compared to the PRA and PAC assay. A tight relation was found between the DRC and the PRA values (concordance correlation coefficient=0.92, p<0.0001) and the PAC values measured with radioimmunoassay and chemiluminescence (concordance correlation coefficient=0.93, p<0.001). For APA identification the AUC of the ARR_{CL} was higher than that of the ARR_{RIA} [0.974 (95% CI 0.940–0.991) vs. 0.894 (95% CI 0.841–0.933), p=0.02].

Conclusions: This rapid automated chemiluminescent DRC/PAC assay performed better than validated PRA/PAC radioimmunoassays for the identification of APA in referred hypertensive patients.

Keywords: aldosterone; aldosteronism; renin assay; secondary hypertension.

Introduction

Potentially curable secondary forms of arterial hypertension (HT) are markedly under diagnosed even though the ESH/ESC guidelines recommend that they be looked for in hypertensive patients fulfilling certain features [1]. This strategy recently led to detect these forms in about half of the patients with resistant HT referred to tertiary centers [2], which is 50-fold more than the current detection rate in common general clinical practice.

The under detection of secondary forms of HT not only results into lifelong costly medical treatment, but more importantly, into an excess damage of the target organs of HT, which translates into an excess rate of otherwise preventable cardiovascular events [3, 4]. Hence, this is a clinical disaster that needs urgent measures.

An accurate assessment of the renin-angiotensin-aldosterone system (RAAS) is key to a successful

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identification of secondary forms of HT [5]. Hence, the development of accurate assays for measuring renin and aldosterone that are simple, fast, and easy to implement in real life practice can represent a major leap forward to accelerate and facilitate diagnosis and decrease the costs of undiagnosed HT. To this aim, nonradioactive methods for the simultaneous measurement of active renin and aldosterone are much desirable, as they are less labor-and time-consuming and thus ultimately cheaper. Moreover, for renin measurement they have the theoretical advantage over plasma renin activity (PRA) of the independence of renin on its substrate (angiotensinogen).

Several studies have evaluated chemiluminescent assays for measuring the plasma concentration of active renin (DRC) and aldosterone in hypertensive patients [6–11]. These studies, albeit showing that compared to the radioimmunometric methods the DRC assay allowed saving labor time and costs, overall had limitations in that most of them were designed to compare PRA to DRC [6, 8–12], rather than to test the diagnostic accuracy of the aldosterone-renin ratio (ARR) based on DRC (ARR_{CL}) and the PRA-based ARR (ARR_{RIA}), for identifying the subtypes of primary aldosteronism that can be diagnosed conclusively. Therefore, no such studies followed the STARD recommendations for estimating diagnostic accuracy [13], which dictate that a mandatory step before any novel test can be introduced in clinical practice entails validation using a "gold standard" as reference. Of note, a conclusive diagnosis of APA (based on the "four corners criteria" [5]), and of FH-1 (based on genetic testing) was never used as reference in these studies. Notwithstanding this, the chemiluminescent assays of renin have rapidly replaced the PRA at many centers, which is much worrying as their superiority, or even equivalence, in terms of diagnostic accuracy over the PRA assay was never proven.

This study was therefore, set up to prospectively evaluate the diagnostic accuracy of a novel chemiluminescent automated assay for DRC and PAC in a cohort of consecutive referred patients with HT in whom a conclusive diagnosis of APA was achieved.

