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Criteria for diagnosing primary aldosteronism on the basis of liquid chromatography–tandem mass spectrometry determinations of plasma aldosterone concentration

Stephanie Baron^{a,b,c}, Laurence Amar^{b,d,e}, Anne-Laure Faucon^{b,d}, Anne Blanchard^{b,c,f}, Laurence Baffalieu^a, Catherine Faucard^a, Simon Travers^h, Jean-Yves Pagnyⁱ, Michel Azizi^{b,d,g}, and Pascal Houillier^{a,b,c}

Background: Primary aldosteronism is affecting about 10% of hypertensive patients. Primary aldosteronism should be diagnosed by screening tests based on plasma aldosterone concentration (PAC) and aldosterone-to-renin ratio (ARR), followed by confirmatory test. The cutoff values for PAC and ARR depend on PAC and plasma renin measurement methods. Liquid chromatography–tandem mass spectrometry (LC-MS/MS), the new gold standard method for aldosterone determination, is now widespread but shows lower values than immunoassays. New cutoff values have yet to be determined with LC-MS/MS PAC.

Methods: In a retrospective cohort, we measured PAC by LC-MS/MS in 93 healthy volunteers, 77 patients with essential hypertension and 82 primary aldosteronism patients (42 lateralized, 24 bilateral, 16 primary aldosteronism without adrenal vein sampling) after 30 min in a seated position.

Results: PAC ranged from 42 to 309 pmol/l in healthy volunteers and from 63 to 362 pmol/l in essential hypertensive patients. A cutoff value of 360 pmol/l for basal PAC had a sensitivity of 90.5% and a specificity of 95.1% to differentiate lateralized primary aldosteronism from essential hypertensive patients. ARR ranged from 2.3 to 22.3 in healthy volunteers and from 3.2 to 55.6 pmol/mU in essential hypertensive patients. Using ROC curves, we selected an ARR of 46 pmol/mU, which provided a sensitivity of 100% and a specificity of 93.4% to distinguish between essential hypertensive and lateralized primary aldosteronism patients (sensitivity 94.4%, specificity 93.9% for the overall primary aldosteronism population).

Conclusion: Criteria for primary aldosteronism screening need to be adapted, given the increasing use of LC-MS/MS to determine PAC. We suggest to use 360 pmol/l and 46 pmol/mU as cutoff values, respectively, for basal PAC and ARR after 30 min of seated rest.

Keywords: adrenocortical adenoma, aldosterone, hypertension

Abbreviations: ARR, aldosterone-to-renin ratio; AUC, area-under-curve; AVS, adrenal vein sampling; CI, confidence interval; CLIA, chemiluminescent immunoassay; LC-MS/MS, liquid chromatography tandem mass spectrometry; NPV, negative predictive value; PAC, plasma aldosterone concentration; PPV, positive predictive value; PRC, plasma renin concentration; RIA, radioimmunoassay; ROC, receiver operating characteristic; SIT, saline infusion test

INTRODUCTION

Primary hyperaldosteronism is the overproduction of aldosterone due to an adrenal disorder reported, in various cross-sectional and prospective studies, to be diagnosed in approximately 10% of hypertensive patients [1–3]. Primary hyperaldosteronism is associated with target organ damage and increased cardiovascular morbidity [4–6]. Clinical practice guidelines for primary hyperaldosteronism diagnosis have recently been published [7–10]. Primary hyperaldosteronism is diagnosed through a multistep procedure, beginning with the measurement of plasma renin (PRC) and plasma aldosterone (PAC) concentrations to compute aldosterone-to-renin ratio (ARR), which is considered to be the most effective

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^aAssistance Publique-Hôpitaux de Paris (AP-HP) Georges Pompidou European Hospital, Physiology Department, ^bParis-Descartes University, ^cINSERM UMR 1138 – CNRS ERL 8228, Paris, ^dAssistance Publique-Hôpitaux de Paris (AP-HP) Georges Pompidou European Hospital, Hypertension Unit, ^eINSERM UMR 970, Paris Cardiovascular Research Center, ^fAssistance Publique-Hôpitaux de Paris (AP-HP) Georges Pompidou European Hospital, Clinical Investigations Center 1418, ^gINSERM, CIC1418, Paris, ^hAssistance Publique-Hôpitaux de Paris (AP-HP), Robert Debré Hospital, Biochemistry Department and ⁱAssistance Publique-Hôpitaux de Paris (AP-HP) Georges Pompidou European Hospital, Interventional Radiology, Paris, France

Correspondence to Stephanie Baron, PharmD, PhD, Physiology Department, Georges Pompidou European Hospital, 20–40 Rue Leblanc, 75908 Paris Cedex 15, France. Tel: +33 156092350; fax: +33 156092675; e-mail: stephanie.baron@aphp.fr

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screening test [11–13]. If ARR exceeds a defined threshold, confirmatory suppression tests have to be performed [7]. Four suppression tests are currently available: the oral sodium loading test, the fludrocortisone suppression test, the captopril challenge test and the saline infusion suppression test (SIT).

These guidelines suggested various cutoff values for ARR, PAC and the suppression tests all derived from studies using immunoassays [14–16]. A small number of immunoradiometric or chemiluminescent (CLIA) assays are now available for PRC determinations [17–19]. All should be standardized against the international standard MRC 68/356, which limits the inter-method variability. PAC is currently measured by numerous radioimmunoassays (RIA) or CLIA assays, but inter-method variability is high. Several factors are responsible for this considerable variability, including a lack of antibody specificity for immunological assays, and a poor inter-method reproducibility due to poor standardization. This variability must be taken into account when defining cutoff values for use in primary hyperaldosteronism screening and confirmatory tests [20–22].

