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Overview and Clinical Application Update of the IDS-iSYS Direct Renin and Aldosterone Immunoassays

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The Renin Angiotensin Aldosterone System (RAAS)

Conventional methods to assess the activity of RAAS

The measurement of plasma renin activity (PRA) has been used for half a century now as a substitute of angiotensin II (AII), the final effector hormone of the RAAS (Fig. 1). The very low concentrations of the octapeptide AII do not allow quantification with direct radioimmunoassay (RIA).

PRA exploits the ability of renin, an acid protease, to generate angiotensin I (AI) from its substrate, angiotensinogen, during an incubation carried out at 37°C and pH5.7-6.0. Depending on the duration of the incubation step (usually 1-3 hours) the amount of generated AI is large enough to be quantified by RIA no matter how low the plasma concentration of renin is.

The basic assumption for using PRA as a marker of RAAS activity "in vivo" is that the enzyme converting AI to AII, angiotensin converting enzyme (ACE), is never a limiting factor. Thus, generated AI mirrors the levels of circulating AII. Values of PRA, being an enzymatic assay, are expressed in ng AI/mL/hr.

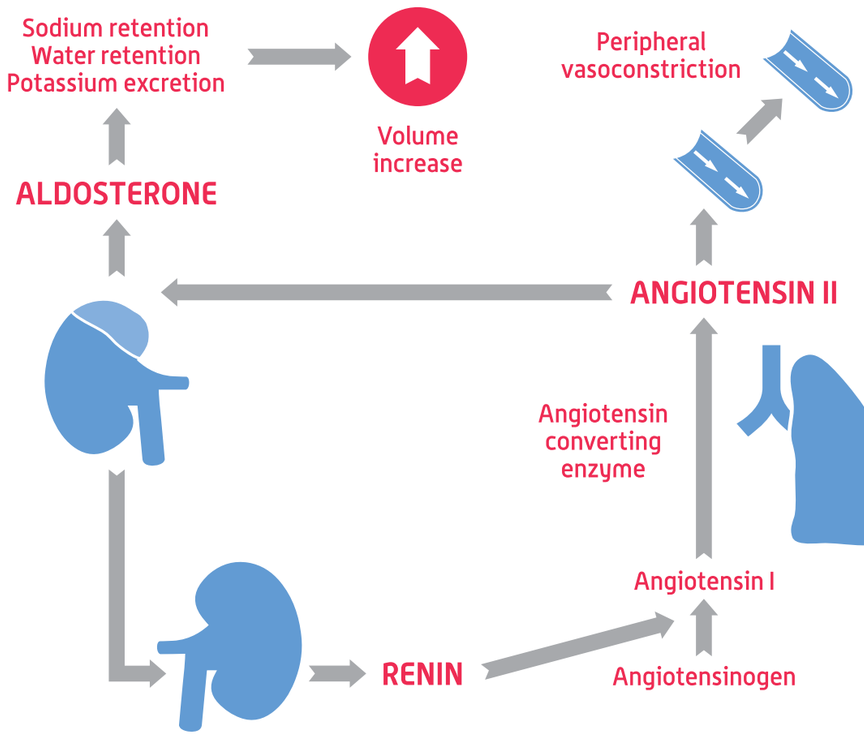

Figure 1. Key factors in the Renin Angiotensin Aldosterone System

PRA is very accurate in detecting low or very low levels of plasma renin[1]. However this assay is obviously complex, time consuming, operator dependent and, as a consequence, results are poorly reproducible between laboratories[2]. In addition, the RIA step requires radioactive material which comes with obvious disadvantages for the end user.

Plasma aldosterone concentration (PAC) is also measurable with RIA, the results being expressed in ng/dL or pmol/L (conversion factor 27.9). However, like PRA, the reproducibility among laboratories is poor due to differences in specificity of aldosterone antibodies, preparation and traceability of reference standards and cross reactivity with soluble aldosterone metabolites[3].

