Combining Molecular Profiling of Hypertension with Advanced Screening for Primary Aldosteronism by RAAS Triple-A Testing

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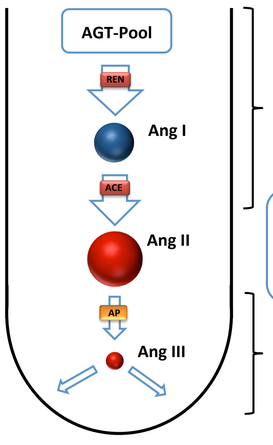
Background

RAS-Equilibrium-Analysis is a novel mass spectrometry based approach providing a comprehensive biochemical evaluation of the circulating renin-angiotensin-system (RAS) on the basis of equilibrium angiotensin levels and circulating aldosterone. In contrast to previous technologies involving complex sampling procedures, RAS-Equilibrium-Analysis combines the robustness and accuracy of LC-MS/MS based quantification with the versatility of serum sample collection to generate a highly reproducible readout containing multiple layers of information regarding the biochemical features of the circulating RAS. Equilibrium levels for Angiotensin I, Angiotensin II and Aldosterone are simultaneously measured in a single sample of 500µl standard collected serum. Sophisticated semi-automatic sample processing in combination with stable-isotope based internal standardization assure highest accuracy of test results and full compatibility with clinical routine. Resulting absolute serum concentrations of these three key molecules of the RAAS are the basis for diagnostic ratios and surrogate markers. Derived from a single blood test, these markers can be used for monitoring of the *in vivo* pharmacologic efficacy of ACE inhibitors, the evaluation of RAAS activity and the diagnosis of primary aldosteronism (PA).

RAS-Equilibrium Analysis and "RAAS Triple-A" Testing



Ang II – Producers
- Angiotensinogen
- Renin
- Soluble ACE, Chymase

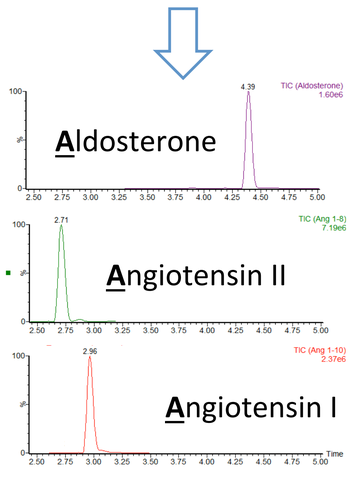
EQUILIBRIUM LEVELS
(FORMATION = DEGRADATION)

Ang II - Utilizers
- Aminopeptidases
- ACE2, PRCP

Sample collection and processing:
- Upright sampling at rest
- Collection of standard serum (fresh or frozen)
- Optional freezing
- Controlled *ex vivo* equilibration
- LC-MS/MS quantification of Angiotensins + Aldosterone