The current gold standard method for PAC determination is liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). This method has now high sensitivity and specificity, making it possible to achieve accurate quantification. Several laboratories have developed their own method for routine measurements [23–27]. PAC values measured with LC-MS/MS have been reported to be lower than those obtained with immunological methods [23,25]. However, none of these studies assessed the consequences of PAC determination by LC-MS/MS in terms of cutoff values used for primary hyperaldosteronism screening.

The aim of this study was to compare our LC-MS/MS method with other currently available PAC assays and to a distinct LC-MS/MS method, to establish reference intervals for both PAC and ARR in normotensive and hypertensive populations and, thus, to propose new cutoff values for primary hyperaldosteronism screening with LC-MS/MS determinations of PAC.

MATERIALS AND METHODS

Participants

This cross-sectional retrospective study included 252 individuals: 93 healthy volunteers, and 159 patients admitted to Georges Pompidou European Hospital between July 2012 and June 2016 for assessment of hypertension; 77 patients with essential hypertension; and 82 patients with primary hyperaldosteronism. All individuals signed informed consent allowing the data collected for routine clinical care to be used for research purposes. The protocol was approved by the 'Comité de Protection des Personnes', Paris-Ile de France III, France. All investigations were performed in accordance with the Helsinki Declaration.

Healthy normotensive volunteers (healthy men and non-pregnant women aged 18–80 years) were recruited by the Clinical Investigations Center using the same sampling procedure as patients (2010-A00365–34). Each individual provided a medical history and underwent a complete physical examination and routine laboratory evaluation, including an electrocardiogram. Individuals taking any medical treatment,

including oral contraceptives, were excluded. This population was previously described [28].

Patients with essential hypertension underwent full investigation at the hypertension reference centre of Georges Pompidou European Hospital. Essential hypertension was diagnosed after extensive evaluations in hospital, including biological tests, plasma hormone determinations, and adrenal and renal artery imaging to exclude secondary hypertension according to 2013 ESH guidelines [29].

Patients with primary hyperaldosteronism were diagnosed in accordance with current guidelines for primary hyperaldosteronism [7]. We used previously validated criteria to define primary hyperaldosteronism [30–32]: ARR higher than 64 pmol/mU on at least two different occasions and a baseline PAC in seated position (30 min) higher than 550 pmol/l on at least two different occasions. If PRC was at least 5 mU/l, the actual PRC value was used to calculate ARR. If PRC was below 5 mU/l, a value of 5 mU/l was used for the calculation to prevent the overestimation of ARR [31,33]. Confirmatory SIT was performed in all patients except those with contraindications (severe symptomatic hypertension, hypokalaemia or congestive heart failure) and those with PAC above 550 pmol/l and PRC below quantification level (<2 mU/l) [7]. Aldosterone secretion was considered to be nonsuppressible if the post-SIT PAC was higher than 139 pmol/l [7]. Patients with primary hyperaldosteronism who desire and who would have benefit from surgery treatment and for whom adrenal venous sampling (AVS) procedure has no contraindications underwent AVS as recommended for primary hyperaldosteronism subtype classification [7]. Briefly, two catheters were inserted into the adrenal veins via the femoral vein; blood was collected simultaneously, without cosyntropin stimulation, from both adrenal veins and a peripheral blood sample (vena cava or femoral vein) was also taken concomitantly [31]. AVS was considered to be selective if the cortisol concentration in both adrenal samples was at least twice that in a peripheral sample taken. This selectivity index cut off value of 2 is widely used by most centres who performed AVS as underlined in AVIS study [34]. The lateralization of aldosterone secretion was confirmed if the lateralization index, aldosterone-to-cortisol ratio on one side was at least four times that on the other side [31]. This stringent lateralization index has been shown to limit interpretation discrepancies [35] and has been widely used [36]. Finally, 35 lateralized patients with primary hyperaldosteronism who underwent unilateral adrenalectomy in our centre had follow-up 6 months after surgery. Complete biochemical success was assessed as recommended by recent guidelines for the outcome after adrenalectomy when kalaemia and ARR were normalized [37]. Among the seven remaining patients, three declined adrenalectomy, and four had no follow-up in our centre after primary hyperaldosteronism diagnosis and subtype classification. For essential hypertensive and primary hyperaldosteronism patients, interfering antihypertensive drugs were discontinued 2 weeks before blood collection for angiotensin 2 receptor antagonists, angiotensin 1-converting enzyme blockers, diuretics and beta-blockers, and 6 weeks before blood collection for mineralocorticoid receptor antagonists and renin inhibitors. Calcium

antagonists, α -blockers and centrally acting antihypertensive drugs were prescribed to patients with severe hypertension if necessary [10]. For all patients and individuals, unrestricted dietary sodium intake was recommended. Patients with persistent hypokalaemia were given oral potassium supplements to try to maintain serum potassium in the normal range. Urinary potassium excretion was assessed by measuring potassium excretion in 24-h urine.

Protocol

The volunteers and patients provided 24-h urine collections. They were admitted in a fasting condition to the hypertension unit (primary hyperaldosteronism and essential hypertensive patients) or the Clinical investigations Center (healthy volunteers). Blood pressure (BP) was measured by trained nurses, with a validated semiautomatic manometer (OMRON 705 CP; Omron Electronics, Rosny sous bois, France). Office BP was determined by calculating the average of three measurements taken in the seated position after a 5-min rest period [38]. Blood samples for the measurement of PAC and PRC were collected into potassium ethylenediaminetetraacetic acid between 0800 and 0900 h after resting 30 min in a seated position [39].

The samples were centrifuged at room temperature; PRC and routine PAC measurements were performed daily using routine immunoassay methods. An aliquot of plasma sample was stored at -80°C until PAC was retrospectively measured by LC-MS/MS.

