



ELSEVIER

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: [www.elsevier.com/locate/clinbiochem](http://www.elsevier.com/locate/clinbiochem)

# Analytical validation and investigation on reference intervals of aldosterone and renin in Chinese Han population by using fully automated chemiluminescence immunoassays

Lingyan Deng<sup>a,1</sup>, Zhigang Xiong<sup>a,1</sup>, Huijun Li<sup>a</sup>, Xiaomei Lei<sup>b</sup>, Liming Cheng<sup>a,\*</sup>

<sup>a</sup> Department of Laboratory Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Jiefang Ave., 1095 Wuhan, Hubei, PR China

<sup>b</sup> Department of Physical Examination Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, PR China

## ARTICLE INFO

### Keywords:

Plasma direct renin concentration  
Aldosterone  
Primary aldosteronism  
Plasma renin activity  
Reference intervals  
Aldosterone renin ratio

## ABSTRACT

**Background:** The aldosterone/renin ratio (ARR) is recommended to screen for primary aldosteronism (PA) in hypertension. We estimated fully automated chemiluminescence immunoassays (CLIA) for plasma aldosterone concentrations (PAC) and plasma direct renin concentrations (PRC) and investigated their reference intervals in Chinese Han population.

**Methods:** PAC and PRC were measured on a fully automated analyzer (LIAISON XL, DiaSorin, Italy). Performance characteristics were estimated according to CLSI approved guidelines. 328 healthy individuals were selected for reference intervals investigation. Results simultaneously tested by CLIA and radioimmunoassays were reviewed from 123 patients with hypertension and/or adrenal space-occupying lesion. PAC/PRC ratio (ARR<sub>prc</sub>) was compared to PAC/plasma renin activity (PRA) ratio (ARR<sub>pra</sub>).

**Results:** Within-laboratory imprecision was 5.6%–6.7% for PAC and 3.0%–3.3% for PRC. The LoQ was 72.2 pmol/L for PAC and 1.27 mIU/L for PRC. Linearity was excellent in the range of concentrations between 94 and 2708 pmol/L for PAC and 1.3–461.8 mIU/L for PRC. Interferences of hemoglobin, unconjugated bilirubin and lipaemia could be acceptable, but not of conjugated-bilirubin when renin and aldosterone at low concentrations. The central 95% reference intervals for males: PAC: 76–722 pmol/L, PRC: 3.3–92.7 mIU/L, ARR: 2.2–46.0 pmol/mIU; for females: PAC: 85–1010 pmol/L, PRC: 3.7–99.8 mIU/L, ARR: 3.6–68.4 pmol/mIU. Upper reference limits for ARR of younger and older men were lower than women. ARR<sub>prc</sub> and ARR<sub>pra</sub> showed almost perfect agreement ( $\kappa = 0.815$ ) for screening PA.

**Conclusion:** The DiaSorin tests are valuable analytical options for PAC and PRC measurements. We recommend sex-specific and age-specific reference intervals of these items should be estimated.

## 1. Introduction

Primary aldosteronism (PA) characterized by elevated secreted aldosterone and suppressed renin secretion is the most frequent cause of secondary arterial hypertension, accounting for > 10% of patients with hypertension. Considering associated cardiovascular risks and the effectiveness of treatment with the correct diagnosis, it is very important to detect the presence of PA [1]. From the Endocrine Society guidelines [1], the plasma aldosterone/renin ratio (ARR) is strongly recommended to screen possible cases of PA in patients with hypertension at risk for

PA: ARR is elevated in PA, and the prediction accuracy depends on the reliability and sensitivity of the aldosterone and renin measurements, especially at low concentrations.

Traditionally, ARR were calculated as plasma aldosterone concentration (PAC)/plasma renin activity (PRA) ratio. Both aldosterone and renin were measured by radioimmunoassays (RIA), with renin being measured as enzymatic activity, based on the conversion of the substrate angiotensinogen to angiotensin I in plasma at 37 °C [2]. Due to the complexity of the renin activity assay, this assay is laborious and hard to be standardized. As the demand for screening patients suspected

**Abbreviations:** PA, primary aldosteronism; ARR, aldosterone/renin ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity; RIA, radioimmunoassays; ARR<sub>prc</sub>, aldosterone/plasma direct renin concentration ratio; ARR<sub>pra</sub>, aldosterone/plasma renin activity ratio; RT, room temperature; PRC, plasma direct renin concentration; CLIA, chemiluminescence immunoassays; LoB, Limit of Blank; LoD, Limit of detection; LoQ, Limit of quantification; CLSI, Clinical and Laboratory Standards Institute; S.D., standard deviation

\* Corresponding author.

