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RESEARCH ARTICLE

Two-site automated chemiluminescent assay for measurement of immunoreactive renin

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Abstract
Measurement of renin is important for the clinical assessment of hypertensive patients and for the screening for primary aldosteronism. The aim of this study was to evaluate the performances of an automated immunoassay for measurement of immunoreactive renin. Functional sensitivity, *in vitro* stability, and reference values were determined. Method comparison with the plasma renin activity assay was also performed. Our results demonstrate that the Liaison® direct renin assay may assist the clinician in the assessment of hypertensive patients and in the screening for primary aldosteronism.

Keywords: Renin, primary aldosteronism, hypertension, sensitivity

Introduction

Measurement of renin is important for the clinical assessment of hypertensive patients and for the screening for primary aldosteronism (PA) (Giacchetti et al. 2008; Campbell et al. 2009). The first case of confirmed PA was presented by Jerome Conn in 1955 and nowadays the prevalence of PA is about 10% among unselected hypertensive patients and reaches 20% among patients with resistant hypertension (Conn 1955; Funder et al. 2008). PA is associated with major adverse cardiovascular outcomes and thus the early diagnosis of PA is important to initiate the appropriate treatment and to improve the patients prognosis and quality of life (Sukor et al. 2010).

The aldosterone to renin ratio (ARR) is currently the most reliable marker used in the screening for PA (Funder et al. 2008; Gruson and Bodovitz 2010). This has led to a marked increase in the detection rate of PA (Mulatero et al. 2004; Olivieri et al. 2004). In addition, a continuous gradient of increasing risk of blood pressure across ARR values has been reported in non-hypertensive individuals and this ratio might therefore play a role in primary prevention (Newton-Cheh et al. 2007).

However, ARR is dependent on the reliability and sensitivity of the aldosterone and renin measurements (Campbell et al. 2009). The reference method for the determination of circulating renin levels remains the plasma renin activity (PRA) enzymatic assay. Nevertheless, this PRA assay is time consuming, labor intensive and lacks appropriate standardization. Assays for measurement of immunoreactive renin are now available and may help to overcome some of the limitations of PRA testing (Campbell et al. 2009; Morganti 2010).

The aim of this study was to evaluate the functional sensitivity of a fully automated chemiluminescent immunoassay for direct renin measurement, the *in vitro* stability of immunoreactive renin and to compare this automated assay with the reference PRA assay. Our study was also aimed at determining reference values for this assay as well as for the ARR to facilitate their application to daily clinical practices.

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Materials and methods

The functional sensitivity of the Liaison® direct renin assay (DiaSorin, Italy), a fully automated immunoassay with chemiluminescence based detection calibrated with the NIBSC 68/356 standard, was defined as the concentration of immunoreactive renin corresponding to a total coefficient of variation (CV) of 20%. Functional sensitivity was determined by performing daily duplicate measurements of five low concentration plasma pools over twelve days and the estimation of the CV was based on the individual measurements.

The *in vitro* stability of the measured immunoreactive renin was investigated using blood samples from 20 hypertensive patients. Blood was collected into potassium ethylenediaminetetraacetic acid (EDTA) tubes. After blood collection, the samples were delivered within 2 min to the laboratory through pneumatic tube system and were kept at room temperature for a time course of 6 h. Baseline samples were directly centrifuged and the time between blood collection and the beginning of the measurement of renin with the automated assay was less than 45 min. After 2, 4 and 6 h, the other tubes were then centrifuged and EDTA plasma tested for immunoreactive renin testing. Sample stability was established by comparing the baseline value of each plasma with values at subsequent time points. The decline of immunoreactive renin was averaged for the different time points after subdividing the results according to tertiles of baseline values of immunoreactive renin and was expressed in absolute values. We also investigated the impact of freezing and thawing on renin concentrations. Thus, ten plasma samples were analyzed on the day of blood collection and after two weeks of storage at –20°C.

The method comparison with the PRA assay was realized using plasma samples of 213 hypertensive patients followed at the Cliniques Universitaires St-Luc, Brussels, Belgium. Blood samples were drawn in potassium EDTA tubes in upright sitting position. Just after the blood collection tubes dedicated to PRA testing were placed in ice and tubes dedicated to immunoreactive renin testing were conserved at room temperature. PRA was measured in EDTA plasma with the DiaSorin RENCTK® assay.