Laboratory data

Potassium and creatinine concentrations were determined by standard methods (DxC800; Beckman Coulter, Villepinte, France). Estimated glomerular filtration rate was calculated with simplified MDRD equation [40]. PRC was determined by the DiaSorin CLIA method on a Liaison (DiaSorin, Saluggia, Italy). This assay specifically determines the concentration of active renin by a sandwich method and is calibrated against NISBC International Standard reference preparation 68/356. The characteristics of this method have been described in detail elsewhere [17,41]. PAC was routinely determined by radioimmunoassay (Coat-a-count; Siemens Medical Solutions Diagnostics, Erlangen, Germany).

Liquid chromatography tandem mass spectrometry measurement of plasma aldosterone concentration

PAC was determined by LC-MS/MS method according to the Waters recommendations [42]. The method is described in detail in the 'supplemental method' section. Briefly, plasma samples in EDTA were deproteinized, and aldosterone was extracted by a solid-phase extraction process, in 96-well plates. The eluate was injected into a Waters Acquity UPLC I-class liquid chromatography system (Waters, Elstree, Hertfordshire, UK) with a Cortecs UPLC C18 2.1 x 100 mm, 1.6 μm column (Waters). Aldosterone was detected with a Waters Xevo TQ-S tandem mass spectrometer operating in the negative electrospray ionization mode. The method was previously validated in accordance with European Medicine Agency and US Food and Drug Administration recommendations [43,44].

Comparison of methods

We compared the PAC values obtained by LC-MS/MS with those obtained with five different immunoassays ($n = 27$ samples): Coat-a-Count Siemens Healthcare Diagnostics (Erlangen, Germany), IBL International (Hamburg, Germany), CisBio (Condolet, France) and Beckman-Coulter (Immunotech, Praga, Czech Republic) RIA methods and the Diasorin CLIA method (Saluggia, Italy). We also tested another LC-MS/MS method (Chromsystem) using a totally distinct process. All assays were performed in accordance with respective manufacturers' instructions.

Statistical analysis

Linear regression (Deming), Bland–Altman analysis (bias and limits of agreement) and Spearman's rank correlation test were used to compare the PAC methods. For each assay, the corresponding low limit of quantification (LLOQ) was used for statistical analysis if the PAC was below the LLOQ. We compared PRC, PAC and ARR between healthy volunteers, essential hypertensive and primary hyperaldosteronism patients by Kruskal–Wallis tests, with *t* Dunn's multiple comparisons test if significant. Receiver-operator curves (ROCs) with area under the curve (AUC) calculations, likelihood ratios, Youden tests and positive and negative predictive values (PPV and NPV) were used to define sensitivity, specificity and to propose cutoff values for ARR and PAC. As the aim was to distinguish between patients with primary hyperaldosteronism and those with essential hypertension, we considered two different situations for ROC analyses using either all primary hyperaldosteronism patients ($n = 82$) or only those with lateralized aldosterone secretion on AVS ($n = 42$) against essential hypertensive patients ($n = 77$). All data analyses were performed with Prism (La Jolla, California, USA) version 7.0 for Mac. Data are expressed as medians [5th–95th percentile] or as percentages with 95% confidence intervals (95% CIs), unless otherwise specified. We considered *P* values below 0.05 to be significant.

RESULTS

Description of the population

We measured PAC and calculated ARR under basal conditions in 252 individuals. The demographic characteristics of the individuals are summarized in Table. Primary hyperaldosteronism patients had lower plasma potassium concentration than essential hypertensive and healthy individuals; 65.3% of them received potassium supplementation, while only 5.1% of essential hypertension patients were supplemented. Primary hyperaldosteronism patients had lower PRC, higher PAC and ARR determined with immune-assays than essential hypertensive patients ($P < 0.0001$). Sodium diets were not significantly different between the three groups.

Among the 82 primary hyperaldosteronism patients, four patients did not undergo AVS, as they were not candidates for surgery; the 78 other patients underwent AVS: 66 AVS were successfully performed. Out of these 66 patients, 42 had lateralized and 24 had bilateral primary hyperaldosteronism. There was no significant difference between lateralized and bilateral primary hyperaldosteronism patients regarding demographic characteristics or biological

parameters except a higher post-SIT PAC in lateralized than in bilateral primary hyperaldosteronism patients ($P < 0.01$). Out of the 42 lateralized primary hyperaldosteronism patients, 35 patients underwent unilateral adrenalectomy and had a complete biochemical success as described in the international consensus [37]. Among the seven remaining patients, three of them finally refused to undergo adrenalectomy, and the four other patients did not have follow-up

in our centre after primary hyperaldosteronism diagnosis and subtype classification.

Comparison of plasma aldosterone concentration methods

Figure 1 and Supplemental Table 1, <http://links.lww.com/HJH/A927> show comparisons of PAC determination with