E-mail address: [chengliming2015@163.com](mailto:chengliming2015@163.com) (L. Cheng).

<sup>1</sup> These authors contributed equally to the work

<https://doi.org/10.1016/j.clinbiochem.2018.04.016>

Received 31 January 2018; Received in revised form 16 April 2018; Accepted 17 April 2018

0009-9120/ © 2018 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.

of PA increases, the less time-consuming and higher throughput methods are preferable.

In recent years, activity assays have been replaced by measurements of plasma renin concentrations on high-throughput automated chemiluminescence platforms in clinical laboratories. Plasma direct renin concentration (PRC) has been compared to PRA in many laboratories, and good consistency of PAC/PRC ratio ( $ARR_{prc}$ ) and PAC/PRA ratio ( $ARR_{pra}$ ) was reported when used for PA screening [3–6].

The purpose of our study was to validate performance characteristics of PAC and PRC by using fully automated chemiluminescence immunoassays (CLIA) on the DiaSorin LIAISON XL platform. We also investigated reference intervals of PAC, PRC and ARR on Chinese Han population.

## 2. Methods and materials

### 2.1. Subjects and sample collection

328 healthy subjects from the Chinese Han population (174 females and 154 males) were selected for the determination of reference intervals; the inclusion criteria were: age  $\geq 18$ , BMI  $< 30 \text{ kg/m}^2$ , blood pressure  $< 140/90 \text{ mmHg}$ , normal liver and kidney function, normal electrolyte, normal glucose (fasting:  $4.11\text{--}6.05 \text{ mmol/L}$ ) and lipid metabolism (fasting: Triglycerides  $< 1.7 \text{ mmol/L}$ , Cholesterol  $< 5.18 \text{ mmol/L}$ , High-density lipoprotein cholesterol  $> 1.04 \text{ mmol/L}$ , low density lipoprotein cholesterol  $< 3.37 \text{ mmol/L}$ ), ultrasound tests of heart, liver, spleen, thyroid and kidney are normal, no family history of hypertension, not pregnant. Because there are many drugs affecting the ARR [7], taking any relevant medications were exclusion criteria. All subjects had unrestricted salt intake. Fasting venous blood samples were collected into  $K_2$ -EDTA tubes (Becton Dickinson, France) between 7:30 am and 10:30 am. Before venipuncture, all individuals had been out of bed for at least 2 h, and then seated for 5–15 min [1]. Samples were transported to laboratory, centrifuged ( $3000 \times g$  for 10 min) and separated at room temperature (RT) within 1 h, then immediately frozen and stored at  $-20^\circ\text{C}$  until tested.

For method comparison, we enrolled 123 hypertensive patients (57 females and 66 males, aged 18–80 years) who received the parallel experiments at Tongji Hospital, from August 2015 to November 2016. All patients had elevated blood pressure (systolic blood pressure  $> 140 \text{ mmHg}$ , and/or diastolic blood pressure  $> 90 \text{ mmHg}$ ). After setting final diagnosis, they were grouped into 2 clinical subgroups: 36 with primary aldosteronism (20 females and 16 males, aged 27–80 years), 87 without primary aldosteronism (37 females and 50 males, aged 18–69 years; 9 with pheochromocytoma, 8 with renovascular hypertension, 1 with reninoma, 3 with adrenal ganglioneuroma, 2 with adrenal myelolipoma, 5 with cushing syndrome, 1 with hyperthyroidism and 58 with essential hypertension). Exclusion criteria were the presence of heart failure, liver cirrhosis, past or present malignancies, renal parenchymal disease, and pregnancy.

$ARR_{pra}$  and  $ARR_{prc}$  were calculated using two different methods (CLIA and RIA) for every patient, and the cutoff values for PA screening were  $25.0 \text{ (ng/dL)/(ng/mL/h)}$  and the 97.5th percentile estimated in our study for a positive ARR. Patients with positive ARR in either of the screening tests underwent confirmatory testing. Case confirmation and subtype identification of PA were carried out according to the Endocrine Society Guidelines [1], as described before [8,9].