Reference values for the Liaison® direct renin assay were determined with EDTA blood samples collected in 100 healthy, normotensive volunteers who were not taking any relevant medications. Aldosterone levels were determined with the Siemens coat-a-count® radioimmunoassay. The sensitivity of this aldosterone radioimmunoassay was 40 pM and the between-run coefficients of variation were 22%, 11% and 8% for aldosterone levels of 175 pM, 270 pM, and 360 pM, respectively.

Statistical analyses, were performed using the MedCalc (Medcalc Software, Belgium). The institutional review board approved the study protocol and all patients gave informed consent.

Results

The immunoreactive renin concentrations of the five pools used for the determination of the functional sensitivity of the Liaison® direct renin assay were 1.2, 1.6, 2.8, 3.7 and 4.6 μIU/mL, respectively. According to the working range of the Liaison assay, extending from 0.53 to 500 μIU/mL, these five pools can be considered as containing very low renin levels. The coefficients of variation for these pools were 28.9, 25.2, 18.4, 12.2 and 10.5%, respectively. The calculated functional sensitivity, defined as the renin concentration at which the CV was 20%, was 2.4 μIU/mL.

The mean renin concentrations of the samples used for the assessment of the *in vitro* stability at room temperature and divided in tertiles were 36 μIU/mL (range: 28–44), 74 μIU/mL (range: 65–84) and 108 μIU/mL (range 97–122). For all group, the decrease of immunoreactivity was not significant after 4 h of storage at room temperature (ANOVA $p > 0.05$) and was significantly higher (ANOVA $p < 0.05$) after 6 h of storage at room temperature for the highest tertile of renin concentrations (Figure 1). The mean renin concentration of the samples used for the assessment of the *in vitro* stability at –20°C were 45 μIU/mL (range: 36–59) and 3.7 ng/mL/h (range: 2.8–4.5) for automated assay and PRA, respectively. After 2 week of storage, the mean renin concentrations for automated assay and PRA were 42 μIU/mL and 3.5 ng/mL/h and these values were not significantly different from baseline.

When comparing measurements of PRA and direct immunoreactive renin, the mean PRA concentration was 2.7 ng/mL/h (range: 0.2–35.9 ng/mL/h) and the mean concentration of immunoreactive renin obtained with the Liaison® assay was 93.1 μUI/mL (range: 0.6–500.0 μUI/mL). An overall significant correlation was obtained between the two assays ($r = 0.92$; $p < 0.001$). The overall inter-rater agreement coefficient (kappa coefficient) between the two assays was 0.87, indicating a very good agreement between the two assays. According to tertiles of baseline PRA values, the correlation coefficients between the two assays were 0.63 ($p < 0.001$), 0.61 ($p < 0.001$) and 0.89 ($p < 0.001$) for PRA concentrations ranging from 0.20 to 0.71, 0.71 to 1.79 and 1.80 to 35.90 ng/mL/h, respectively (Figure 2A–C). The agreement between the methods was better for the samples with the highest renin concentrations.

The 95% confidence intervals reference intervals calculated with the healthy volunteers were 4–56 μIU/mL for immunoreactive renin and 6–64 pM/μIU/mL for the ARR (Figure 3). The range of values of plasma aldosterone was between 28 and 580 pM for healthy volunteers and 32 to 4515 pM for hypertensive patients. For ARR, the ranges were comprised between 1.5 and 79.4 pM/μIU/mL and between 0.2 and 901.7 pM/μIU/mL for normal subjects and hypertensive patients, respectively.

Discussion

Our study has provided valuable new information about the performances of the Liaison® direct renin automated assay. Thus, we have observed for this assay a functional