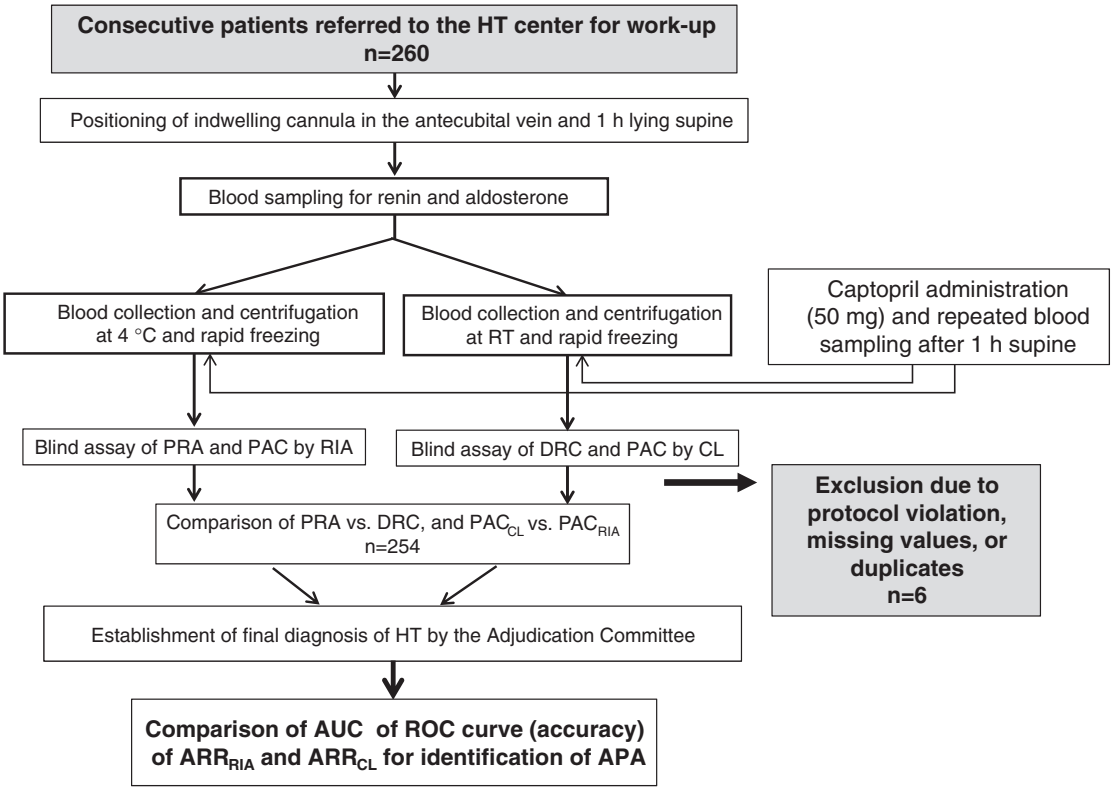

Figure 1: Flow-chart of the study.
After exclusion of six patients due to various reasons 254, were analyzed. For the assays the plasma samples were assigned to different technicians who were blind to the clinical diagnosis and to the results of the other tests. See text for explanations. HT, hypertension; RT, room temperature; PRA, plasma renin activity; PAC, plasma aldosterone concentration; RIA, radioimmunoassay; CL, chemiluminescence; ARR, aldosterone renin ratio; APA, aldosterone producing adenoma.

Materials and methods

Study design

The study protocol followed the requirements of the Declaration of Helsinki and the Statement for reporting Studies of Diagnostic Accuracy (STARD) recommendations [5, 13]. The flow-chart of the study is shown in Figure 1: in brief, paired plasma samples from each patients were collected and submitted to pre-analytical handling as required for the DRC and the PRA assays**.** Two technicians, each kept totally blind to clinical data, the conclusive diagnosis of the type of HT, and to the results of the other renin and PAC measurements, independently performed the assays.

Based on previous experience [14] we determined beforehand that at least 230 patients were needed to achieve acceptable results; given an anticipated attrition rate a 10% we enrolled 260 consecutive consenting patients referred to the Clinica dell'Ipertensione Arteriosa, Centro Regionale Specializzato, Regione Veneto, Center of Excellence of the ESH, for the evaluation of HT. Exclusion criteria entailed heart failure, liver cirrhosis, type 1 diabetes mellitus, and any other major illness that could affect life expectancy and/or the renin angiotensin-aldosterone system. An Adjudication Committee made by experienced clinicians (GPR, TMS and GM) made the final diagnosis of the cause of HT.

Subjects and methods

For a detailed description of the pharmacological preparation of the patients and the conditions for testing, please refer to the Supplemental Material.

Biochemical measurements

All biochemical measurements were centralized and performed in the ISO 9001-certified central laboratory of the Azienda Università-Ospedale-Padova as detailed in the Supplemental Material.

DRC and PAC were measured shortly after blood sampling in the ad hoc collected samples using an automated system (DiaSorin, LIAISON® XL instrument), the LIAISON® Direct Renin kit (DiaSorin, Saluggia, Italy) and the LIAISON® XL Aldosterone kit. Normal ranges and antibody cross-reactivity for the hormonal measurements have already been reported [5].

ARR_{RIA} and the ARR_{CL} calculation and diagnostic criteria

The ARR was first calculated using PAC (in ng/dL) as numerator, and either DRC (in mIU/L, ARR_{CL}) or PRA (in ng/mL/h, ARR_{RIA}) values as denominator. The ARR value was also recalculated after setting the lowest possible value of the denominator to 0.6 mIU/L (corresponding to the 25th percentile) and to 0.2 ng/mL/h for the DRC and PRA, respectively, as described [14], to avoid over inflation due to low renin levels.

Primary aldosteronism (PA) was diagnosed in the presence of biochemical evidence of PA, as shown by an elevated ARR_{RIA}: for the diagnosis of APA lateralized aldosterone excess at adrenal vein sampling (AVS); identification of APA at surgery and/or pathology; demonstration of correction of the hyperaldosteronism and cure, or marked improvement of the hypertension post-adrenalectomy, e.g. the "four corner criteria", were also required [5]. Patients with a high ARR_{RIA}, who showed no lateralized aldosterone excess, were presumed to have idiopathic hyperaldosteronism (IHA). Renovascular hypertension was diagnosed based on demonstration of a hemodynamically significant renal artery stenosis at angioCT using the METRAS Study criteria [15], and a fall greater than 20 mmHg systolic and diastolic blood pressure on the same or lowered antihypertensive drugs (dose and/or number) after renal revascularization [15]; familial hyperaldosteronism type 1 (FH-1) was diagnosed by long PCR [16]; renin-producing tumor (RPT) was diagnosed based on biochemistry, imaging and renal venin sampling.

