DIRECT RENIN TESTING

Analytical advantages

Clinical usefulness

Endocrine Society Guidelines for Primary Aldosteronism (PA)

Dr Margherita Banci
Key factors of the Renin-Angiotensin-Aldosterone System

- Angiotensin I
- Aldosterone
Relationships between Angiotension II levels, Direct Renin levels (IrR) and PRA

\[ n = 27 \]
\[ r = 0.93 \]

\[ n = 27 \]
\[ r = 0.79 \]

Morganti, 1990
Key factors of the Renin-Angiotensin-Aldosterone System

- Sodium Retention
- Water Retention
- Potassium Excretion

Aldosterone

- Volume Increase
- Peripheral Vasoconstriction

Angiotensin II

Angiotensin Converting Enzyme

Angiotensin I

Renin

Angiotensinogen
Factors Hampering the Effective Evaluation of the RAAS through *in vitro* assays

**CLINICALS**
- Poor understanding of the pathophysiology of the secondary forms of hypertension
- Multiple factors affecting the activity of the RAAS (posture, physical activity, sodium intake)
- Drugs interference

**ANALYTICALS**
- Angiotensin II is a very small peptide
- It circulates in minute concentrations (pg/mL)
- Its measurement requires plasma extraction
- Angiotensin II determination can be substituted with Renin assessment
- The PRA method to test Renin is definitely cumbersome to be performed
Laboratory point of view: PRA method

- Liver
  - Angiotensinogen
  - Prorenin
  - Renin
  - Inhibitor
  - Angiotensin I
  - Angiotensin II

- Kidney

- PRA
  - 2 runs
  - 37°C 1 hr
  - 4°C 1 hr
  - pH 5.7
  - Generated
  - Endogenous
  - Angiotensin I
  - RIA 1
  - RIA 2

PRA = Ag I (RIA 1) - Ag I (RIA 2) (ng/mL/h)
Limiting factors in the Renin Angiotensin I generation system

Renin

Angiotensinogen → Angiotensin I

Angiotensinogenases → Angiotensinogenases
Bacteria → Bacteria

Degradation fragments

Converting Enzyme

Angiotensin II

from Sealey and Laragh
PRA assays

Completely manual

\[ \text{PRA ng/mL/h} = \frac{\text{RIA generated} - \text{RIA endogenous}}{\text{incubation time (> 60' incubation)}} \]

\[ \text{Time to first result: + 300'} \]

2 analytical series

\[ \text{37°C} + \text{4°C} \]

\[ \text{pH 5.6} \]

inhibitor of the Angio I to Angio II transformation

generation (> 60' incubation)

RIA assay for generated Angio I

RIA assay for endogenous Angio I

RIA generated - RIA endogenous divided by incubation time
EDTA plasma sample
tracer (MoAb)
catcher (MoAb)
incubation
Wash
Measuring the bound fraction

Direct Renin assays
RIA or fully automated*

*Fully automated
Time to first result: 40’
Throughput: 170 tests/hour
PRA measurement issues

Performance of the enzymatic assay for renin (PRA) depends on

✓ pH 5.7 (to enhance AI production)
✓ Incubation time for generating AI
✓ Level of substrate (angiotensinogen)
✓ Inhibitor added to prevent AI to be transformed to AII
✓ Manual ability of the operator

PRA is calculated through a mathematical expression

✓ Difference between AI produced in plasma incubated at 37°C and at 4°C for a fixed time
PRA measurement issues

Under these circumstances the PRA analytical method shows:

✓ Poor standardization among Labs
✓ High variability due to the complicated analytical steps

In addition to this:

✓ No International Standard is available
✓ In vitro conditions used in PRA methods to optimize AI generation (pH around 6) are definitely not physiological
✓ Clinicians claim PRA levels are poorly accurate among different Labs and interchangeability of results at Congresses is limited
Direct Renin Assays

Levels of Plasmatic Renin Concentration tested with the Direct Renin assay are:

- Indipendent by pH, by time of AI generation, by substrate level, no inhibitor is needed
- Obtained with a completely automated procedure on the LIAISON instrument, with short incubation time
- Calculated through the working curve calculated on the master curve
- Referenced to the WHO IS 68/356
- Interchangeable as reproducibility among different centre is maximized
Performance of the enzymatic assay for renin (PRA) depends on

- pH 5.7 (to enhance AI production)
- Incubation time for generating AI
- Level of substrate (angiotensinogen)
- Inhibitor added to prevent AI to be transformed to AII
- Manual ability of the operator

PRA is calculated through a mathematical expression

- Difference between AI produced in plasma incubated at 37°C and at 4°C for a fixed time
Values of Direct Renin (IrR) and PRA in 8 Pool Samples Measured in 8 Labs

IrR (pg/ml) measured with the Pasteur IRMA assay

Morganti et al. 1990
PRA and Direct Renin reproducibility
CV interLabs

A. Morganti et al. 2009, going to be submitted to CiChem
Correlations of PRA and Direct Renin levels

The switch between concentrations of Renin tested with the PRA assay to Direct Renin levels deserves a clarification

- it is to be considered ONLY from the Clinical point of view

- NO FACTOR is to be used to calculate levels of PRA representing an activity from levels of Direct renin representing a concentration and viceversa

- Each PRA assay show different slope versus the respective PRC levels estimated with Direct Renin assays
PRA vs LIAISON® Direct Renin

X axis
REN CTK levels
Y axis
LIAISON® Direct Renin levels

Prof Ketelsleger, UCL, Bruxelles, 2007
Prof Morganti, University of Milan, 2007

Lonati C et al., ESH Meeting, Milan, June 2007

y = 2.7 + 26.1x

r = 0.89   n = 102

PRA (ng/ml/h)

LSN (µU/mL)

X axis
RENT CK modified levels

Y axis
LIAISON Direct Renin levels

Prof Morganti, University of Milan, 2007

Lonati C et al., ESH Meeting, Milan, June 2007
PRA vs LIAISON® Direct Renin

\[ y = 24.183x - 4.0214 \]

\[ R^2 = 0.9877 \]

X axis
REN CTK

Y axis
LIAISON® Direct Renin

Confidential data

*European Multicentric evaluation of Direct Renin against PRA*

*Going to be submitted to CI Chem*
From PRA to Direct Renin testing

Summary

During a symposia dedicated to the effectiveness of renin testing in monitoring the new anti-hypertensive therapies at the last Meeting of the European Society of Hypertension
Milan, June 2009

Prof J Nussberger, University of Lausanne
supporter of the ‘trapping method to test PRA’
SAID
these new methods are

FAST AND PRACTICAL
WHY we test Renin?

• To address the pharmacological treatment of the hypertensive patient

• To monitor effectiveness of the therapy with Renin inhibitors (Aliskiren by Novartis)

• To assess the risk of cardiovascular events

  LEVELS OF RENIN ARE FUNCTION OF THE NUMBER OF CARDIOVASCULAR EVENTS

• To DIAGNOSE Primary Aldosteronism*
Hypertension Simply is...

- Normal Volume
- Normal Constriction
  - Normal BP

- Increased Volume
- Normal Constriction
  - Increased BP

- Normal Volume
- Increased Constriction
  - Increased BP
# Classification of Blood Pressure

<table>
<thead>
<tr>
<th>Category</th>
<th>SBP mmHg</th>
<th>DBP mmHg</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>&lt; 120</td>
<td>and</td>
</tr>
<tr>
<td>Pre-Hypertension</td>
<td>120 to 139</td>
<td>or</td>
</tr>
<tr>
<td>Hypertension, Stage 1</td>
<td>140 to 159</td>
<td>or</td>
</tr>
<tr>
<td>Hypertension, Stage 2</td>
<td>≥ 160</td>
<td>or</td>
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</table>

Key: SBP = Systolic Blood Pressure  DBP = Diastolic Blood Pressure

**Target Blood Pressure:** < 130 / 80 mmHg
Primary and Secondary Hypertension

Primary (Essential)
-Unknown Cause
-Genetic Factor for the MOST (90%)
  over 80% of all High Blood Pressure Cases

Secondary

- High Blood Pressure caused by different Disorder
  (e.g., Hyperaldosteronism, Renal Artery Disease, etc.)

