Measurement of Immunoreactive Plasma Renin Concentration by an Automated Method and its Correlation with Plasma Renin Activity.

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ABSTRACT

It is debatable whether the results of immunoreactive renin concentration (RC) provide the same information as plasma renin activity (PRA). Additionally, it has been suggested that the determination of the aldosteronemia / PRA ratio (ARR) has a higher positive predictive value than isolated determinations of PRA and aldosterone (A) for screening of primary hyperaldosteronism (PHA). We designed an experimental and prospective study of 227 consecutive samples from ambulatory individuals, none of them with a definitive diagnosis of PHA. Our objective was to evaluate: 1) the correlation between results from an automated RC measurement method (LIAISON, DiaSorin) and PRA. (RIA, DiaSorin); 2) the presumptive diagnostic concordance (PDC) between the results of RC and PRA; 3) the correlation between the results of the A/CR ratio (ACR) and ARR. Results: There is a high significant correlation (p<0.0001) between results of PRA and RC, with a PDC = 83\% for regular and high PRA. However, PDC is low and not acceptable for low levels of PRA (<1.3 ng/mL/hr). The correlation between the results of A/RC ratio (ACR) and ARR is highly significant (p<0.0001). Conclusions: RC methodology would be useful for an initial study of a patient, in order to rule out PHA. For low RC levels, PRA should also be measured, because of its higher sensitivity for low Renin (R) concentrations. Larger population studies are required to confirm this preliminary data. Rev Argent Endocrinol Metab 50:71-77, 2013

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Key words: renin, aldosterone, aldosterone/renin index, methods, primary hyperaldosteronism.

INTRODUCTION

The Renin (R)-Angiotensin (Ang) -Aldosterone (A) system is a proteolytic cascade associated with a signal transduction system involved in the regulation of blood pressure and water and salt homeostasis.

In plasma, circulating renin is present in an active molecular form (renin proper) and in an inactive form of higher molecular weight, prorenin (proR). The relative proportions of renin and prorenin vary widely according to different pathophysiologic situations \textsuperscript{(1)}. Renin is a protease that catalyzes the cleavage of the N-terminal end of angiotensinogen (liver protein), forming the decapeptide angiotensin I (ANG I). The angiotensin converting enzyme (ACE), mainly secreted by pulmonary and renal endothelial cells, converts ANG I into angiotensin II (Ang II), which has vasoactive and antidiuretic properties and stimulates aldosterone secretion.

Aldosterone is synthesized in the glomerular area of the adrenal cortex. A regulates the fluid-electrolyte balance, favoring sodium reabsorption in the distal tubule and collecting duct and simultaneously increasing osmotic water reabsorption, which increases the extravascular space, systolic-diastolic blood pressure and secretion of potassium and hydrogen ions \textsuperscript{(2)}.

Primary aldosteronism (PA) is defined as a group of disorders in which aldosterone production is inappropriately high, relatively autonomous of the R-Ang system, in which aldosterone secretion is not suppressed by sodium loading. This disease is
strongly associated with hypertension, and it is considered as one of the major public health problems in developed countries, affecting near 1000 million people worldwide.

In recent decades, there has been a marked increase in the prevalence of PA, currently reaching 10%, and 20% in cases of resistant hypertension (3). PA is currently considered as the most common cause of endocrine hypertension. It has been demonstrated that many patients diagnosed with PA are normokalemic. Therefore, an adequate screening of this condition is of utmost importance. Detection of this condition has increased as a result of the implementation of a sensitive and useful laboratory method, i.e. aldosterone / plasma renin activity ratio (ARR), reported in 1981 by Hiramatsu et al. (4). The measurement of this ratio has been shown to have a higher positive predictive value than separate measurements of A and plasma renin activity (PRA) (5,6). As this ratio is highly denominator dependent, R assays should be sensitive enough to detect low activity values.

Measurement of PRA by radioimmunoassay (RIA) is the most widely used method for diagnostic and follow-up purposes. In the absence of a gold standard method for measuring R, PRA measurement, of great clinical usefulness, is used as the reference method. This is a labor-intensive, complex, and time-consuming method, and as it is operator-dependent, it is difficult to compare results obtained by different laboratories.

Even if the ARR ratio has been successfully used by many investigators, there is no agreed cut-off value to date, given its variability throughout different studies. The cutoff value has to be defined by each research team for their population depending on the laboratory technique used. In a study conducted in our country, an ARR of 36 has been established as cut-off value for normality, using the RIA method (Diasorin) to measure PRA and RIA, DPC to measure A (7).