Further tests

The patients with an ARR exceeding the cutoff values at baseline and post-captopril (27 and 13 ng/dL/ng/mL/h, respectively) [5] were submitted to AVS to identify a lateralized aldosterone excess production, and to an imaging test for identification of adrenocortical nodule [5, 17, 18]. Only bilaterally selective AVS were used to demonstrate lateralization of hyperaldosteronism [18]. As the bilateral simultaneous blood sampling technique was used, we did not perform AVS with cosyntropin because we previously showed that despite improving the assessment of selectivity of catheterization this stimulation does not enhance the diagnostic accuracy [19].

Statistical analysis

DRC, PRA, PAC, and ARR values were skewed and therefore, were analyzed after a normal distribution was achieved by log transformation. One-way ANOVA with Bonferroni's post-hoc test was used to compare quantitative variables across groups. Distribution of categorical variables was investigated by χ^2 analysis. Bland-Altman plots and Deming regression analysis were used to assess the within-patient relationship between DRC and PRA, and between PAC_{RIA} and PAC_{CL} values [20]. Bland-Altman plots were used to detect systematic error, proportional error, or a magnitude-dependent bias. For the Deming regression, the concordance correlation coefficient, which evaluates the degree to which pairs of observations fall on the 45° line through the origin, was calculated. This coefficient is a measure of accuracy and was estimated by the following formula: $\rho c = \rho\, Cb$, with ρ indicating the Pearson correlation coefficient and Cb indicating a bias correction factor that measures how far the best-fit line deviates from the 45° line through the origin. Given the different ranges of values furnished by these assays the Z score were calculated from the raw values and used for the latter analyses.

The receiver operator characteristics (ROC) curves were employed to assess the accuracy of the ARR_{CL} and the ARR_{RIA} for identifying APA [21]. The area under the ROC, used as an estimate of diagnostic accuracy, was compared between the ARR_{CL} and the ARR_{RIA} with the method of Hanley [22]. The Youden index (J), a main summary statistic of the ROC curve defined as J=max (c) [sensitivity (c)+specificity (c)–1], was employed to determine the optimal cutoff (c^*). This was defined as the value that optimizes the ARR's discriminating ability in that it corresponds to the highest average of sensitivity and specificity [23]. The significance was set at $p<0.05$. SPSS™ for Mac (vers. 22.0) was used for all but the ROC curve analyses, which were performed with the MedCalc™ software (vers. 15.6 MedCalc Software, Mariakerke, Belgium).

Results

Baseline characteristics

Between April 2014 and April 2015 260 consecutive newly referred patients with HT were recruited. Of them six were excluded due to protocol violations, missing data or duplicated (n=1). Of the remaining 254 patients, 67.3% had primary hypertension (PH) and 32.7% secondary HT. In the latter group, 17.3% (of the total) had an APA, 11.4% IHA, 2.4% RVH due to atherosclerotic renal artery stenosis, 0.8% (two patients) FH-1, 0.4% (one patient) apparent mineralocorticoid excess due to licorice abuse and 0.4% (one patient) renin-producing tumor; 3.9% (ten patients) were adrenalectomized APA patients studied at follow-up after surgery. The demographic and clinical data of the entire cohort of consecutive hypertensive patients (Table 1) showed that the patients were middle aged, overweight, with normal sodium intake and renal function. Table 2 shows the clinical and the raw biochemical data of the patients in the three main groups of diagnosis; the adrenalectomized PA patients were not included as PA in the overall analysis.

The PA patients showed the lowest values of serum K^+, PRA and DRC; both the PA and the RVH showed high PAC; however, only the former exhibited overtly elevated values of the ARR, regardless of the assays used for its determination. When the PA, which comprised the largest group with secondary HT, was examined, the APA patients showed slightly lower serum K^+, and higher PAC and ARR values,

Table 1: Baseline characteristics of the entire cohort of consecutive hypertensive patients.

Variable	All patients (n=254)
Age, years	46.1±13.5
Gender (m/f, %)	57/43
BMI, kg/m^2	28.4±9.7
Systolic BP, mmHg	146±17
Diastolic BP, mmHg	91±13
Serum Na^+, mmol/L	141.2±2.0
Serum K^+, mmol/L	3.9±0.5
Urinary Na^+ excretion, mmol/24 h	171±77
Urinary K^+ excrextion, mmol/24 h	65±23
Serum creatinine, µmol/L	69±22
eGFR, mL/min/1.73 m^2	110±64

Table 2: Anthropometric and clinical features of the patients in the main diagnosis groups.