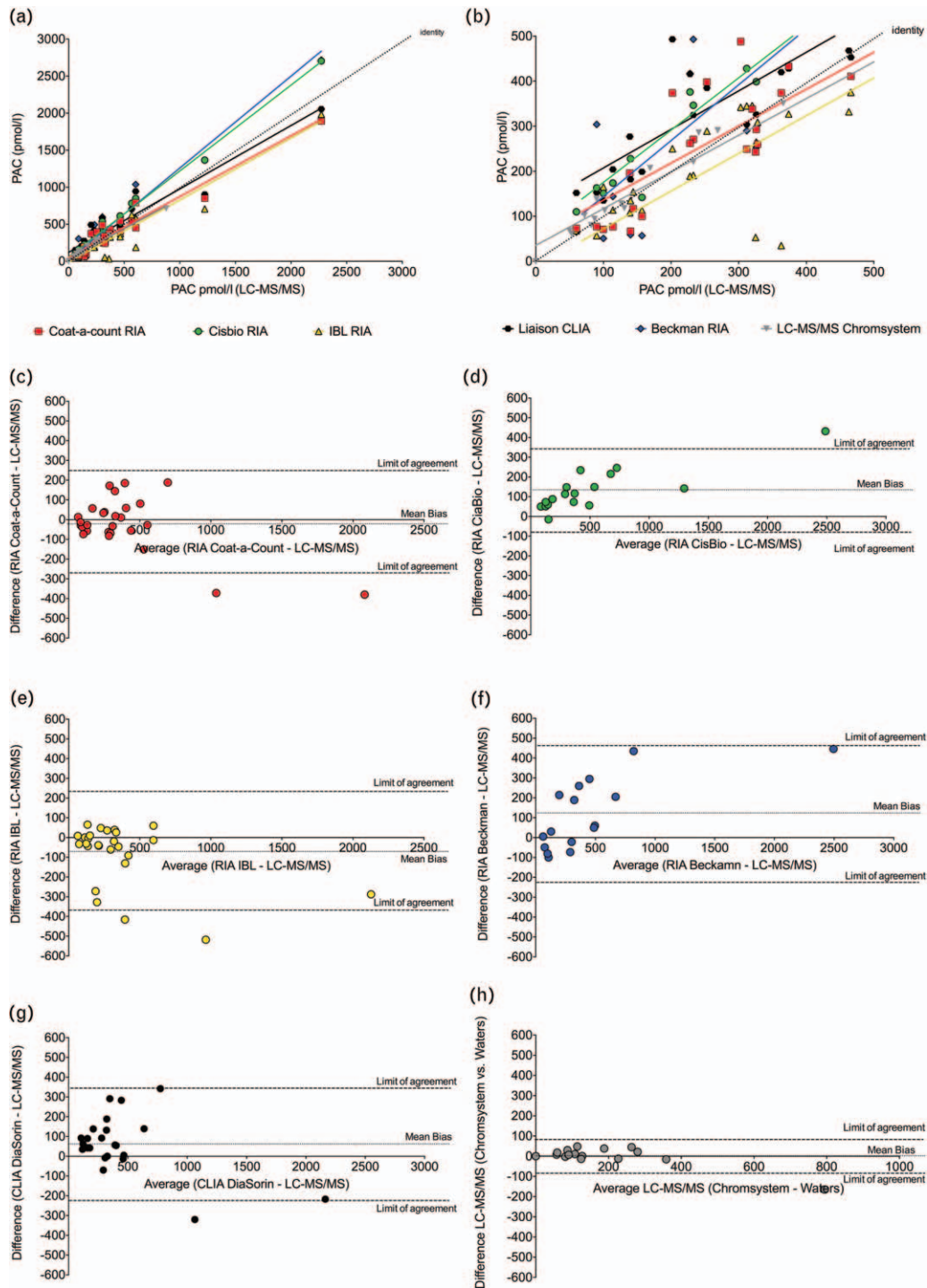


FIGURE 1 Comparison of methods measuring plasma aldosterone concentration: Linear regressions between various immunoassays (RIA and CLIA) and two different LC-MS/MS methods: (a) all range, (b) PAC below 500 pmol/l; Bland–Altman representation of (c) Coat-a-Count RIA versus Waters LC-MS/MS, (d) CisBio RIA versus Waters LC-MS/MS, (e) IBL RIA versus Waters LC-MS/MS, (f) Beckman RIA versus Waters LC-MS/MS, (g) DiaSorin CLIA versus Waters LC-MS/MS and (h) Chromsystem LC-MS/MS versus Waters LC-MS/MS.

our LC-MS/MS method with five marketed immunoassays and another commercially available LC-MS/MS method. PAC ranged from 60 to 2272 pmol/l with our LC-MS/MS method. The slopes of the regression lines differed significantly between the five assays ($P < 0.0001$ for all) (Fig. 1a and b, Supplemental Table 1, <http://links.lww.com/HJH/A927>). For values below 500 pmol/l (Fig. 1b), PAC varied by a factor of two to three, depending on the method used. The mean bias was between -17 and $+131$ pmol/l on Bland–Altman plots (Fig. 1c–h and Supplemental Table 1, <http://links.lww.com/HJH/A927>). The comparison of our LC-MS/MS method to Chromsystem LC-MS/MS showed a linear regression with Aldosterone_{LC-MS/MS} = 0.816 Aldosterone_{LC-MS/MS Chromsystem} + 35.5 with $R^2 = 0.968$. Bland–Altman analysis showed a mean bias of $+1.3$ pmol/l, with -94.0 and 96.6 pmol/l as the limits of agreement (Fig. 1h and supplemental Table 1, <http://links.lww.com/HJH/A927>).

Determination of reference intervals for plasma renin concentration, plasma aldosterone concentration and aldosterone-to-renin ratio under basal conditions, and new cutoff values for the screening of primary hyperaldosteronism by liquid chromatography–tandem mass spectrometry

Plasma renin concentration measurements

The reference intervals (5th–95th percentile) for seated PRC in healthy volunteers and essential hypertensive patients were 5.0 to 37.4 and 2.0 to 33.2 mU/l, respectively (Table 1, Fig. 2a). In primary hyperaldosteronism patients, PRC ranged from 2.0 to 7.9 mU/l (5th–95th percentile). PRC values were significantly lower in primary hyperaldosteronism patients than in healthy volunteers and essential hypertensive patients, even though there was some overlap ($P < 0.0001$ for both comparisons; Table 1 and Fig. 2a).