An automated chemiluminescent method is currently available for quantification of immunoreactive rennin concentration (RC). This immunoassay is easier to implement in the clinical laboratory, has better reproducibility and requires lower processing times than the method used for PRA measurement. While PRA measures enzymatic activity, the RC method determines the mass of rennin present in the sample. As these immunoassays do not measure the same variable, only the Presumptive Diagnostic Concordance (PDC) of results can be evaluated. In other words, we can only determine if both methods lead to the same presumptive biochemical diagnosis.

In recent years, different research teams have conducted studies comparing RC vs. PRA. Some authors have begun to study the diagnostic efficiency of the A/RC ratio (ARC) for PA and to determine the cutoff value.

In a population of subjects from the City of Buenos Aires and the province of Buenos Aires attending Hospital Ramos Mejía, we decided to:

**OBJECTIVES**

1) Evaluate the correlation of results from an automated method to measure RC in relation to PRA results. 2) To determine the PDC between RC and PRA results. 3) To determine the correlation between ARC and ARR results.

**MATERIALS AND METHODS**

We conducted an experimental, prospective study in 227 consecutive samples from ambulatory subjects that attended our laboratory between 12-MAY-11 and 30-MAY-12, which included a wide range of PRA values. Parallel measurements of RC, PRA and A were performed in those samples. The sample was collected between 8 and 9 AM(9), after ambulation. The sample was obtained in 6% EDTA-K$_3$ tube that inhibits
conversion of Ang I into Ang II by chelating the cofactors necessary for ACE activity \(^{(9)}\). Centrifugation was performed within 10 minutes of sample collection to ensure the quality of measurement \(^{(10,11)}\). Plasma samples were aliquoted and immediately stored at -20°C until assayed. Each aliquot was thawed only once. Plasma A was measured by solid-phase competitive RIA (RIAzenco, Zentech). Measurement of PRA was performed by solid-phase RIA (DiaSorin) and RC measurement was performed by a chemiluminescent method under an automated platform (LIAISON), DiaSorin. The characteristics of each assay, according to the manufacturer's specifications, are detailed in Table I. In our laboratory, the interassay CV% was < 7.8 % for RC and < 17.4 % for PRA. Intra-assay CV% was < 6.2 % for RC and < 7.0% for PRA.

The method for measuring PRA consists of two steps: in the first step, Ang I is generated in vitro by the enzymatic action of R present in the patient's sample. In the second step, the Ang I generated is measured by a solid-phase RIA.

First, the plasma sample is mixed with maleate buffer (pH 6.0) - which optimizes the enzymatic reaction - and then phenylmethanesulfonyl fluoride (PMSF) is added to block conversion of Ang I into Ang II and avoid proteolytic degradation during generation of Ang I. The sample is subsequently divided into 2 aliquots: one is incubated for 90 minutes at 37°C to generate Ang I and the other is kept in an ice bath. Ang I is then quantified in both aliquots by a RIA including incubation at room temperature for 3 hours. PRA in each sample is calculated based on the difference between the values of Ang I generated at 37°C and the corresponding baseline (Ang I at 4°C).

Quantitative measurement of RC was performed by a sandwich chemiluminescence immunoassay that uses two anti-renin monoclonal antibodies: one bound to magnetic particles (solid phase) that recognized both R and proR and another monoclonal antibody specific for rennin that is linked to an isoluminol derivative that generates a measurable signal. According to the manufacturer's specification, this method is calibrated so that PRA = 1 mg/mL/h corresponds to 8 µIU/mL of RC.

The A/PRA (ARR) and A/RC (ARC) ratios were estimated.

Based on the lower limit of the manufacturer's reference range for PRA, the population studied \((n = 227)\) was divided into 2 subgroups according to the levels of PRA observed: GI \((n = 104)\): samples with low PRA (\(\leq 1.3 \text{ ng/mL/hour}\)) and GII \((n = 123)\): samples with PRA > 1.3 ng/mL/hour, i.e. normal and elevated.

None of the patients whose samples were compared with this study has a confirmatory diagnosis of PA yet. The RC and PRA methods measure different parameters but their results would evaluate the same clinical suspected diagnosis. For this reason, in order to be able to compare such results, we decided to use the presumptive diagnostic concordance (PDC) as a parameter, which is defined as the agreement between presumptive diagnoses from each biochemical finding according to each manufacturer's reference range. We calculated PDC as the percentages of samples that, based on their laboratory data, are equally classified within, below or above the reference range established by the manufacturer of the each of the two methods studied. We have used these reference ranges because, to date, no in-house references ranges are available at our laboratory for the methods studied.

**STATISTICS**

Paired data were analyzed using the Spearman’s correlation method (statistical software MedCalc, version 9.3).
TABLE I. Characteristics of assays according to the manufacturers’ specifications.

