Cluster Analysis of Renin-Angiotensin-Aldosterone System (RAAS) hormones in general population individuals under anti-hypertensive treatment

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Introduction

Hypertension is a leading cause of death worldwide (1). Renin-Angiotensin-Aldosterone System (RAAS) is a critical pathway responsible for long-term control of blood pressure (**Figure 1**). The main hormones of RAAS are angiotensin II, generated from angiotensin I by angiotensin-converting enzyme (ACE). Aldosterone is stimulated by the binding of angiotensin II to angiotensin II type 1 receptor. RAAS is therapeutically targeted by anti-hypertensive drug (AHD), including ACE inhibitors (ACEi), angiotensin II receptor blockers (ARB) or combinations of an ACEi or an ARB with a diuretic.

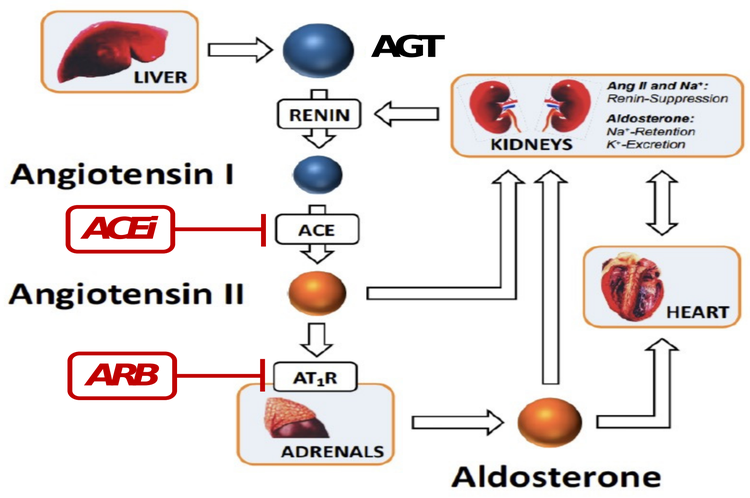

Figure 1: RAAS functional steps to regulate blood pressure.

The aims of this study are: (1) jointly characterize angiotensin I, angiotensin II and aldosterone using cluster analysis. (2) evaluate if certain RAAS clusters are associated with responsiveness to AHD and clinical variables.

Study Design:

The Cooperative Health Research in South Tyrol (CHRIS) study consists of 13, 393 participants from the general population recruited between 2011 and 2018 (2). We considered 800 participants (age: 43-years; 54% females) divided into 8 age- and gender-balanced groups: each group corresponded to a different AHD treatment (**Table 1**).

AHD group	**Gender** (M/F)	**Age**:Mean(sd)
Normotensive	46/55	65(7)
Hypertensive	46/54	62(8)
Drug not AHD	46/54	69(10)
ACEi	46/54	69(10)
ARB	46/54	69(10)
ACEi + Diuretic	46/53	65(9)
ARB + Diuretic	46/54	69(10)
Beta Blocker	46/54	66(9)
Total (800)	368/432	67(10)

Table 1: Distribution of AHD groups by age and gender

We used the novel RAAS Triple-A assay to obtain high quality RAAS hormones data from clinical serum samples. The three RAAS hormones Angiotensin I, Angiotensin II and Aldosterone were simultaneously quantified by liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) analysis.

Angiotensin I was higher in participants under ACEi or ARB, regardless whether they were combined with a diuretic or not (**Figure 2**). Angiotensin II was highest within participants under ARB. Aldosterone didn't show appreciable variations across groups.

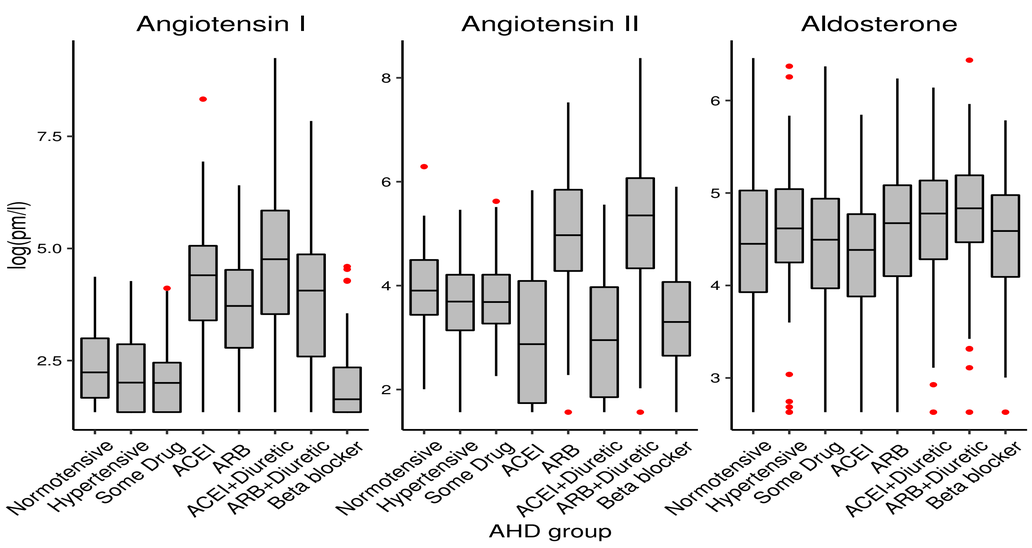

Figure 2: Distribution of RAAS hormones by AHDs. Red dots indicate ouliers outside lower and upper quartiles.