The new direct chemiluminescence immunoassays (CLIA) for renin and aldosterone

The availability of highly specific monoclonal antibodies against renin and aldosterone molecules has made the direct quantification of both hormones possible, circumventing the cumbersome complexities of RIA.

In the CLIA for direct renin, 200 µl of plasma are incubated with a mixture of a biotinylated capture antibody and a second acridinium-conjugate tracer detection antibody against renin (Fig. 2a). In the assay for aldosterone an aldosterone-acridinium-conjugate tracer competes with native aldosterone for binding to a biotinylated anti-aldosterone antibody (Fig. 2b). Streptavidin magnetic particles are added in each assay to capture the immune complexes. Trigger solutions are then added to the cuvette to activate the chemiluminescent reaction whereby the light signal is directly proportional to the amount of renin and indirectly proportional to aldosterone present in the sample.

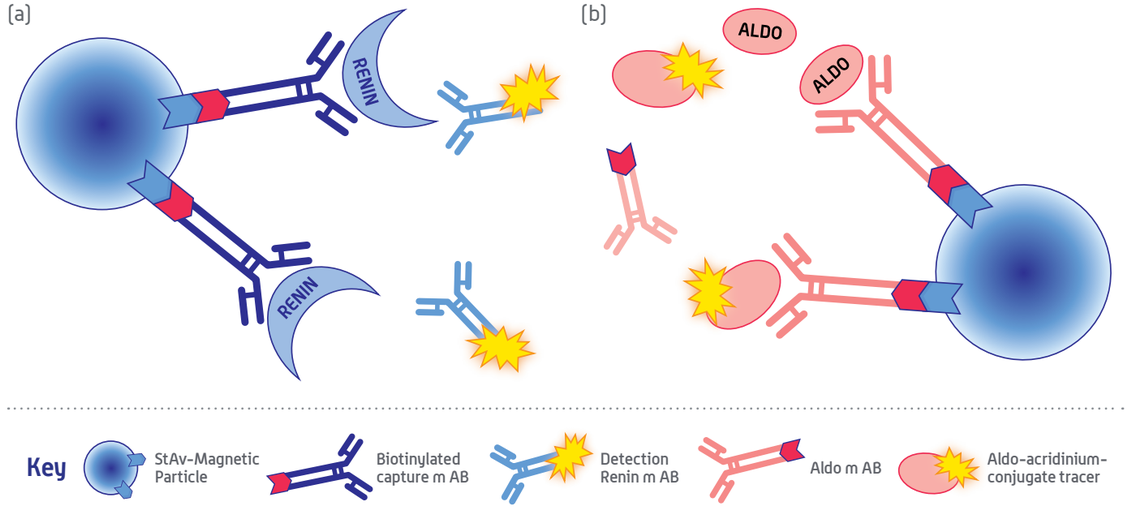

Figure 2. Schematic representation of the IDS-iSYS Direct Renin (a) and IDS-iSYS Aldosterone (b) assays

In both CLIAs International Standard Reference Preparations and gold-standard calibration methods are used. Values of plasma renin concentration (PRC) are expressed in µU /mL or pg/mL.

Both CLIAs have higher inter-laboratory reproducibility than conventional RIAs, do not require radioactive material and have the additional advantage of being performed in a fully automated analyzer platform that allows the simultaneous determination of hundreds of samples in less than one hour.

The technical features of renin and aldosterone CLIA are reported in the tables shown in the indicated reference[4].

Comparison of RIA and CLIA methods in human plasma samples

PRA and PAC measured with a commercial kit (DiaSorin, Saluggia, Italy) using conventional RIA methods were compared with the IDS CLIAs in a cohort of 393 hypertensive patients. Samples were collected after 10 minutes in a sitting position. Correlation studies showed a highly significant statistical R^2 value of 0.71 between PRA and PRC. In the low range the correlation was poorer, a finding observed also by other investigators[5] (Fig. 3a). Likewise the correlation between PAC measured with RIA and CLIA was statistically significant with an R^2 value of 0.72 (Fig. 3b). As for renin the dispersion of data was greater for low values.