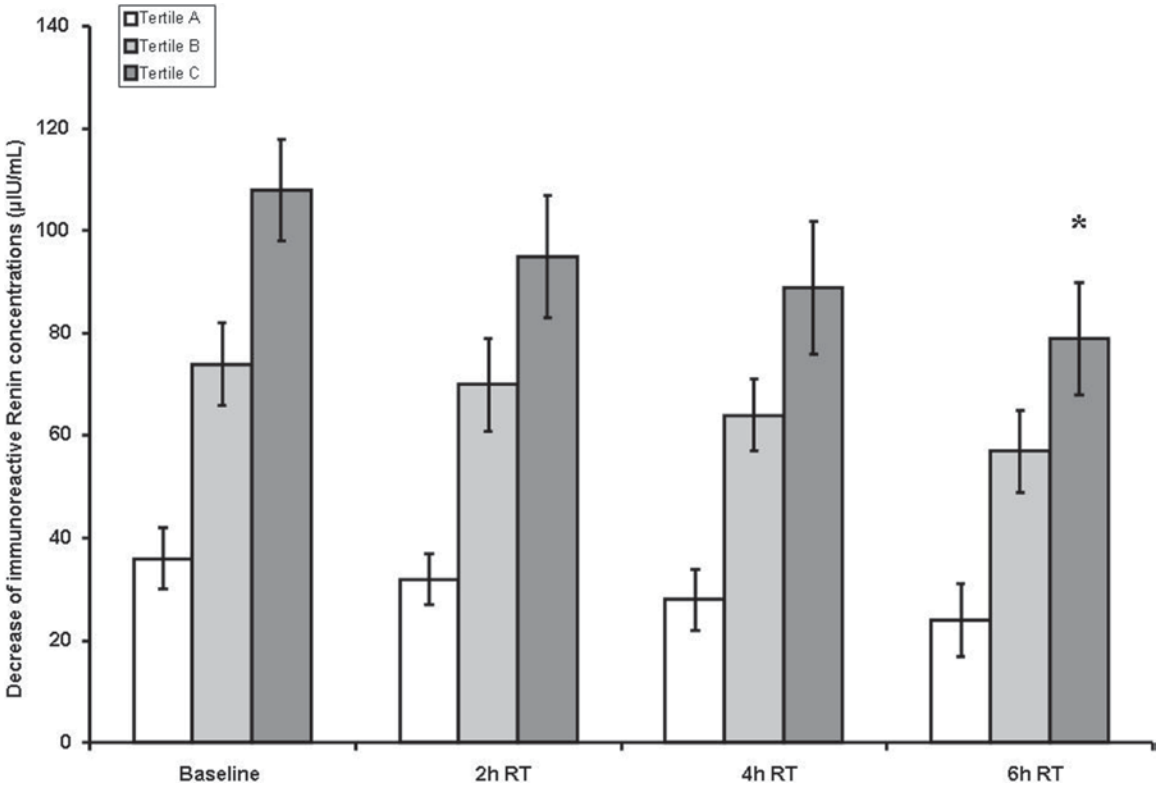

Figure 1. *In vitro* stability of renin concentrations presented as the decrease of renin immunoreactivity over a time course of 6 hours at room temperature (RT). Data were subdivided according to tertiles of baseline direct renin concentrations (tertile A, 28–44 µIU/mL; tertile B, 65–84 µIU/mL and tertile C, 97–122 µIU/mL). *$p < 0.05$ in comparison to baseline value.

sensitivity of 2.4 µIU/mL and an *in vitro* stability of renin immunoreactivity that lasted for at least 4 hours at room temperature. Our results have also demonstrated a good agreement between the immunoreactive renin assay and the PRA reference assay and also established the reference values for this immunoreactive renin automated assay as well as for the ARR ratio. Of note, the upper value of our reference interval for this ration is in agreement with the cut-off of 91 pM/µIU/mL proposed by recent Endocrine Society clinical practice guideline (Funder et al. 2008).

The aldosterone to renin ratio is a reliable screening tool for primary aldosteronism. However, the accuracy of this ARR ratio is strongly dependent of the quality of the renin assay (Campbell et al. 2009). As the PRA reference assay may have some limitations, automated measurement of renin may facilitate the measurement of renin in population-based studies and offers advantages with regard to processing and standardization (de Bruin et al. 2004; Hartman et al. 2004; Unger et al. 2004). Our study demonstrates an overall good agreement between PRA and immunoreactive renin testing over a broad spectrum of values, including low renin concentrations. However, according to tertiles of baseline renin values the relationship between the two methods is better for plasmas with high renin levels than for plasmas with low and intermediate renin. This has been already reported previously (de Bruin et al. 2004; Hartman et al. 2004).