Liquid chromatography–tandem mass spectrometry plasma aldosterone concentration measurements

The reference interval (5th–95th percentile) for seated PAC was 42–309 pmol/l in healthy volunteers and 63–362 pmol/l

in essential hypertensive patients (Fig. 2b). PAC was significantly higher in patients with primary hyperaldosteronism than in healthy volunteers or essential hypertensive patients ($P < 0.0001$; Fig. 2b) but with some overlap.

The ability of PAC to discriminate between essential hypertensive ($n = 77$) and overall primary hyperaldosteronism patients ($n = 82$) was assessed by a ROC curve analysis. The AUC was 0.9695 ($P < 0.0001$). A PAC cutoff value of 362 pmol/l yielded a sensitivity of 82.2% (95% CI: 71.5–90.2) and a specificity of 95.1% (95% CI: 88.0–88.7) for primary hyperaldosteronism screening (Table 2). The Youden index for this cutoff value was 77.3, and the positive likelihood ratio (LR+) was 16.8. A PAC cutoff value of 502 pmol/l showed a 100% specificity for primary hyperaldosteronism screening with a 75.3% sensitivity.

When essential hypertensive patients were plotted against lateralized primary hyperaldosteronism patients ($n = 42$), the AUC was of 0.9882 ($P < 0.0001$). A cutoff value of 360 pmol/l yielded a sensitivity of 90.5% (95% CI: 77.4–97.3) and a specificity of 95.1% (95% CI: 88.0–98.7) to discriminate between lateralized primary hyperaldosteronism and essential hypertensive patients (Table 3). The Youden index for this cutoff was 85.6, and the LR+ was 18.5.

Liquid chromatography–tandem mass spectrometry aldosterone-to-renin ratio

The reference interval for LC-MS/MS ARR was 2.3 to 22.3 pmol/mU in healthy volunteers and 3.2 to 55.6 pmol/mU in essential hypertensive patients (Fig. 2c). LC-MS/MS ARR values were significantly higher in primary hyperaldosteronism patients than in healthy volunteers and patients with essential hypertension ($P < 0.0001$).

The ability of ARR to discriminate between essential hypertensive ($n = 77$) and overall primary hyperaldosteronism patients ($n = 82$) was assessed by a ROC curve analysis. The AUC was 0.9877 ($P < 0.0001$). An ARR cutoff value of 46 pmol/mU yielded a sensitivity of 94.4% (95% CI: 86.2–98.4) and a specificity of 93.9% (95% CI: 86.3–98.0) for primary hyperaldosteronism screening (Table 4). The Youden index for this cutoff value was 82.3, and the LR+ was 15.5. ARR value of 64.5 pmol/mU showed a 100%

TABLE 1. Demographic characteristics of individuals studied for the definition of cutoff values for basal plasma aldosterone concentration and aldosterone-to-renin ratio

	Healthy volunteers	Essential hypertension	Primary aldosteronism
<i>n</i> individuals (% male)	93 (54.8)	77 (58.4)	82 (59.7)
Age (years)	50.0 [21.9–73.0]	41.0 [18.0–67.0]	49.0 [34.0–65.4]**
BMI (kg/m ²)	24.2 [20.1–28.7]	26.2 [19.7–38.3]	28.3 [19.6–37.7]*
SBP (mmHg)	122 [99–150]	138 [117–165]	147 [110–179]*
DBP (mmHg)	71 [57–89]	88 [66–110]	90 [68–108]
Potassium supplementation (%)	0 (0)	4 (5.1)	47 (65.3)****
Plasma potassium (mmol/l)	4.1 [3.5–4.6]	3.8 [3.3–4.4]	3.2 [2.6–4.1]****
24-h urine potassium excretion (mmol/day)	48 [18–90]	59 [24–107]	59 [25–140]*
eGFR (ml/min per 1.73 m ²)	92.0 [63.8–118.1]	98.0 [59.0–138.0]	90.5 [51.3–154.0]
PRC (mU/l)	16.0 [5.0–37.4]	9.9 [2.0–33.2]	2.0 [2.0–7.9]****
IA PAC (pmol/l)	124 [42–374]	282 [125–481]	658 [286–1754]****
IA ARR (pmol/mU)	8.7 [3.1–20.9]	25.5 [7.9–87.7]	128.2 [47.7–353]****

The data shown are medians, percentiles [p5–p95] or (percentages).

ARR, aldosterone to renin ratio; eGFR, estimated glomerular filtration rate; IA, immunoassay; PAC, plasma aldosterone concentration; PRC, plasma renin concentration.

* $P < 0.05$.