Minireview: Primary Aldosteronism-Changing Concepts in Diagnosis and Treatment
...a joke?

...if you are 50 years old:

OR

YOU SUFFER FROM HYPERTENSION

OR

YOU WILL SUFFER FROM HYPERTENSION
Hypertension Statistics
Europe and North America
(2008 estimation)
population
\[ \approx 1,150 \text{ Million People} \]

- 1/3 at risk of developing Hypertension = 380 Million
- 20% secondary Hypertension
- 10% Primary Aldosteronism* = around 7.6 Million
- Plus other mild forms of hypertension
Hypertension Statistics
Europe and North America
(2008 estimation)

population
≈ 1,150 Million People

- 1/3 at risk of developing Hypertension = 380 Million
- ‘cured’ subjects = around 40%
- effectively cured subjects = 50% of the ‘cured’

76 million persons in Europe and North America
USING the ratio

\[
\frac{\text{Aldosterone}}{\text{Renin}}
\]

to assess Primary Aldosteronism*

ARR=aldosterone to renin ratio
Number of Diagnosed Cases of PA per Year Before and After Using ARR for Screening

Before ARR

After ARR

Torino

Rochester

Brisbane

Singapore

Santiago

Mulatero P et al., J Clin Endocrinol Metab 2004; 89: 1045-1050
Which is the reason of the increasing number of cases?

aldosterone or renin alone: overlap
ARR: separation

(healthy vs. PA)

We recommend case detection of primary aldosteronism be sought in higher risk groups of hypertensive patients and those with hypokalemia by determining the aldosterone-renin ratio under standard conditions and....
Who should be submitted to the ratio:

ES guideline recommends screening of all cases of:

- stage 2 (>160/100 mmHg) and stage 3 (>180/110 mmHg) hypertension
- drug resistant hypertension (>3 AHTs)
- hypertension plus hypokalemia (spontaneous or diuretics induced)
- hypertension with adrenal incidentaloma
- hypertension with family history high BP or cerebrovascular event occurring <40 years
- hypertensive first degree relatives of PA patients
1.2 Values

In particular, this recommendation acknowledges the costs currently associated with ARR testing of all patients with essential hypertension. Against this recommendation for selective testing, however, must be weighed the risk of missing or at least delaying the diagnosis of PA in some hypertensive individuals.

The consequences of this may include the later development of more severe and resistant hypertension resulting from failure to lower levels of aldosterone or to block its actions.

Furthermore, duration of hypertension has been reported by several investigators to be a negative predictor of outcome after unilateral adrenalectomy for APA (46, 47), suggesting that delays in diagnosis may result in a poorer response to specific treatment once PA is finally diagnosed.
Case Detection, Diagnosis, and ..........  

Assay reliability

Although newer techniques are evolving, we prefer to use validated immunometric assays for plasma renin activity (PRA) or direct renin concentration (DRC); PRA takes into account factors (such as estrogen-containing preparations) that affect endogenous substrate levels. Laboratories should use aliquots from human plasma pools, carefully selected to cover the critical range of measurements, rather than the lyophilized controls provided by the manufacturer to monitor intra- and interassay reproducibility and long-term stability.

