Hypertension is becoming more and more common in the general population, and the identification of its cause allows it the initiation of the appropriate treatment and the prevention of adverse cardiovascular events. Hypertension is a complex condition with multifactorial causes and renin-angiotensin-aldosterone system (RAAS) plays a key role in the regulation. Recent investigations have demonstrated that within the normal range both a higher aldosterone level and a higher aldosterone-to-renin-ratio (ARR) serve as markers of increased risk for hypertension (1-3). However, the distribution of the ARR in the general population is largely unknown. Automated assays have been developed for aldosterone and renin testing and might facilitate the accessibility and reliability of renin and aldosterone assays. We aim to provide a sex-specific distribution and reference ranges of plasma aldosterone concentration (PAC), plasma renin concentration (PRC) and its ratio (ARR) in a large population sample.

Methods

- **Study Sample and Study Design:** A cohort of 7,584 male and 7,426 female randomly selected subjects participated in the population-based Gutenberg Health Study (GHS). A reference population was selected by excluding all participants with hypokalemia, hypertension, renal insufficiency, and intake of antihypertensives. The reference interval was defined as the central 95% range between the 2.5th and 97.5th percentiles.

- **Venous Blood Sampling and Processing:** Venipunctures were performed in a supine position after a fasting period of 5 h with 1.5 mg EDTA EDTA-plastic Petri dish (BD, Franklin Lakes, USA). All samples were centrifuged at 10 min with 4,000 rpm, 2-D barcoded polypropylene tubes (Microtronic B.V., Leidestad, The Netherlands) were used for aliquoting of 300 µL EDTA plasma. All samples were frozen within 2 hours directly at -80°C. For marker analyses, frozen samples were thawed in a water bath at 37°C for 10 min and turned 10 times carefully upside down by hand.

- **Standardized Measurement of Aldosterone and Renin:** PAC and PRC were measured by chemiluminescence immunoassay (CLIA) (LIAISON®; DiaSorin). Both aldosterone and renin measurements were performed with a single EDTA tube. According to the manufacturer's instructions the measurable range for PAC is 1.9 - 100ng/mL and any value that reads below 1.9ng/mL (competitive assay). The measurable range for PRC is 1.96 – 500ng/mL.

Results

**Study Sample and Distribution of Aldosterone, Renin and its ratio**

In total, 7,474 (50.7%) men and 7,266 (49.3%) women were analyzed. The median age of the sample population was 55 years (interquartile range: 46-65 years). Both, PAC and PRC were available from 10,992 participants. Distribution of CVRFs and CVD was representative for an European population and was characterized by high quality control metrics, below run coefficients of variation (CV) for PAC were 9.7% at 11.6ng/dL (n=75) and 7.8% at 29.6ng/dL (n=75) with the Liaison® assay. For PRC between run CVs were 7.8% at 21.0ng/mL (n=75) and 7.7% at 100ng/mL (n=75). Log-transformed distribution of aldosterone (left) and renin (right) in the study sample is presented in Figure 1.

**Relation of Aldosterone and Aldosterone/Renin-Ratio to stage of hypertension**

In the population circulating aldosterone concentration and ARR increased according to the stage of hypertension in both sexes. For women the hypertension associated increase was more obvious in ARR.

**Conclusions and Outlook**

- **Our study contributed to establish age and sex-dependent reference values for the immunoassay-based aldosterone and renin automated assay, as well as for the related ARR values.**

- **The use of nomograms (stratified by sex and age) should be part of interpretation of ARR to enable accurate diagnosis and management of hypertension in daily clinical practice.**

- **Additionally, the use of automated aldosterone and renin assays might also participate to the overall assays standardization and facilitate the laboratory accreditation process.**

Reference Ranges for Aldosterone, Renin and Aldosterone/Renin Ratio

Sex differences were found for PAC with lower PRC and higher ARR in females than in males in both sexes. The ratio was increased in males and females whereas PAC decrease was significant in females only. Increase in ARR with age was comparable in both sexes (δARR (ARR) per decade: 0.11 [95% CI 0.09:0.13]; p<0.0001). A sex-specific reference range was defined by excluding all participants with hypokalemia, hypertension, renal insufficiency, and intake of antihypertensives (ACTC code: C02, C03, C07, C08, C09) (n=4821). Sex-specific reference limits and categories indicating the grade of deviation from the reference were calculated by quintile regression (Table 2-4, for PAC, PRC and the ARR).

A nomogram for the ARR is shown in Figure 3 (for the sample population with and without medication). The regression lines refer to the median of the reference category (aged line), followed by the 95th percentile for the reference sample and the 95th and 99th percentiles for males and females. Points are assigned to the dependent variable according to the sex-specific percentile limits.