PRA: Plasma Renin Activity.
RC: Renin Concentration

<table>
<thead>
<tr>
<th>Method</th>
<th>PRA</th>
<th>RC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rationale</td>
<td>RIA</td>
<td>QLIA</td>
</tr>
<tr>
<td>Processing Time</td>
<td>12 h</td>
<td>1 h</td>
</tr>
<tr>
<td>Intra-assay CV%</td>
<td>&lt; 10%</td>
<td>&lt; 7%</td>
</tr>
<tr>
<td>Inter-assay CV%</td>
<td>&lt; 10%</td>
<td>&lt; 7%</td>
</tr>
<tr>
<td>Functional sensitivity</td>
<td>0.2 ng/tube</td>
<td>1.96 µIU/mL</td>
</tr>
<tr>
<td>Reference range (ambulation)</td>
<td>(1.3-3.3) ng/mL/h</td>
<td>(4.4-46.0) µIU/mL</td>
</tr>
</tbody>
</table>

RESULTS

Table II shows results of A, PRA, RC, ARR and ARC in the whole population and in each of the groups studied.

Fig. 1a, b, c shows a very good correlation between results of RC vs. PRA, in the whole population (n = 227, Spearman r = 0.936; p < 0.0001) and GI (n = 104, Spearman r = 0.791, p < 0.0001) as well as in GII (n = 123, Spearman r = 0.829, p < 0.0001). Likewise, a highly significant correlation was found between ARC and ARR (n = 227, r Spearman = 0.9000, p < 0.0001) (Fig. 2).

The results obtained (Table III) show a PDC of 83% in GII, but the PDC was 12% in GI, a group corresponding to samples with PRA ≤ 1.3 ng/mL/h.

TABLE II. Circulating levels of Aldosterone (A), Plasma Renin Activity (PRA), Renin Concentration (RC), A/PRA ratio (ARR), A/RC ratio (ARC).

A in pg/mL, PRA in ng/mL/h, RC in µIU/mL, ARR, ARC in the whole population and in subgroups: GI: samples with low PRA (≤ 1.3 ng/mL/hour); GII: samples with normal (1.3 to 3.3 ng/mL/hour) and high PRA (> 3.3 ng/mL/hour).

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>N</th>
<th>Median</th>
<th>Range</th>
<th>25th- 75th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI + GII</td>
<td>A</td>
<td>227</td>
<td>23.3</td>
<td>2.8-127.0</td>
<td>15.1-31.9</td>
</tr>
<tr>
<td></td>
<td>PRA</td>
<td>1.6</td>
<td>0.2-40.0</td>
<td>0.7-3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>21.7</td>
<td>0.9-361.8</td>
<td>10.2-44.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ARR</td>
<td>13.8</td>
<td>0.3-635.0</td>
<td>7.0-30.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ARC</td>
<td>1.0</td>
<td>0.02-50.8</td>
<td>0.5-1.8</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>A</td>
<td>104</td>
<td>19.6</td>
<td>2.8-127.0</td>
<td>13.7-27.1</td>
</tr>
<tr>
<td></td>
<td>PRA</td>
<td>0.6</td>
<td>0.2-1.3</td>
<td>0.3 - 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>9.8</td>
<td>0.9-26.5</td>
<td>5.3 - 14.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ARR</td>
<td>31.9</td>
<td>7.4-635.0</td>
<td>18.5 - 53.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ARC</td>
<td>1.8</td>
<td>0.4-50.8</td>
<td>1.1-3.2</td>
<td></td>
</tr>
<tr>
<td>GII</td>
<td>A</td>
<td>123</td>
<td>27.0</td>
<td>3.4-120.0</td>
<td>18.3-35.3</td>
</tr>
<tr>
<td></td>
<td>PRA</td>
<td>3.2</td>
<td>1.4-40.0</td>
<td>2.1-6.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>41.9</td>
<td>7.4-361.8</td>
<td>29.0-70.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ARR</td>
<td>8.2</td>
<td>0.3-31.7</td>
<td>3.7-12.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ARC</td>
<td>0.6</td>
<td>0.02-5.55</td>
<td>0.3-1.0</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1 Correlation between Renin Concentration (RC) and Plasma Renin Activity (PRA). Spearman correlation test.

Fig 1a. RC (µU/mL) vs. PRA (ng/mL/h) with data corresponding to the whole population. (n = 227), r = 0.936, p < 0.0001.

Fig. 1b. RC (µU/mL) vs. PRA (ng/mL/h) with data corresponding to Group I: samples with low PRA (< 1.3 ng/mL/hour), n = 104, r = 0.791; p < 0.0001.