Variable	PA (n=69)	p-Value	RVH (n=6)	p-Value	PH (n=171)	p-Value
		PA vs. RVH		RVH vs. PH		PH vs. PA
Age, years	48.9±11.4	ns	39.4±15.1	ns	45.2±14.1	ns
Gender (m/f, %)	62/38	ns	50/50	ns	55/45	ns
Systolic BP, mmHg	150±19	ns	147±18	ns	143±15	ns
Diastolic BP, mmHg	93±15	ns	93±14	ns	90±12	ns
Serum K^+, mmol/L	3.6±0.5	ns	3.9±0.2	ns	4.0±0.4	<0.0001
PRA_b, ng/mL/h	0.34 (0.26–0.41)	<0.0001	1.87 (0.56–3.19)	0.001	0.80 (0.68–0.91)	<0.0001
PRA_c, ng/mL/h	0.45 (0.32–0.59)	<0.0001	6.52 (0.39–15.80)	<0.0001	1.38 (1.13–1.63)	0.003
DRC_b, mIU/L	5.34 (4.07–6.60)	<0.0001	32.87 (16.43–49.30)	0.03	17.54 (15.04–20.03)	<0.0001
DRC_c, mIU/L	8.30 (5.75–10.85)	<0.0001	88.74 (9.98–167.50)	<0.0001	26.69 (22.38–31.00)	<0.0001
$PAC_{b_{RIA}}$, ng/dL	19.6 (16.1–23.1)	ns	21.1 (9.1–33.1)	0.01	10.1 (9.4–10.9)	<0.0001
$PAC_{c_{RIA}}$, ng/dL	15.8 (12.9–18.7)	ns	12.9 (5.1–19.9)	ns	6.9 (6.5–7.3)	<0.0001
$PAC_{b_{CL}}$, ng/dL	18.2 (14.7–21.7)	ns	22.7 (11.6–33.9)	0.002	9.6 (9.0–10.3)	<0.0001
$PAC_{c_{CL}}$, ng/dL	16.6 (12.7–20.4)	ns	16.4 (9.7–23.1)	ns	7.6 (7.2–8.0)	<0.0001
$ARR_{b_{RIA}}$ (ng/dL)/(ng/mL/h)	99.6 (76.3–123.0)	<0.0001	12.8 (6.9–18.8)	ns	22.2 (19.0–25.6)	<0.0001
$ARR_{c_{RIA}}$ (ng/dL)/(ng/mL/h)	76.3 (53.5–99.0)	0.004	6.3 (1.3–11.2)	ns	14.3 (11.1–17.4)	<0.0001
$ARR_{b_{CL}}$ (ng/dL)/(mIU/L)	7.5 (5.3–9.7)	0.005	0.9 (0.4–1.3)	ns	1.0 (0.8–1.1)	<0.0001
$ARR_{c_{CL}}$ (ng/dL)/(mIU/L)	5.2 (3.5–6.9)	0.009	0.4 (0.1–0.6)	ns	0.7 (0.5–0.8)	<0.0001

Median and 95% CI range, as appropriate. PA, primary aldosteronism; RVH, renovascular hypertension; PH, primary (essential) hypertension; BP, blood pressure; PRA_b, plasma renin activity (PRA) at baseline; PRA_c, PRA after captopril; DRC_b, direct renin concentration (DRC) at baseline; DRC_c, DRC_{RIA} after captopril; $PAC_{b_{RIA}}$, plasma aldosterone concentration (PAC) measured with radioimmunoassay (RIA)-based technique at baseline; $PAC_{c_{RIA}}$, PAC measured with RIA after captopril; $PAC_{b_{CL}}$, PAC measured with chemiluminescent assay at baseline; $PAC_{c_{CL}}$, PAC measured with chemiluminescent assay after captopril; $ARR_{b_{RIA}}$, PAC-to-PRA ratio calculated using PAC measured with RIA as numerator and PRA value as denominator, at baseline; $ARR_{c_{RIA}}$, PAC-to-PRA ratio calculated using PAC measured with RIA as numerator and PRA value as denominator, after captopril; $ARR_{b_{CL}}$, PAC-to-DRC ratio calculated using PAC measured with chemiluminescent assay as numerator and DRC as denominator, at baseline; $ARR_{c_{CL}}$, PAC-to-DRC ratio calculated using PAC measured with chemiluminescent assay as numerator and DRC as denominator, after captopril.

albeit only for the ARR_{CL}. The blood pressure values were significantly lower in the adrenalectomized APA than in the APA studied before adrenalectomy; moreover, their PRA and DRC values increased, and the PAC values fell, as compared to the pre-adrenalectomy group (Table S1). The baseline and post-captopril values of the ARR_{RIA} and ARR_{CL} in the patients divided by final diagnosis (Figure 2) evidenced a remarkable similarity between values determined with either method across all groups. The corresponding values of PRA, DRC, and PAC also showed prominent similarity (Figures S1 and S2), as assessed by the concordance correlation coefficient, which was 0.92 and 0.93 for renin and PAC, respectively (Figure S3).