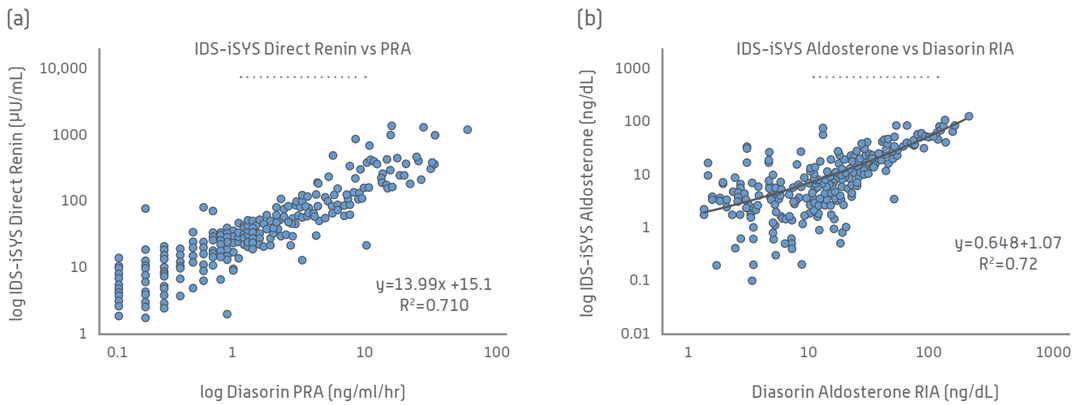

Figure 3. Correlation of Direct Renin to PRA (a), and Aldosterone CLIA to RIA shown in panel (b)

Clinical Application

When measuring renin and aldosterone in hypertensive patients

The measurements of renin and aldosterone are mandatory for the diagnosis of the two most frequent forms of secondary hypertension i.e. primary aldosteronism (PA) and renovascular hypertension (RVH).

Renin and aldosterone should also be evaluated in less frequent cases of licorice and contraceptive (pill) hypertension and in patients with resistant hypertension. In addition evaluation of the RAAS is useful for selecting the most appropriate antihypertensive treatment for individual patients[6-8].

Primary aldosteronism

Among hypertensive patients referred to hypertension centers the prevalence of PA is estimated in the order of 10%, with 40% of these cases being due to an aldosterone producing adenoma (APA) and 60% to bilateral adrenal hyperplasia (BAH)[9].

Due to the aldosterone mediated volume expansion and ensuing elevation of blood pressure, renin secretion is suppressed. Thus the combination of high plasma aldosterone with low renin is recognized as the hallmark of PA, often but not always associated with low plasma potassium.

Accordingly, the recent guidelines published by the Endocrine Society[10] have reinforced the recommendation of evaluating the aldosterone to renin ratio (ARR) in blood collected in the seated position in all groups of hypertensive patients with high prevalence of PA. ARR is calculated as the ratio between aldosterone, expressed in ng/dL, to renin expressed in µU/mL using the CLIA methods (or ng/mL/hr using PRA).

Caution must be applied in interpreting the ARR values since numerically they differ greatly depending on the units used to express renin and aldosterone concentration.

The Endocrine Society Guidelines for Primary aldosteronism recommend screening using the ARR in patients with:

- Moderate/severe hypertension
- Drug resistant hypertension
- Hypertensive patients with spontaneous or diuretic induced hypokalemia
- Hypertension with adrenal incidentaloma
- Hypertension with obstructive sleep apnea

Table 1. Hypertensive groups with a high prevalence of PA recommended for screening with the ARR

Many factors may lead to false-positive or false-negative ARR results[10] but the most relevant among the antihypertensive drugs are ß-blockers and anti-aldosterone medication that should be stopped before blood collection for ARR determination.

Effect of gender and postural changes on PRC, PAC and the ARR

Gender may affect renin and aldosterone levels due to a number of humoral factors. Therefore, we evaluated both parameters as well as the ARR in an unselected cohort of 106 hypertensive patients (37 male, 69 female). The median values of PRC measured in blood collected in sitting position were similar in males (10.7 µU/mL) and females (11.3 µU/mL) (Fig. 4a). The corresponding median values of PAC were also similar in the two genders (male 5.0 ng/dL, female 6.3 ng/dL; Fig. 4b). Consequently there was no significant difference in the median ARR value of the two groups [0.39 in males and 0.60 in females, Fig. 4c].