** $P < 0.01$.

**** $P < 0.0001$.

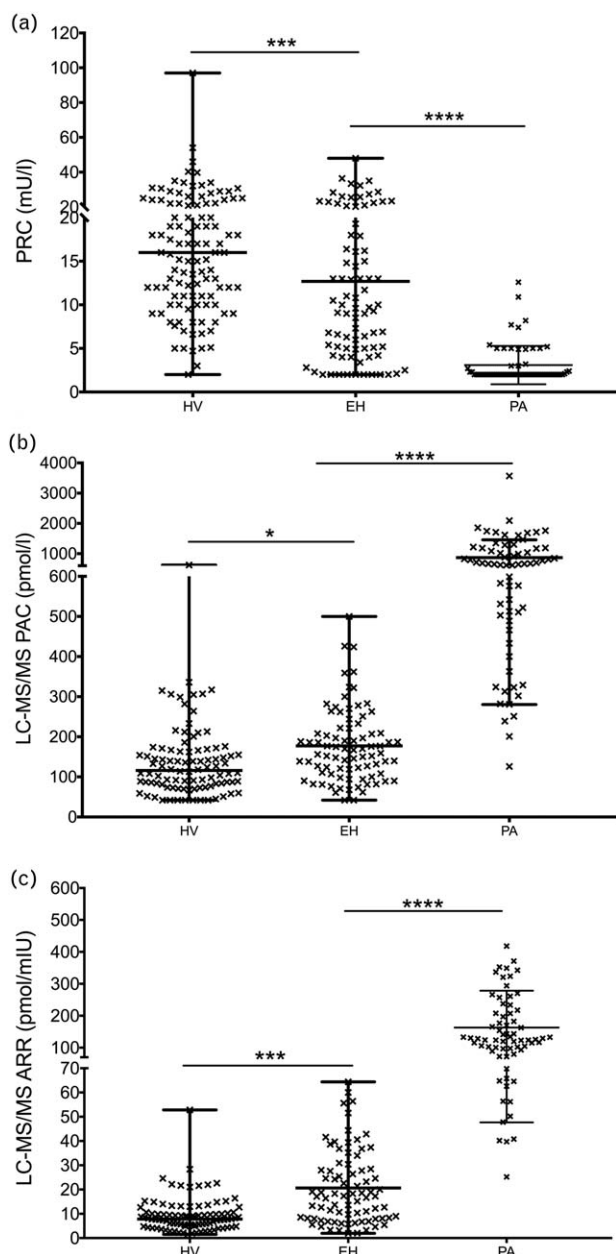


FIGURE 2 Plasma renin concentration (PRC) (a), LC-MS/MS plasma aldosterone concentration (PAC) (b) and LC-MS/MS aldosterone-to-renin ratio (ARR) (c) in healthy normotensive volunteers (HV), patients with essential hypertension (EH) and primary aldosteronism patients (PA) (median with 5th–95th percentile).

specificity to screen primary hyperaldosteronism with an 87.3% sensitivity.

When essential hypertensive patients were plotted against lateralized primary hyperaldosteronism patients ($n = 42$), the AUC was 0.9983 ($P < 0.0001$). An ARR cutoff value of 46 pmol/mU yielded a sensitivity of 100% (95% CI: 91.6–100) and a specificity of 93.9% (95% CI: 86.3–98.0) for primary hyperaldosteronism screening (Table 5). The Youden index for this cutoff value was 93.9, and the LR+ was 16.4. ARR value of 64.5 pmol/mU showed a 100% specificity to screen primary hyperaldosteronism with an 87.3% sensitivity. Using only lateralized primary hyperaldosteronism patients as reference for primary hyperaldosteronism, AUC reached 0.991 ($P < 0.0001$) with a 100% sensitivity and a 92.3% specificity.

Patients misclassifications

Using previously suggested cutoff values for PAC (360 pmol/l) and ARR (46 pmol/mU) for the diagnosis of primary hyperaldosteronism, four patients among the 159 patients were considered as misclassified using LC-MS/MS (Supplemental Table 2, <http://links.lww.com/HJH/A927>). Three of them had hypokalaemia at the time of this one sampling, and PRC and PAC would have need to be reassessed after potassium supplementation. Among these three patients, only one had a diagnosis of lateralized primary hyperaldosteronism. One patient was classified as primary hyperaldosteronism with LC-MS/MS, as the final diagnosis was essential hypertension.

Eleven other patients with a final diagnosis of primary hyperaldosteronism did not reach the cutoff values for primary hyperaldosteronism diagnosis either using LC-MS/MS PAC or immunoassay. Ten of them had hypokalaemia at the time of the assessment of PRC and PAC.

DISCUSSION

Primary hyperaldosteronism diagnosis is based on a first step screening that implies PRC and PAC determination to compute ARR. Few methods are available for PRC measurements, with an international standardization and few inter-assay discrepancies [16–19]. Thus, primary hyperaldosteronism screening mainly depends on PAC determination. PAC assays have to be accurate, precise and able to detect low levels of PAC, because excluding the diagnosis of primary hyperaldosteronism may require suppression tests. Numerous immunoassays (RIA or CLIA methods) are

TABLE 2. Performance of basal liquid chromatography–tandem mass spectrometry plasma aldosterone concentration cutoff values for primary aldosteronism screening: primary aldosteronism versus essential hypertension patients

LC-MS/MS PAC (pmol/l)	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	Youden index	LR+	LR–	PPV (%)	NPV (%)
> 125	100 [95.1–100]	31.7 [21.9–42.9]	31.7	1.5	0.00	14.0	100
> 202	97.3 [90.5–99.7]	69.5 [58.4–79.2]	66.8	3.2	0.04	26.2	99.6
> 257	94.5 [86.6–98.5]	80.5 [70.3–88.4]	75.0	4.8	0.07	35.0	99.2
> 308	90.4 [82.1–96.0]	91.5 [83.2–96.5]	81.9	10.6	0.11	54.1	98.8
> 362	82.2 [71.5–90.2]	95.1 [88.0–98.7]	77.3	16.8	0.19	65.2	98.0
> 425	79.5 [63.9–84.7]	97.6 [91.5–99.7]	77.0	32.6	0.21	78.3	97.7
> 502	75.3 [63.9–84.7]	100.0 [95.6–100]	75.3	0.25	100	97.3	97.3
> 559	67.1 [55.1–77.7]	100.0 [95.6–100]	67.1	0.33	100	96.5	96.5

Primary aldosteronism: $n = 82$; essential hypertensive patients: $n = 77$. CI, confidence interval; LR+, positive likelihood ratio; LR–, negative likelihood ratio; NPV, negative predictive value; PAC, plasma aldosterone concentration; PPV, positive predictive value.