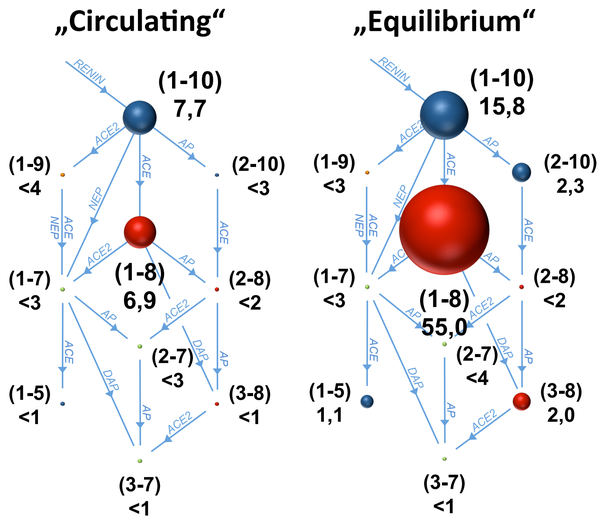
UPLC-MS/MS Multiplexing

AB Sciex 6500 *Xevo™ TQ-S*

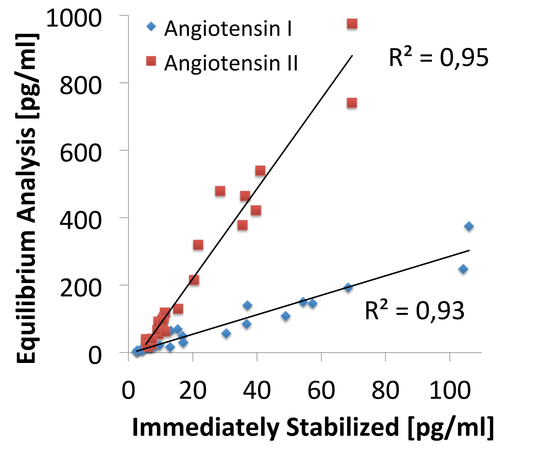


Circulating vs. Equilibrium Angiotensin Levels

Healthy Volunteers at Baseline



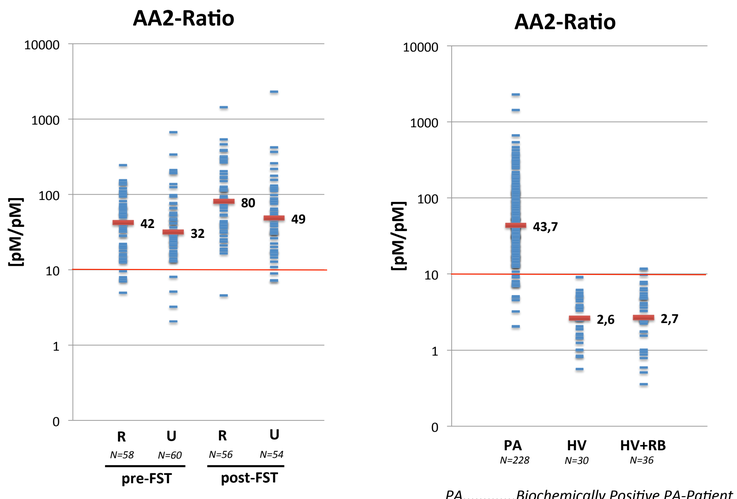
Healthy Volunteers +/- ARB Treatment



➔ **Equilibrium-Angiotensin-Levels highly correlate with in vivo circulating levels**

PA-Screening: The AA2-Ratio

PA-Patients during FST Confirmation Testing

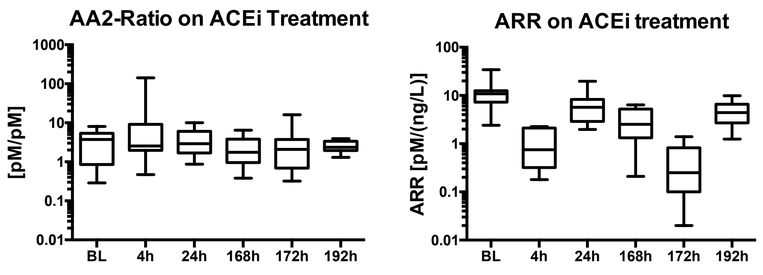


R....Recumbent
U.....Upright
FST.....Fludrocortisone + Salt Loading (4 days)

PA.....Biochemically Positive PA-Patient
HV.....Healthy Volunteers
HV+RB.....HV on RAS-Blockade (ACEi, RENi)

Unlike ARR, the AA2-Ratio is unaffected by ACE-Inhibitor Treatment

Healthy Volunteers: 10 mg Enalapril per Day, Single Dose, 8 Days, N=12

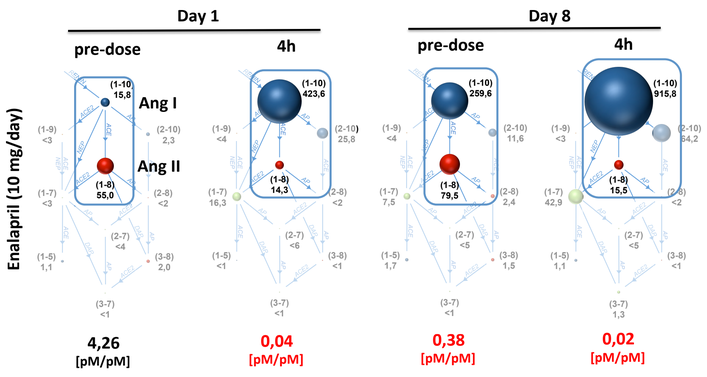


Suggests the possibility of PA-Screening on ACE-Inhibitors!

ACE: The eqAng II/eqAng I-Ratio

Healthy Volunteers on ACE-Inhibitor Treatment

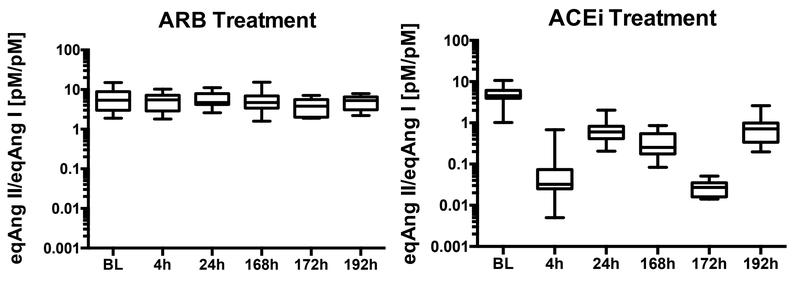
10 mg Enalapril per Day, Single Dose, 8 Days, N=11



EQ-(Ang II/Ang I)– Ratio suppressed by ACEi !