Relationship between the DRC and the PRA and PAC_{RIA} and PAC_{CL}

The DRC and PRA values showed a significant within–patient correlation in the whole cohort, both at baseline (r=0.91 (0.89–0.93), p<0.0001 and post-captopril (r=0.88 (0.85–0.91), p<0.0001), thus indicating a high between-method concordance in the all range of plasma renin levels (Figure S3). The PAC_{RIA} and PAC_{CL} values also showed a significant within–patient correlation in the whole cohort, both at baseline (r=0.93 (0.91–0.94), p<0.0001) and post-captopril (r=0.88 (0.84–0.90), p<0.0001). Therefore, there was a high between-method concordance in a wide range of PAC levels spanning from those found in patients with low PAC (AME and FH-1 under dexamethasone treatment) and high PAC values (PA and RVH patients).

Diagnostic accuracy of the ARR_{RIA} and the ARR_{CL}

Figure 3 shows the Bland-Altman plot of the ARR_{RIA} and the ARR_{CL}. The Z scores were used for this plot to avoid creating an artificial proportional error (due to the different

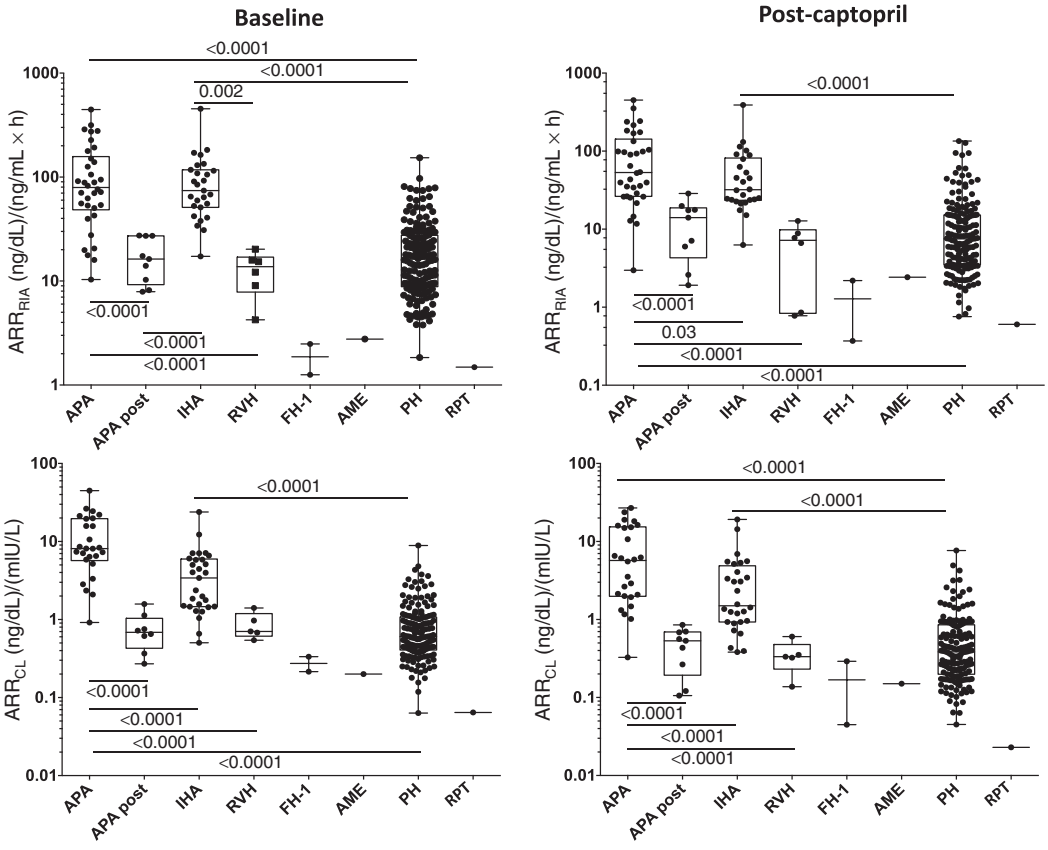

Figure 2: Box and whisker plots of the aldosterone-renin ration (ARR) values calculated using PAC and DRC measured with the automated chemiluminescent method (ARR_{CL} lower panels) or with radioimmunoassay of PAC and PRA (ARR_{RIA} upper panels) in the 254 patients divided according to the final diagnosis.

The left panels show the baseline values while the right panels depict the values obtained after captopril challenge. Please note the remarkably similar results obtained with the different methods under both conditions, the ARR values higher in the APA group than in the PH and RVH groups, and the markedly lower values in the adrenalectomized APA patients and in the patient with a renin-producing tumor.

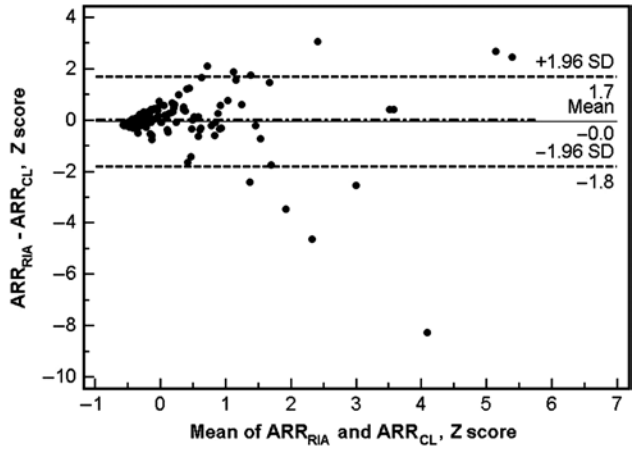

Figure 3: Bland-Altman plot showing the comparison of the ARR_{RIA} and the ARR_{CL} values (plotted as Z scores of the raw values). See text for explanation.

units of measure of the assays). Although eyeball examination suggested a 'funnel effect', e.g. a magnitude-related (proportional) difference between the methods, no evidence for systematic biases was detected: only 10 of the 254 values fell out of the 95% confidence interval.