Because the ARR is mathematically highly dependent on renin (49), renin assays should be sufficiently sensitive to measure (PRA) levels as low as 0.2–0.3 ng/ml/h DRC 2 mU/liter (10, 16).
Interpretation

There are important and confusing differences between laboratories in the methods and units used to report values of renin and aldosterone. For aldosterone, 1 ng/dl converts to 27.7 pmol/liter in System International (SI) units. For immunometric methods of directly measuring renin concentration, a PRA level of 1 ng/mL/h (12.8 pmol/L in SI units) converts to a DRC of approximately 8.2 mU/L when measured by either the Nichols Institute Diagnostics automated chemiluminescence immunoassay (previously widely used but recently withdrawn) or the Bio-Rad Renin II RIA.

Because DRC assays are still in evolution, these conversion factors may change. For example, 1 ng/mL/h PRA converts to a DRC of approximately 12 mU/L (7.6 ng/L) when measured by the recently introduced and already widely used Diasorin automated chemiluminescence immunoassay.
• on top of assay variability is the confusion with units:
  - aldosterone in ng/dL, pg/mL or pmol/L
  - renin activity ng/mL*h or pmol/L*min
  - renin concentration in mU/L or ng/L

• ES guideline: You have the choice...

| TABLE 5. ARR cutoff values, depending on assay and based on whether PAC, PRA, and DRC are measured in conventional or SI units |
|---|---|---|---|---|
| PAC (ng/dl) | PRA (ng/mL/h) | PRA (pmol/liter/min) | DRC (mU/liter) | DRC (ng/liter) |
| 20 | 1.5 | 3.7 | 2.4 | 3.8 |
| 30 | 2.5 | 4.9 | 5.7 |
| 40 | 3.1 | 91 | 7.7 |
| PAC (pmol/liter) | 750 | 60 | 3.1 | 144 |
| 1000 | 80 | 122 | 192 |
Aldosterone and Renin in PA, diagnostic algorithm

Aldosterone/PRC ratio > 37

Retest and confirm the ratio

Confirmation tests

Saline infusion SLT (less cumbersome)  Fludrocortisone FST (4 days hospitalization)

Cut-off: aldo levels <40 pg/mL
Aldosterone and Renin in PA, treatment algorithm

Aldosterone/PRA(PLC) ratio CONFIRMED

Adrenal CT

No surgery

Mineralcorticoid Receptor antagonists

surgery
desired applicable

AVS (adrenal venous sampling)

bilateral

monolateral

Laparoscopic adrenalectomy
Patients

- 77 essential hypertensive pts
- Age 13-81 years
- 42 pts not treated (no ACEI / ARB)
- 35 pts under treatment (ACEI / ARB)
- Assays used: LIAISON Direct renin
  RenCTK
  AldoCTK
- Drawing: supine and after 60’ active orthostatism
- Collection of the samples:
  - RT till centrifugation
  - Quick freezing at -20°C till the measurement
- Exclusion criteria:
  - PRA clinostatism < 0.2 ng/ml/h
  - Lack of orthostatism increase of PRA
  - Morphological findings for adrenal masses
PRA levels (clino and ortho) in essential hypertensive pts treated and not treated with ACEI/ARB

![Bar graph showing PRA levels (ng/ml/h) for No-ACEI/ARB and ACEI/ARB treated groups.](image)

- **No-ACEI/ARB (n = 42)**
  - Clino: [Bar height]
  - Orto: [Bar height]

- **ACEI/ARB (n = 35)**
  - Clino: [Bar height]
  - Orto: [Bar height]

* P < 0.05
Direct Renin levels (clino and ortho) in essential hypertensive pts treated and not treated with ACEI/ARB

** P < 0.05

* P < 0.05

LSN Direct Renin (mU/dL)

- ** No-ACEI / ARB (n = 42)
- * ACEI / ARB (n = 35)

Clino Orto
Aldosterone levels (clino and ortho) in essential hypertensive pts treated and not treated with ACEI/ARB

* P < 0.05
Correlations among PRA, LSN (CliR), Aldosterone and orthostatic stimulus in essential hypertensive pts treated and not treated with ACEI/ARB