A previously published multicentric study has demonstrated that the measurement of immunoreactive renin with a chemiluminescent automated method appears as a reliable alternative to PRA and that the inter- and intra-laboratory reproducibilities were improved (Morganti 2010). Indeed, the coefficients of variation of renin concentrations of the six plasma pools evaluated among the participating laboratories found for PRA were always higher than those of automated assay. These coefficients of variation were 59.4, 30.2, 36.0, 15.5, 20.0 and 21.8% with the PRA assay for mean concentrations of 0.14, 0.55, 1.3, 1.7, 3.2 and 18.9 (ngAI/mL per h) and were 41.0, 24.7, 18.1, 10.7, 11.5 and 10.7% with the chemiluminescent immunoreactive renin assay for concentrations of 4.2, 12.4, 29.8, 35.1, 50.5 and 436 µIU/mL).

Our results also demonstrate that the functional sensitivity of the Liaison® direct renin assay is compatible with the clinical practice guideline of the Endocrine Society for case detection, diagnosis and treatment of patients with PA, requiring that direct renin assays should accurately measure levels as low as 2 µIU/mL (Funder et al. 2008).

Our in vitro stability study indicates also that plasma samples should be sent to the laboratory within the first 6 h after blood collection, because a decrease of immunoreactivity occurs after this time point. Our results are contrasting with those obtained by Locsei et al., reporting a significant decrease of renin immunoreactivity when the tubes were kept 2 h at room temperature (Locsei et al. 2009). It can be hypothetized that denaturation, reduction