Fig. 1c. RC (µU/mL) vs. PRA (ng/mL/h) with data corresponding to Group II: samples with normal (1.3 to 3.3 ng/mL/hour) and elevated PRA (3.3 ng/ml/hour), n = 123, r = 0.8929, p < 0.0001.

Referencias de la figura:
CR = RC
ARP = PRA
Figure 2. Aldosterone / Renin Concentration Ratio (ARC) vs. Aldosterone / Plasma Renin Activity ratio (ARR) in the whole population (n = 227), r = 0.900, p <0.0001. Spearman correlation test.

TABLE III. Presumptive Diagnostic Concordance (PDC) between biochemical results of Plasma Renin Activity (PRA) and Renin Concentration (RC) according to each manufacturer's reference values.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>PDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>G I</td>
<td>104</td>
<td>12%</td>
</tr>
<tr>
<td>G II</td>
<td>123</td>
<td>83%</td>
</tr>
</tbody>
</table>

DISCUSSION

In this study, we evaluated the correlation and PDC between two immunoassays: an automated assay for measuring RC and a manual assay for measuring PRA, in a group of samples with a wide range of PRA levels.

We found a highly significant correlation and a good PDC (83%) between results obtained with PRA and RC in samples with PRA > 1.3 ng/mL/h (G II). Therefore, both methods would be equally useful for this range of PRA. However, when analyzing samples with low PRA (G I), which are the concentrations usually found in PA, we found a low PDC (12%) despite the highly significant correlation.

Other authors have compared PRA and CR (IRMA), showing a good correlation (12,13), although such correlations were also concentration-dependent, with difficulties in analytical concordance when concentrations were low(14). Our results agree with those findings and confirm the limitation of the method that measures RC for the diagnosis of PA.

In the absence of a gold standard method for measuring R, PRA measurement, of great clinical usefulness, is used as the reference method. As an advantage of this method we should highlight the capacity for a wider range of measurement, changing
the times of Ang I generation for interpolation in the RIA calibration curve in better zones of linearity according to the higher or lower PRA found in the sample.

However, several limitations of this assay (it is labor-intensive, requires prolonged incubations, it is operator-dependent, difficult interlaboratory comparison and dependent on angiotensinogen concentration) have created a need for a new method such as measurement of renin mass concentrations.

The methods used for measuring RC and PRA greatly differ. PRA measures the enzymatic activity of R, while the RC immunoassay measures renin mass or concentration, therefore detecting R immunoreactivity. Results are expressed in different units in the two methods: PRA is expressed as ng of Ang I generated in vitro per milliliter and per hour, expressing the enzymatic activity of renin, while RC is expressed in mass per volume units (microU/mL). Therefore, results of these two methods can only be compared in relation to their PDC.

RC measurement may be performed using an automated method; it has higher intra- and interlaboratory reproducibility and is applicable to routine lab testing. RC results are standardized against the WHO International Standard 68/356 (NIBSC), which ensures traceability, allowing comparison among various laboratories, while PRA measurements are not traceable to any standard \(^{13}\).

Both immunoassays are prone to overestimation of R because of cryoactivation of proR, which requires careful handling in the preanalytical stage.

In 2010, a guideline for A/R ratio measurement and management of patients with PA was published, which contains preanalytical and analytical considerations for the determination of the A/R ratio \(^{15}\).

To demonstrate how complex the current situation is, it is worth mentioning that an important research team \(^{16}\) has incorporated as from 2002, the measurement of RC (Liason; DiaSorín) as routing testing for PA. These same authors have recently reported \(^{17}\) false positive values in the A/R index in normal women evaluated during the luteal phase when using RC and this could account for the higher incidence of impaired A/R ratios in hypertensive women vs. men, which would lead to unnecessary repeat testing. For this reason, these authors suggest that PRA is preferable to RC for measuring the quotient, at least when evaluating women.

To sum up, in the studied population: 1) a highly significant correlation is observed between PRA and RC results. 2) Even if the PDC for PRA and RC is high, it is lower and unacceptable when R concentrations are low. 3) A highly significant correlation is observed between PRA and ARR results.

CONCLUSIONS

RC measurement would be useful for the initial study of the patient in order to rule out PA. If RC is low, PRA should also be measured, as it has a higher sensitivity for low values of R. Further population studies with a larger number of subjects, including patients with a clinical diagnosis of PA, are required to confirm our preliminary results.

Ethical considerations: we have taken into account the criteria approved by the ethics committee at Hospital Ramos Mejía GCBA, adhering to the guidelines for human subject research of the Declaration of Helsinki of 1975, revised in 2000. All patients signed an informed consent.
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