The ROC curves AUC for ARR_{CL} and ARR_{RIA} for the unambiguously diagnosed APA, used as reference, differed significantly from that under the identity line, for both ARRs indicating that they provided a diagnostic gain in over tossing a coin (Figure 4). However, the AUC for

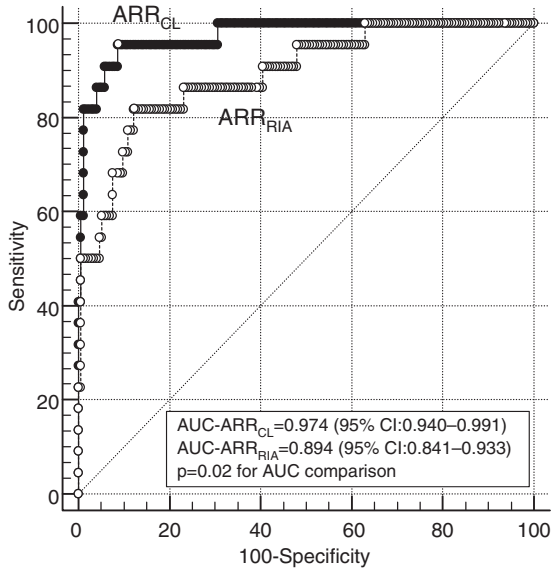

Figure 4: The comparison of the ROC curves for the ARR_{CL} and the ARR_{RIA} measured under baseline conditions for the identification of APA, as conclusively established by using the 'four corners' criteria, shows a higher area under the curve (AUC) for the ARR_{CL} than for the ARR_{RIA}, indicating a better performance of the former over the latter.

ARR_{CL} was higher than that for ARR_{RIA} (p=0.02). Results were similar when the analysis was repeated after constraining the lowest value of DRC to 6 mIU/L, and the PRA to 0.2 ng/mL/h, the values used in clinical practice when calculating the ARR to avoid over-inflating its values due to exceedingly low renin values [5, 24].

Optimal cutoff of the ARR_{CL} for identification of APA

The Youden index, used to identify the optimal cutoff for the raw and corrected ARR_{CL}, was 2.06 for the raw ARR_{CL} expressed as PAC in ng/dL and DRC as mUI/L, which corresponded to a sensitivity of 92% and a specificity of 91.6% (Table 3). For the ARR_{RIA} the optimal cutoff value (in ng/dL/ng/mL/h) was 38.7, corresponding to a sensitivity of 80% and a specificity of 92%. Results were similar when the analysis was repeated after constraining the lowest DRC value to 6 mIU/L, and the PRA to 0.2 ng/mL/h.

Discussion

The direct immunochemical measurement of active renin is rapidly replacing PRA for several potential advantages including independence on renin substrate availability, more consistent results, handling of plasma at room temperature, and the possibility of performing the assay with automation, thus saving time, costs and human resources. Accordingly, the replacement of the PRA with the chemiluminescent renin assay has rapidly occurred at many centers in spite of lack of any proof for superiority of the former over the latter in terms of diagnostic accuracy. The available studies mostly investigated the correlation of DRC with DRA [6–11]; only one retrospective study validated a chemiluminescent assay in PA patients, but did not compare the ARR_{CL}, with the ARR_{RIA} [12].

We planned this study in 2013 to prospectively compare head-to-head the ARR obtained from an automated simultaneous chemiluminescent measurement of PAC and DRC (ARR_{CL}) with the ARR based on established and clinically validated PRA and PAC radioimmunoassay (ARR_{RIA}) in a sizable cohort of patients. According to the protocol, we selected no specific forms of HT as we wished to test the performance of these assays in a real life situation entailing a cohort of consecutively referred hypertensive patients. Hence, we recruited mostly patients with essential HT, but also, consistently with the referral

Table 3: Area under the ROC Curve (AUC) for the aldosterone-to-renin ratio (ARR) measured with the chemiluminescence-based technique and the optimal criterion value.

ROC curve	
Disease prevalence, %	12.3
Area under the ROC curve (AUC)	
Area under the ROC curve (AUC)	0.971
Standard error[a]	0.014
95% Confidence interval	0.937–0.989
Z statistic	32.71
Significance level p (Area=0.5)	<0.0001
Youden index	
Youden index J	0.8362
Associated criterion	>2.057
Sensitivity	92.00
Specificity	91.62
Optimal criterion[b]	
Optimal criterion[a]	>5.029
Sensitivity	80.00
Specificity	98.88

[a]Taking into account disease prevalence (12.3%) and estimated costs: cost false positive: 1; cost false negative: 1; cost true positive: 0; cost true negative: 0. [b]The optimal criterion value takes into account not only sensitivity and specificity, but also disease prevalence and costs of various decisions.