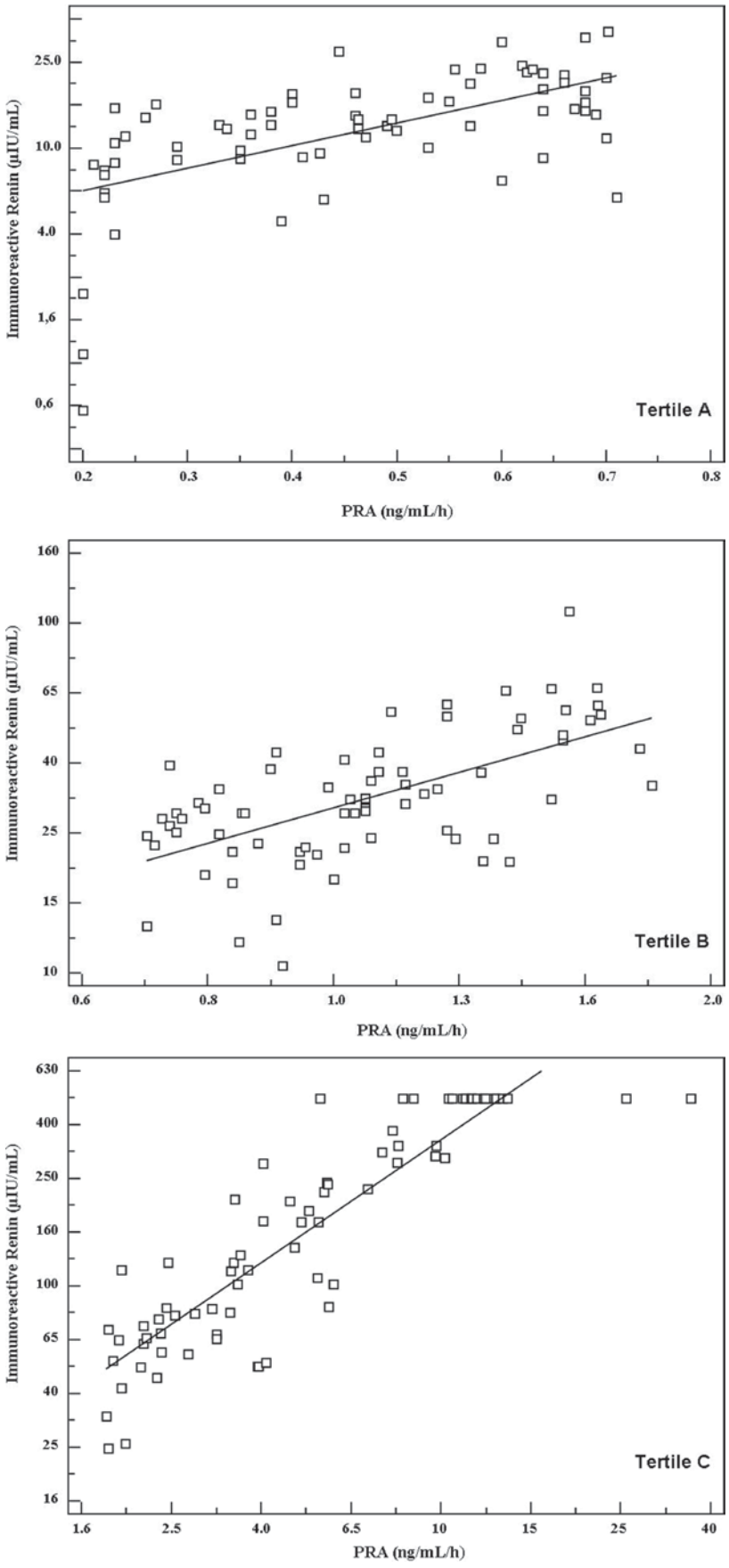

Figure 2. Method comparison between the Liaison® direct renin assay and the plasma renin activity (PRA) reference assay. Data are subdivided according to tertiles of baseline PRA concentrations (A, 0.20–0.71 ng/mL/h; B, 0.71–1.79 ng/mL/h and C, 1.80–35.90 ng/mL/h).

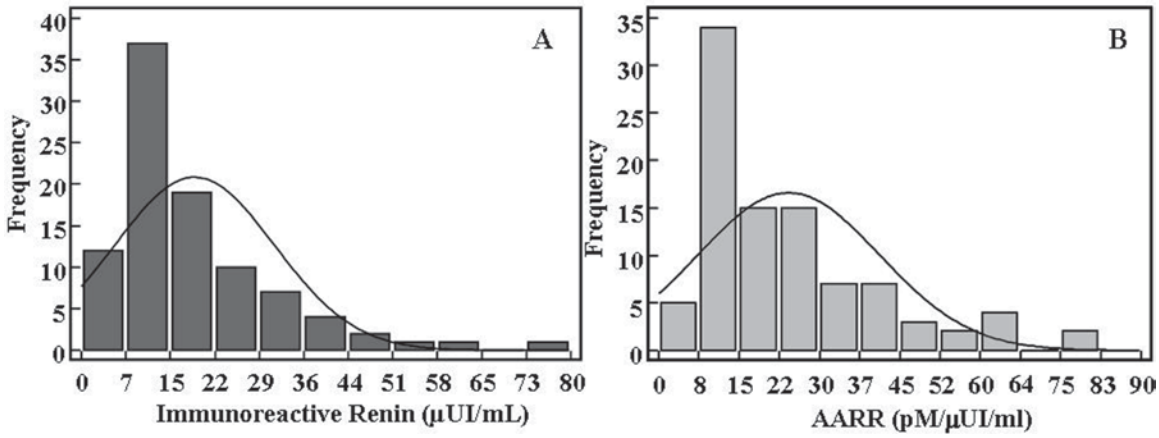

Figure 3. Reference intervals for immunoreactive renin automated assay (A) and for the ratio between aldosterone and immunoreactive renin, AARR (B).

and oxidation of renin but also change in the conformation of the enzyme may decrease the renin concentrations after prolonged exposure to room temperature. In contrast, when the samples were stored at −20°C for two weeks we did not observed any significant reduction of renin concentrations which is in agreement with the previous results of Locsei et al. (Locsei et al. 2009) and compatible with batch analysis in laboratories.

Although helpful in clinical practice, the use of direct renin assays may have some pitfalls and physicians should remain aware that a treatment with the new renin inhibitors such as Aliskiren may interfere with renin measurements, increasing direct renin concentrations while decreasing plasma renin activity (Campbell 2008; Nussberger et al. 2007). Many other drugs may also affect renin secretion, leading to false positive or negative results, and should also be avoided during the measurement of renin levels, particularly when investigating for PA (Funder et al. 2008).

In summary, the Liaison® direct renin assay appears as an easy-to-use, accurate and high throughput assay that may assist the clinical assessment of hypertensive patients and for the screening for primary aldosteronism.

Declaration of interest

The authors report no declarations of interest.

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