<table>
<thead>
<tr>
<th></th>
<th>No ACEI / ARB (n = 42)</th>
<th>ACEI / ARB (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>r</td>
</tr>
<tr>
<td>PRA vs CliR clino</td>
<td>0.90*</td>
<td>0.99*</td>
</tr>
<tr>
<td>PRA vs CliR ortho</td>
<td>0.92*</td>
<td>0.89*</td>
</tr>
<tr>
<td>PRA vs CliR clino + ortho</td>
<td>0.92*</td>
<td>0.90*</td>
</tr>
<tr>
<td>Δ PRA vs/ Δ CliR</td>
<td>0.88*</td>
<td>0.78*</td>
</tr>
<tr>
<td>PRA vs Aldo clino</td>
<td>0.51*</td>
<td>0.26</td>
</tr>
<tr>
<td>PRA vs Aldo ortho</td>
<td>0.52*</td>
<td>0.03</td>
</tr>
<tr>
<td>CliR vs Aldo clino</td>
<td>0.44*</td>
<td>0.17</td>
</tr>
<tr>
<td>CliR vs Aldo ortho</td>
<td>0.42*</td>
<td>0.09</td>
</tr>
<tr>
<td>Δ PRA vs Δ Aldo</td>
<td>0.46*</td>
<td>0.04</td>
</tr>
<tr>
<td>Δ CliR vs Δ Aldo</td>
<td>0.42*</td>
<td>0.26</td>
</tr>
</tbody>
</table>

* P < 0.05
ARR levels in Essential hypertensive pts Clino&Ortho, treated and not treated with ACEI/ARB

ARR PRA

No-ACEI / ARB (n = 42) ACEI / ARB (n = 35)

ARR LSN

No-ACEI / ARB (n = 42) ACEI / ARB (n = 35)

ARR PRA = aldosterone renin ratio con PRA
ARR LSN = aldosterone renin ratio con LSN
Cut-off ranges for ARR
Cut-off Advice by the Endocrine Society Guideline (ESGL)

<table>
<thead>
<tr>
<th></th>
<th>PRA (ng/ml/h)</th>
<th></th>
<th>PRC (mU/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>ESGL</td>
<td>Range</td>
</tr>
<tr>
<td>PAC (ng/dl)</td>
<td>20-40</td>
<td>30</td>
<td>24-49</td>
</tr>
</tbody>
</table>

*Funder SW et al., J Clin Endo Metab 2008; online*
Range and average levels for ARR PRA/ARR LSN Clino&Ortho in Essential Hypertensive pts treated and not treated with ACEI/ARB

<table>
<thead>
<tr>
<th></th>
<th>Clino</th>
<th>Ortho</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Average</td>
</tr>
<tr>
<td>ARR PRA NoTr</td>
<td>1-47</td>
<td>18</td>
</tr>
</tbody>
</table>
| ARR LSN NoTr     | 0.5-30.3 | 7.1     | 1.1-33.6 | 9.8
| ARR PRA Treated  | 0-40  | 11      | 0.74* | 0-70  | 14      | 0.54* |
| ARR LSN Treated  | 0.1-27.3 | 4.2     | 0.2-77.6 | 7.7

* P < 0.05
Conclusions

Direct Renin assay is a valid alternative to PRA as it reflects the RAS effects

Aldosterone/Renin ratio (ARR) calculated with the Direct Renin assay gives levels statistically correlated to those obtained with PRA

Average levels for ARR calculated with the Direct Renin assay stay in the normal limits showed by the Endocrine Society GuideLine and obtained with different direct Renin techniques

Similar levels obtained for ARR regardless from posture and ACEI/ARB treatment suggest the possibility of lowering the degree of attention to those conditions when the ARR test is performed
Ongoing studies

• To monitor effectiveness of the therapy with Renin inhibitors (Aliskiren by Novartis)

• To assess the involvement of Renin level risk of cardiovascular events

• To DIAGNOSE Primary Aldosteronism
TODA

GRAZIE

THANKS