Secondary causes of hypertension other than PA were diagnosed based on computed tomography, laboratory analyzes of plasma metanephrines and norepinephrines, renal artery angiography, the histological findings of the affected adrenal gland, and overnight dexamethasone suppression testing and so on, as clinically indicated.

For all diagnostic procedures, antihypertensive medications known to affect the renin–aldosterone axis were withheld or switched to calcium channel blockers at least 2 weeks before any adrenal hormones were measured. Hypokalemia was corrected with oral potassium

supplementation.

This research was approved by the Institutional Review Board Approval of Tongji Hospital, Tongji Medical College, HUST (IRB ID: TJ-C 20160203).

### 2.2. Laboratory measurements

Measurements of PAC and PRC were performed on a fully automated chemiluminescent analyzer (LIAISON XL, DiaSorin, Italy) with dedicated reagents according to the manufacturer's instructions, which were described in details previously [5,10]. The DiaSorin PRC assay is calibrated to the WHO reference material (National Institute for Biological Standards and Control code 68/356). Samples were collected in  $K_2$ -EDTA tubes.

For method comparison, aldosterone and angiotensin I were measured by RIA using commercially available kits purchased from North Institute of Biological Technology (NIBT, Beijing, China), which are permitted by China Food and Drug Administration (CFDA) and widely used in China. For PAC measurement, intra- and inter-assay coefficients of variation (CV) were 8.2% and 9.7% at  $385 \text{ pmol/L}$  ( $13.9 \text{ ng/dL}$ ), 3.5% and 5.5% at  $1091 \text{ pmol/L}$  ( $39.4 \text{ ng/dL}$ ). PRA was calculated by subtracting angiotensin I measured at  $4^\circ\text{C}$  from that determined at  $37^\circ\text{C}$ , and the intra- and inter-assay coefficients of variation of angiotensin I were 8.91% and 14.38% at  $0.35 \text{ ng/mL}$ , 6.8% and 11.94% at  $4.8 \text{ ng/mL}$  (intra- and inter-assay coefficients of variation were below 10% and 15% from the manufacturer's instructions, respectively).

### 2.3. Performance characteristics

#### 2.3.1. Imprecision

Imprecision of PAC and PRC was verified in accordance with Clinical and Laboratory Standards Institute (CLSI) document EP05-A2 [11], on the LIAISON XL platform. Briefly, two plasma pools indicated two different concentration levels were prepared with fresh plasma for each biomarker, split to aliquots, kept at  $-70^\circ\text{C}$ , and then thawed immediately in the  $37^\circ\text{C}$  water until measurement. Samples were measured in duplicate, two runs per day, over a minimum of 20 days, generating 80 replicates for each plasma pool. During the imprecision verification, one lot of reagent was used by one operator for each biomarker respectively.

#### 2.3.2. Sensitivity

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were verified based on guidance from CLSI document EP17-A2 [12]. For the LoB verification, two diluents free of aldosterone and renin supplied by DiaSorin were tested four times per day for three days. Similarly, for LoD verification, two plasma samples with very low levels of aldosterone and additional two samples with very low levels of renin were tested four times per day for three days, respectively. For LoQ verification, two samples with very low levels of aldosterone and four samples with very low levels of renin were tested six to eight replicates per sample per day for three days. Mean values were calculated to yield the target values.

#### 2.3.3. Linearity

Linearity of the two assays was confirmed according to CLSI document EP6-A [13]. Series dilutions of different concentrations were prepared from two plasma samples, one with very low concentration and another with high concentration, which were mixed together with the specific ratios and measured in duplicate.

The mean observed values were also compared to the expected values. The observed value/expected value (OBS/EXP) was calculated for the intermediate dilutions, to provide the percent recovery, determined using the following formula: Recovery = (Mean observed value/Expected value)  $\times 100\%$ .

Download English Version:

<https://daneshyari.com/en/article/8316914>

Download Persian Version:

<https://daneshyari.com/article/8316914>

[Daneshyari.com](https://daneshyari.com)