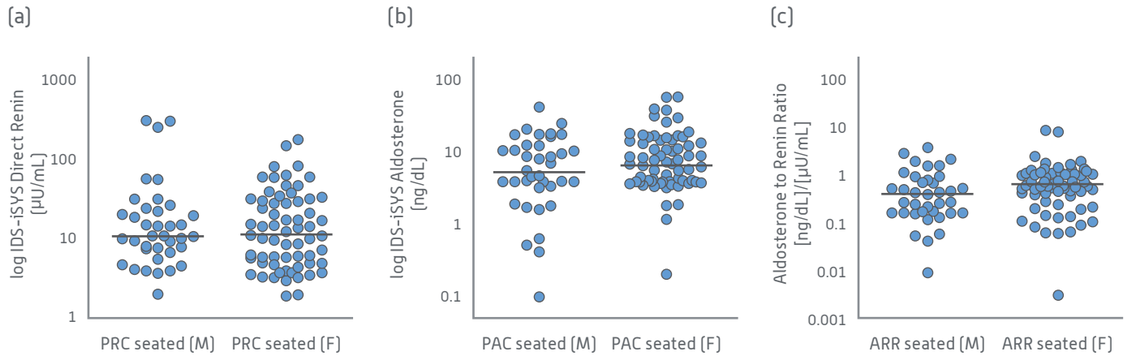

Figure 4. Plasma renin, aldosterone concentrations and ARR in male and female patients in a seated position

Postural changes are commonly used to assess the integrity of RAAS response to blood volume movement following the assumption of upright posture. Failure of renin and aldosterone to increase in response to standing is one of the confirmatory tests of PA.

In a sub cohort of 22 patients with essential hypertension (15 female) PRC and PAC responded physiologically to one hour of active standing increasing respectively from (mean, SEM) 21.4 ± 3.5 µU/mL to 36 ± 5.3 µU/mL (Fig. 5a) and from 10.8 ± 1.1 ng/dL to 23.7 ± 3.9 ng/dL (Fig. 5b). As a result of the concomitant increments of PRC and PAC values of ARR were similar in supine and standing positions (0.69 and 0.82 respectively) (Fig. 5c).

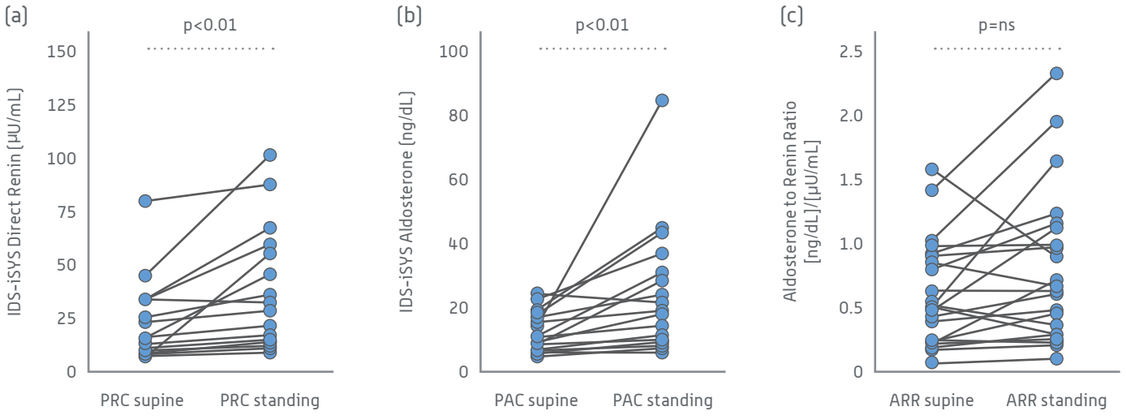

Figure 5. Plasma Renin and Aldosterone concentrations and ARR values in supine and standing position