Criterion values and coordinates of the ROC curve

	Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	–LR	+PV	–PV	Cost
	≥0.063	100.0	86.3–100.0	0.0	0.0–2.0	1.00		12.3		0.88
	>0.905	100.0	86.3–100.0	69.8	62.5–76.5	3.31	0.00	31.6	100.0	0.27
	>0.915	96.0	79.6–99.9	69.8	62.5–76.5	3.18	0.057	30.8	99.2	0.27
	>1.461	96.0	79.6–99.9	83.2	76.9–88.4	5.73	0.048	44.4	99.3	0.15
	>1.467	92.0	74.0–99.0	83.2	76.9–88.4	5.49	0.096	43.4	98.7	0.16
→	**>2.057**	**92.0**	**74.0–99.0**	**91.6**	**86.6–95.2**	**10.98**	**0.087**	**60.5**	**98.8**	**0.08**
	>2.094	88.0	68.8–97.5	91.6	86.6–95.2	10.50	0.13	59.5	98.2	0.09
	>2.827	88.0	68.8–97.5	94.4	90.0–97.3	15.75	0.13	68.7	98.3	0.06
	>2.829	84.0	63.9–95.5	94.4	90.0–97.3	15.04	0.17	67.7	97.7	0.07
	>3.285	84.0	63.9–95.5	96.1	92.1–98.4	21.48	0.17	75.0	97.7	0.05
	>3.328	80.0	59.3–93.2	96.1	92.1–98.4	20.46	0.21	74.1	97.2	0.06
	>5.029	80.0	59.3–93.2	98.9	96.0–99.9	71.60	0.20	90.9	97.3	0.03
	>7.079	60.0	38.7–78.9	98.9	96.0–99.9	53.70	0.40	88.2	94.7	0.06
	>7.125	60.0	38.7–78.9	99.4	96.9–100.0	107.40	0.40	93.7	94.7	0.05
	>8.572	40.0	21.1–61.3	99.5	96.9–100.0	71.60	0.60	90.9	92.2	0.08
	>8.888	40.0	21.1–61.3	100.0	98.0–100.0		0.60	100.0	92.3	0.07
	>44.88	0.0	0.0–13.7	100.0	98.0–100.0		1.00		87.7	0.12

The arrow and bold indicate the cutoff value that meets the optimal sensitivity and specificity. +PV, positive predictive value; –PV, negative predictive value; +LR, positive likelihood ratio; –LR, negative likelihood ratio [25].

nature of our center, a notable proportion with secondary forms of HT, among which primary aldosteronism largely prevailed, but other forms, including renovascular HT, FH-1, renin-producing tumor, and apparent mineralocorticoid excess were also represented. Moreover, as a result of our policy of systematically assessing the patients post-adrenalectomy to prove correction of the PA, there were also APA patients who were studied at follow-up post-adrenalectomy. Altogether these features testified the 'real life' setting of this study and allowed to compare the two assays over a wide range of renin and PAC values.

Of note, the secondary forms of HT were diagnosed unambiguously by state-of-the art criteria: for APA and FH-1, the subtypes of PA that can be unequivocally diagnosed, by the "four corners criteria" and long PCR, respectively [5, 17]. Since, as expected FH-1 was found only in two

cases, we used APA as reference index to determine the accuracy of either ARR following the STARD recommendations [13]. The diagnostic accuracy of the ARR_{CL} and the ARR_{RIA} for identifying APA could then be compared using the ROC curve analysis.

When the entire cohort was examined, both the PRA and DRC, and the PAC values, and consequently the ARR_{RIA} and ARR_{CL} (Figure 2) showed remarkably similar results and, more importantly, the expected differences across diagnosis groups (Table 2 and Figures S1 and S2). The renin values were lower in the PA group than in the PH and the RVH group with both assays; moreover, both PAC_{RIA} and PAC_{CL} values were higher in the PA and RVH patients than in the PH patients, as expected. Of note, the highest DRC value was observed in the patient with the renin-producing tumor, in which the PRA values were not so prominently elevated. This finding, along with the fact that another such case was identified at our center in the past few months after the introduction of the DRC assay, suggests that this assay, by circumventing the underestimation of renin with the PRA assay (due to dependency on limiting) substrate availability), can improve the detection of rate of this rare cause of HT.

We found that the DRC and PRA values were tightly within-patient correlated in the whole cohort, both for the baseline and for the post-captopril stimulation (Figure S3). This finding is at variance with what we found in a smaller retrospectively investigated cohort of the PAPY study, where only the post-captopril PRA and DRC values correlated strongly [14]. We speculated at that time that the stronger correlation post-captopril could be because of the higher precision of both renin assays when renin values are raised. Instead the present findings showed that the PRA and DRC correlated well also in the low renin range, e.g. in PA patients under unstimulated conditions. Likely this different better outcome originated from several factors: the very rigorous control of the pre-analytical phase that avoided inadvertent alteration of renin measurement due to cryoactivation [6, 26], use of an automated method in a centralized laboratory, along with a storage time duration much shorter than in the PAPY Study [14].