Healthy Volunteers on ARB or ACE-Inhibitor Treatment

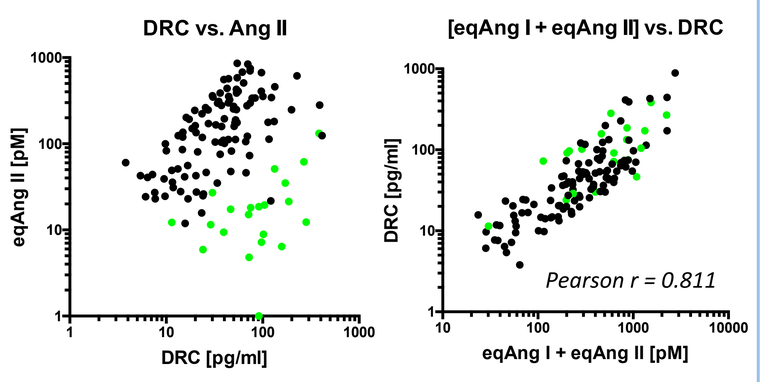
10 mg Enalapril or 50mg Losartan per Day, Single Dose, 8 Days, N=12



Renin: eqAng I + eqAng II

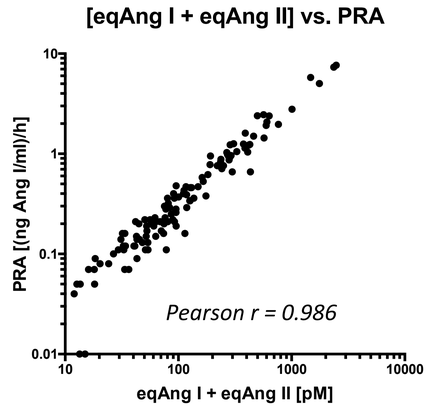
Healthy Volunteers on ACEi or ARB Treatment

10 mg Enalapril or 50mg Losartan per Day, Single Dose, 8 Days, N=12, 6 Time Points, 144 Data Pairs (green: 4h following oral ACEi)

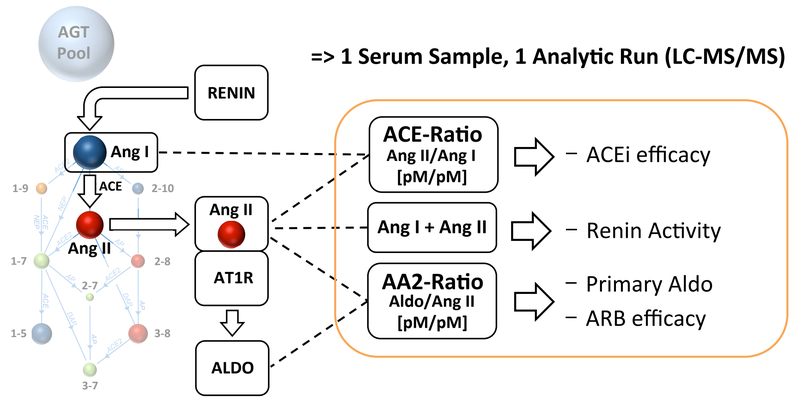


Strong correlation between PRA and [eqAng I + eqAng II] !

Correlation with PRA in Hypertensive Patients (N=124)

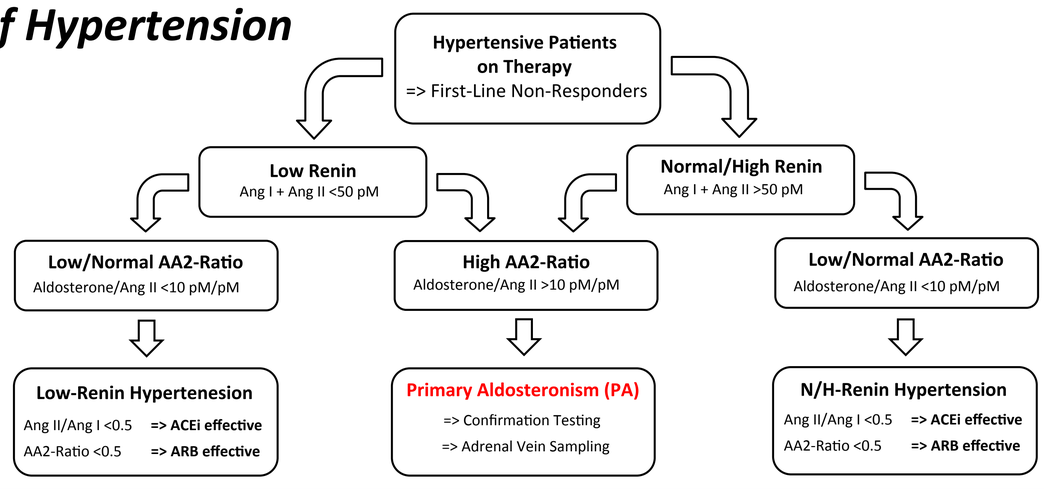


Summary



MDx-Based Treatment of Hypertension

=> „RAAS Triple A Testing"
of uncontrolled hypertensive patients on therapy as a molecular basis for early therapeutic decisions to increase treatment efficacy?



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