ARR cut-off values for PA diagnosis

Values of PRC and PAC measured with the IDS CLIA and the calculated ARRs were evaluated in 93 patients with PA, in 152 with essential hypertension and in 147 healthy controls[4]. At a cut-off of 1.12(ng/dL)/(µU/mL) the sensitivity and specificity of ARR for detecting PA were respectively 98.9% and 78.95%. In patients with an APA, ARR values ranged from 1.1 to 52.1 with an average of 6.8, significantly greater than that observed in patients with a BAH (2.1, range 1.1-11.2; Fig. 6).

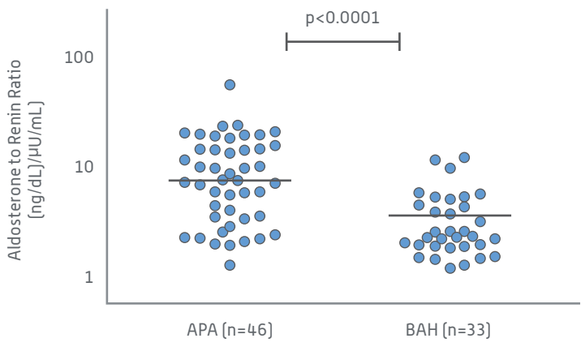

Figure 6. The aldosterone to renin ratio in primary aldosteronism patients with aldosterone producing adenoma (APA) and bilateral adrenal hyperplasia (BAH)

Confirmatory test for PA

Patients with a positive ARR should undergo confirmatory testing, the most popular being the saline infusion test (SIT) which evaluates the suppression of aldosterone in response to the i.v. infusion of 2 liters of saline over 4 hours. In non-PA patients PAC should decrease to values below 5ng/dL. Figure 7 shows that with the IDS CLIA in all 46 patients with previous diagnosis of PA, the SIT failed to suppress aldosterone whereas in all 73 non-PA patients PAC decreased below 5ng/dL.

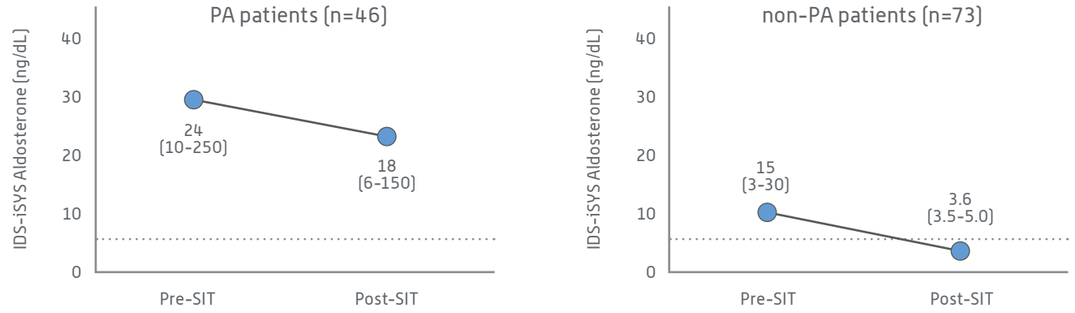

Figure 7. PACs during the saline infusion suppression test in primary aldosteronism (PA) and essential hypertensive (non-PA) patients

Conclusions

- Conventional RIA methods for measuring renin and aldosterone have a number of limitations that can be circumvented with the novel CLIAs
- CLIAs are fast, reliable, reproducible between laboratories and don't involve radioactive material
- PRC and PAC measured in hypertensive patients in the seated position with the new direct methods are similar in males and females. In addition, with the new assays the PRC and PAC response to the postural stimulus is similar to that described with conventional methods
- In a large cohort of hypertensive patients the determination of ARR with the new CLIA has allowed the diagnosis of PA with a high degree of sensitivity and specificity
- The lack of aldosterone suppression in response to volume expansion with saline infusion reliably confirms the diagnosis of PA in patients with high ARR

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