The notable precision of the automated DRC assay to measure the low renin levels of PA patients agrees with the findings of a highly significant correlation between PRA and DRC in plasma samples with low renin concentration (mean value of 1.63 mIU/L, range 0.8–11.7) in PA patients, as well as with the manufacturer's claim that the LIAISON Direct Renin kit has a functional sensitivity of 2 (1.60–1.96 IFU DS) mIU/L. This finding is obviously relevant from the practical standpoint and supports the contention that the ARR_{CL} could be at least as accurate as ARR_{RIA} in identifying PA patients [18].

To further investigate the accuracy of the two ARR we used the AUC under the ROC curves, which estimates the overall diagnostic accuracy because it provides an index of the average sensitivity for all values of specificity, and vice versa. For the ARR_{CL} and the ARR_{RIA} the AUC was higher than 0.50, indicating that both tests are useful for the screening of APA over "tossing a coin". More importantly, the accuracy of the ARR_{CL} was higher than that of the ARR_{RIA}: a formal comparison of the ARR_{CL} and the AUC ARR_{RIA} showed that the AUC for the former test (0.974 (95% CI 0.940–0.991) was higher than that of the latter (0.894 (95% CI 0.841–0.933, p=0.02) (Figure 4). Identical conclusions were reached when the ROC analysis was repeated after constraining the lowest limit of DRC- and PRA measured renin values at the aforementioned minimum values.

The optimal cutoff for the raw ARR_{CL} (expressed as PAC in ng/dL and DRC as mIU/L, Table 2), was 2.06; it corresponded to a sensitivity of 92% and a specificity of 92% (Table 3); for the ARR_{RIA} the optimal cutoff value was 38.7 (expressed as PAC in ng/dL and PRA as ng/mL/h) corresponding to a sensitivity of 80% and a specificity of 92%. This ARR_{CL} cutoff is higher than the 1.2 reported for another chemiluminescent assay in a retrospective study, in which, however, the diagnosis of APA was not unambiguously confirmed by the four corner criteria (12). With that cutoff, however, in spite of slightly higher sensitivity (98.9% vs. 92%) the specificity was much lower compared to that found in the present study (79% vs. 92%). Moreover, to enable readers to select the ARR_{CL} cutoff values that best meet their desired combination of sensitivity and specificity we have provided a table with the sensitivity, specificity, positive (+PV) and negative predictive value (–PV), positive likelihood ratio (+LR), negative likelihood ratio (–LR), and also the optimal criterion value, which takes into account not only sensitivity and specificity, but also disease prevalence, and costs of various decisions (Table 3).

It might be argued that extrapolation of these findings to the general population of hypertensives is not warranted because we examined patients referred to a specialized hypertension center, who show a prevalence of secondary forms of hypertension much higher than that found in the hypertensive patients commonly seen in general practice. However, the prevalence of secondary forms of HT was lower than the 50% reported in a recent multicenter study of patients with resistant HT [2]; moreover, our cohort had an overall prevalence of PA of 27%, higher than that (11.2%) found in the all PAPY Study

[5]. Nonetheless, the rate of the different subtypes was similar, which would speak against a selection bias and overall support the generalizability of our findings to the population of referred hypertensive patients.

Strengths of this study include its prospective design, a careful standardization of the conditions for patient's preparation, blood sampling and biochemical assay (see Supplemental Material), and, moreover, se of an accepted methodology based on the conclusive diagnosis of secondary HT, namely of APA, as reference index for the diagnosis of PA [5, 17, 27].

These results allow, in our view, the following conclusions: in patients adequately prepared from the pharmacological standpoint, when samples are properly collected and handled under carefully standardized conditions, the diagnostic performance of the ARR based on DRC is equivalent to that based on a clinically validated PRA assay in a wide range of clinical conditions. Moreover, for the detection of PA, a common cause of secondary HT that is most challenging for the renin assays because of its low or very low renin values, the ARR_{CL} obtained from PAC and DRC measured with an automated chemiluminescent method performed better than the ARR_{RIA}. Major advantages of the this method for estimating active renin over the PRA assay include a simpler pre-analytical handling with blood collection and at room temperature and automation, which translated in more reproducible results and in less hands-on use of human resources. Finally, lack of use of radioactivity and radioactive waste make these assays environment friendly. Based on these considerations we have now introduced the chemiluminescence-based assays instead of the PRA and PAC radioimmunoassay at our institution. This has allowed a marked shortening of the time from blood sampling to results, with the benefits of speeding up reaching the diagnostic conclusions.

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Note: An App for the calculation of the ARR_{RIA} and ARR_{CL} has been developed by the authors and it is available at the Apple Store.

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