TABLE 3. Performance of basal liquid chromatography–tandem mass spectrometry plasma aldosterone concentration cutoff values for primary aldosteronism screening: lateralized primary aldosteronism versus essential hypertensive patients

LC-MS/MS PAC (pmol/l)	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	Youden index	LR+	LR–	PPV (%)	NPV (%)
> 236	100 [91.6–100]	78.1 [67.5–86.4]	78.1	4.6	0.00	33.6	100
> 272	97.6 [87.4–99.9]	85.4 [75.8–92.2]	93.0	6.7	0.03	42.6	99.7
> 292	97.6 [87.4–99.9]	90.3 [81.7–95.7]	97.9	10.0	0.03	52.6	99.7
> 323	95.2 [83.8–99.4]	92.7 [84.8–97.3]	87.9	13.0	0.05	59.1	99.4
> 361	90.5 [77.4–97.3]	95.1 [88.0–98.7]	85.6	18.5	0.10	67.3	98.9
> 446	88.1 [74.4–96.0]	98.8 [93.4–100]	86.9	72.2	0.12	88.9	98.7
> 502	83.3 [66.7–93.0]	100.0 [95.6–100]	83.3		0.17	100	98.2
> 559	71.4 [55.4–84.3]	100.0 [95.6–100]	71.4		0.29	100	96.9

Lateralized primary aldosteronism: $n=42$; essential hypertensive patients: $n=77$.

CI, confidence interval; LR+, positive likelihood ratio; LR–, negative likelihood ratio; NPV, negative predictive value; PAC, plasma aldosterone concentration; PPV, positive predictive value.

TABLE 4. Performance of aldosterone-to-renin ratio cutoff values for primary aldosteronism screening: primary aldosteronism versus essential hypertension patients

LC-MS/MS ARR (pmol/mU)	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	Youden index	LR+	LR–	PPV (%)	NPV (%)
> 24.9	100.0 [94.9–100]	68.3 [57.1–78.1]	68.3	3.2	0.00	25.9	100
> 29.4	98.6 [92.4–99.9]	75.6 [70.3–88.4]	74.2	4.0	0.02	31.0	99.8
> 36.0	98.6 [92.4–99.9]	80.5 [70.3–88.4]	79.1	5.1	0.02	36.0	99.8
> 40.4	95.8 [88.1–99.1]	87.8 [78.7–94.0]	83.6	7.9	0.05	46.6	99.5
> 46.1	94.4 [86.2–98.4]	93.9 [86.3–98.0]	82.3	15.5	0.06	63.2	99.3
> 50.9	91.6 [82.5–96.8]	93.9 [86.3–98.0]	85.5	15.0	0.09	62.5	99.0
> 56.3	90.1 [80.7–95.9]	96.3 [89.7–99.2]	86.5	24.6	0.10	73.2	98.9
> 64.5	87.3 [77.3–94.0]	100 [95.6–100]	87.3		0.13	100	98.7
> 76.1	78.9 [67.6–87.7]	100 [95.6–100]	78.9		0.21	100	97.7
> 91.3	76.1 [64.5–85.4]	100 [95.6–100]	76.1		0.24	100	97.4

Primary aldosteronism: $n=82$; essential hypertension patients: $n=77$.

ARR, aldosterone-to-renin ratio; CI, confidence interval; LR+, positive likelihood ratio; LR–, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

currently available, but no internal standard is yet available. As shown in our study and those from other groups, immunoassays exhibited huge inter-assay discrepancies, with different reference ranges from one assay to another [22]. These discrepancies could be the consequence of the lack of specificity and cross-reactivities of most immunoassays with excessive aldosterone measurement as already underlined in several studies [45–47].

LC-MS/MS is the new gold standard method for PAC determination, as it is more specific than immunoassays with high reproducibility and accuracy of the measurements. As already shown in several studies, we found lower PAC with LC-MS/MS methods than with different immunoassays [23–25,47]. Moreover, our data showed that PAC measured with different LC-MS/MS methods exhibited few discrepancies and negligible bias [23]. These data suggested that inter-laboratory variability of PAC should decrease, as

more and more laboratories will implement LC-MS/MS method for PAC determination. Moreover, regarding cost-effectiveness, our PAC LC-MS/MS method has an equivalent cost as fully automated immunoassays [48] and thus could not be responsible for a limitation of LC-MS/MS use. These methodological changes would imply a need to reassess guideline recommendation cutoff values.

The aim of this study was to define criteria for distinguishing between patients with primary hyperaldosteronism and essential hypertension. Various studies have tried to define cutoff values for primary hyperaldosteronism screening with each immunoassay [16,19,49], but none of them defined until now cutoff values for PAC measurement by LC-MS/MS. We studied a large cohort of fully phenotyped individuals including healthy volunteers, patients with essential hypertension and patients with primary hyperaldosteronism.

TABLE 5. Performance of aldosterone-to-renin ratio cutoff values for primary aldosteronism screening: lateralized primary aldosteronism versus essential hypertension patients

LC-MS/MS ARR (pmol/mU)	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	Youden index	LR+	LR–	PPV (%)	NPV (%)
> 46.1	100.0 [91.6–100]	93.4 [86.3–98.0]	93.9	16.4	0.00	64.6	100
> 49.7	97.6 [87.4–99.9]	93.9 [86.3–98.0]	91.5	16.0	0.03	64.0	99.7
> 56.0	97.6 [87.4–99.9]	96.3 [89.7–99.2]	94.0	26.7	0.03	74.8	99.7
> 61.3	97.6 [87.4–99.9]	98.8 [93.4–99.9]	96.4	80.0	0.02	89.9	99.7
> 64.6	95.2 [83.8–99.4]	100 [95.6–100]	95.2		0.05	100	99.5
> 81.5	88.1 [74.4–96.0]	100 [95.6–100]	88.1		0.10	100	99.0
> 101.4	80.9 [65.9–91.4]	100 [95.6–100]	80.9		0.19	100.0	97.9

Lateralized primary aldosteronism: $n=42$; essential hypertension patients: $n=77$.