Statistical Methods

The K-means unsupervised clustering technique was adopted to explore subgroups of participants based on age and gender corrected RAAS. The clustering algorithm assigns each data point to one of K groups based on a similarity feature computed from the covariance matrix. The hormones were first turned into three principal components (PCs), which were then used to stratify the 800 participants into the K clusters. Clinical and laboratory variables were evaluated according to the cluster analysis. ANOVA and Chi-square tests were used to assess the heterogeneity of continuous and categorical variables across clusters at a significance level of 0.05.

Results

The PC analysis (**Figure 3**), indicated that the three components explained 62%, 28% and 10% of the variation of the RAAS variation, respectively. All the individuals were eventually stratified into K=3 clusters.

The widest cluster comprised 55% of all participants, whereas the remaining clusters included 30% and 15% participants. The cluster analysis identified subgroup of individuals based on responsiveness to AHD. The first cluster comprised participants with no active AHDs treatment and participants taking plain beta blocker. The second cluster comprised participants with a single ACEi and a combination of ACEi with Diuretic, while Participants with a single ARB and a combination drug ARB with Diuretic characterized the third cluster. The distributions of the various AHD across the clusters were statistically significant (χ^2 = 10.5, P-value = 0.0001).

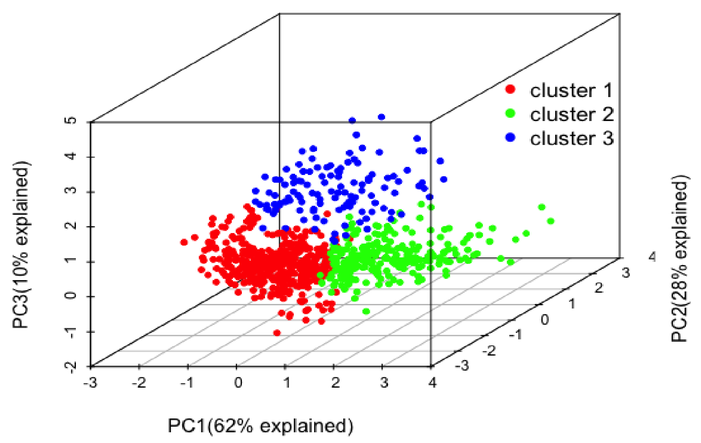

Figure 3: RAAS Clusters based on three PCs

Cluster 1 was also characterized by a higher systolic (138 mm/Hg) and diastolic (85.27 mm/Hg) blood pressure (**Table 2**). Cluster 2 individuals had higher and statistically significant BMI than those in cluster 1 and cluster 3. Clusters 2 and 3 individuals had mean glycated hemoglobin (HbA1c) level greater than 6%, substantially higher than for individuals in cluster 1 (mean=5.7%, P-value=0.0028). Age and gender were not significantly different across the three clusters, mainly due to the fact that RAAS hormones were corrected for age and gender before the clustering.

Variables	**Cluster 1** Mean(sd)	**Cluster 2** Mean(sd)	**Cluster 3** Mean(sd)	Test	P-value
Age (Year)	66.57(9.07)	65.71(9.67)	68.60(10.48)	F=3.72	0.1826
Gender F: n(%)	240(54)	129(55)	63(52)	χ^2=0.26	0.8789
BMI (kg/m^2)	27.38(4.53)	28.99(4.98)	29.32(4.53)	F=13.50	0.0021
HbA1c(%)	5.70(0.47)	6.04(0.63)	6.04(0.52)	F=5.92	0.0028
Systolic BP (mm/Hg)	137.99(18.46)	132.40(14.63)	137.54(17.9)	F=8.34	0.0434
Diastolic BP (mm/Hg)	85.27(9.40)	83.56(8.50)	83.96(9.42)	F=3.02	0.0019

Table 2: The distribution of clinical and laboratory variables by clusters. The tests used to examine difference between the cluster subgroups are: F (F-test) for continuous and Chi-square (χ^2) for categorical variables.

Conclusions

- Cluster analysis based on RAAS system is a feasible approach for investigating the heterogeneity of participants with hypertension in a population-based study.
- The cluster analysis identified subgroups of individuals based on responsiveness to various AHD treatment.
- The RAAS cluster groups indicated heterogeneity based on relevant clinical and laboratory characteristics

References

1 Chockalingam A et al. (2006). Worldwide epidemic of hypertension. Canadian journal of cardiology, 22(7), 553-555.

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