ARR, aldosterone-to-renin ratio; CI, confidence interval; LR+, positive likelihood ratio; LR–, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

In healthy volunteers, the reference interval for PAC determined between 0800 and 0900 h after 30 min of rest in a seated position was 42–309 pmol/l. Only one previous study has defined the reference interval for PAC (51–618 pmol/l) in a group of 35 healthy volunteers after 10 min in a seated position [23] exhibiting an important inter-individual variation. In this study, PAC was measured after 30 min in a seated position. Indeed, several studies suggest that PRC and PAC are subject to significant changes during the first 30 min after turning into seated position. Sympathetic system and beta-adrenergic receptors are differently stimulated leading to distinct kinetics of variations between renin and aldosterone during those first 30 min [50,51]. Zoccali *et al.* even showed a slight increase in norepinephrine secretion 15 min after turning into supine position [52]. Moreover, Shirreffs and Maughan [53] also showed significant changes in serum potassium concentration and blood volume during the first 15 min in the supine position. Sancho *et al.* [54] (in adults) and Fukushima *et al.* [55] (in children) had observed important changes in plasma renin activity and PAC during the 30 min following the change in position, whereas PRA and PAC remained steady thereafter. Overall, those data suggest that a 30-min lag-time is needed for PRA and PAC to reach a steady level after a change in position. Accordingly, several authors have suggested to compute ARR after a 30-min rest [56,57]. Japanese guidelines recommended to privilege a 30-min rest when ARR is to be computed in a specialized centre [58].

In essential hypertension patients, the upper 95th percentile of PAC after 30 min-rest in a seated position was 360 pmol/l. We suggest to use this value as cutoff to distinguish between essential hypertensive and primary hyperaldosteronism patients. The 2016 Endocrine Society guidelines suggest that the combination of a PAC above 550 pmol/l (based on immunoassays data) and a suppressed PRC (<2 mU/l) is sufficient for primary hyperaldosteronism diagnosis with no need for further confirmatory tests [7]. This decrease in PAC cutoff value for primary hyperaldosteronism screening mostly depends on the use of LC-MS/MS.

ARR is recommended for primary hyperaldosteronism screening [7,11,13,59], even though some studies showed that ARR could be questionable [60–62]. Our LC-MS/MS ARR AUC are better than those reported in recent studies using immunoassays for PAC [16,49] and similar to that from Burrello *et al.* [15]. Various ARR cutoff values have been reported for different methods for determining aldosterone concentration. Current guidelines suggest the use of ‘the most commonly adopted cutoff values’ based on immunoassays PAC [7]. Moreover, ARR is not perfectly standardized, as values vary with techniques and kits for PAC determination [10]. Most of the previously proposed ARR cutoff values would lead to miss primary hyperaldosteronism diagnosis [7,15,16] and lead to poor diagnostic performance when PAC is determined with LC-MS/MS method.

When PAC is measured by LC-MS/MS, we suggest an ARR cutoff value of 46 pmol/mU for distinguishing between patients with primary hyperaldosteronism and those with essential hypertension. This cutoff determined after 30 min in a seated position yielded a 100% sensitivity for the screening of lateralized primary hyperaldosteronism

patients and a 93.4% specificity. Indeed, lateralized aldosterone secretion primary hyperaldosteronism patients can be the surgically biochemically curable, which confirmed retrospectively primary hyperaldosteronism diagnosis [37].

In this study, LC-MS/MS based cutoff values lead to some discrepancies between LC-MS/MS based conclusion and final diagnosis with this one-point hormonal assessment.

Most of these LC-MS/MS false negatives were performed while patients had hypokalaemia. Indeed, for these patients, primary hyperaldosteronism diagnosis were not made on this samples but on posterior one for whom potassium supplementation was sufficiently increased to maintain serum potassium in the normal range. In our cohort, 50% of primary hyperaldosteronism patients had potassium concentration below 3.3 mmol/l at the time of venipuncture despite potassium supplementation. PAC and ARR ROC curve analysis were reassessed with only normokalaemic patients (Supplementary Tables 3 and 4, <http://links.lww.com/HJH/A927>). When plasma potassium level was normal, the sensitivity of ARR was slightly better (97.4 versus 93.4% overall, Supplemental Table 4, <http://links.lww.com/HJH/A927>). Moreover, hypokalaemic patients had a slight increase in ARR when compared with normokalaemic ones (Supplemental figure 1, <http://links.lww.com/HJH/A927>). As recommended in guidelines, primary hyperaldosteronism diagnosis has to be reassessed after correction of plasma potassium concentration (when possible) if the diagnosis cutoffs are not met when the patient has hypokalaemia [7].

This study also highlighted few discrepancies between immunoassay and LC-MS/MS with one-point hormonal assessment. Indeed, even if LC-MS/MS PAC determination improves accuracy and selectivity of the measurement, LC-MS/MS cannot suppress the fluctuations of the secretion and the impact of the environmental conditions such as hypokalaemia. As recommended in guidelines, in such cases, ARR computation has to be repeated in cases of suspected false-negative ARR [7].

To conclude, our study shows that the use of LC-MS/MS PAC instead of previous PAC immunoassays improves PAC measurement analytical performances (accuracy and selectivity), decreases inter-assay discrepancies, shows lower PAC values than those obtained with many immunoassays, and maintain the sensitivity of primary hyperaldosteronism screening with a significant improvement in specificity when appropriate cutoff values determined after 30 min of seated rest are used (we suggest 46 pmol/mU for ARR and 360 pmol/l PAC).

Obviously, further studies especially prospective ones are required to confirm our findings and to validate these cutoffs before they can be widely used. Furthermore, cutoff values for confirmatory tests should probably also be reassessed regarding the increasing importance of LC-MS/MS for PAC determination.

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Conflicts of interest

There are no conflicts of interest

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