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ORIGINAL RESEARCH ARTICLE

# Clinical determinants and prognostic implications of renin and aldosterone in patients with symptomatic heart failure

Masatake Kobayashi[1], Susan Stienen[1], Jozine M. ter Maaten[2], Kenneth Dickstein[3,4], Nilesh J. Samani[5], Chim C. Lang[6], Leong L. Ng[5], Stefan D. Anker[7], Macro Metra[8], Gregoire Preud'homme[1], Kevin Duarte[1], Zohra Lamiral[1], Nicolas Girerd[1], Patrick Rossignol[1], Dirk J. van Veldhuisen[2], Adriaan A. Voors[2], Faiez Zannad[1] and João Pedro Ferreira[1]*

[1]*INSERM, Centre d'Investigations Cliniques Plurithématique 1433, Inserm U1116, CHRU de Nancy and F-CRIN INI-CRCT, Université de Lorraine, Nancy, France;* [2]*Department of Cardiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands;* [3]*Department of Internal Medicine, University of Bergen, Bergen, Norway;* [4]*Department of Cardiology, Stavanger University Hospital, Stavanger, Norway;* [5]*Department of Cardiovascular Sciences, University of Leicester, NIHR Leicester Biomedical Research Centre, Glenfield Hospital, Leicester, UK;* [6]*Division of Molecular and Clinical Medicine, School of Medicine, University of Dundee, Ninewells Hospital & Medical School, Dundee, UK;* [7]*Department of Cardiology (CVK), Berlin, Institute of Health Center for Regenerative Therapies (BCRT); German Centre for Cardiovascular Research (DZHK) partner site Berlin; Charité Universitätsmedizin Berlin BerlinGermany;* [8]*Department of Cardiology, University and Civil hospitals of Brescia, Brescia, Italy*

## Abstract

**Aims** Activation of the renin–angiotensin–aldosterone system plays an important role in the pathophysiology of heart failure (HF) and has been associated with poor prognosis. There are limited data on the associations of renin and aldosterone levels with clinical profiles, treatment response, and study outcomes in patients with HF.

**Methods and results** We analysed 2,039 patients with available baseline renin and aldosterone levels in BIOSTAT-CHF (a systems BIOlogy study to Tailored Treatment in Chronic Heart Failure). The primary outcome was the composite of all-cause mortality or HF hospitalization. We also investigated changes in renin and aldosterone levels after administration of mineralocorticoid receptor antagonists (MRAs) in a subset of the EPHESUS trial and in an acute HF cohort (PORTO). In BIOSTAT-CHF study, median renin and aldosterone levels were 85.3 (percentile$_{25–75}$ = 28–247) μIU/mL and 9.4 (percentile$_{25–75}$ = 4.4–19.8) ng/dL, respectively. Prior HF admission, lower blood pressure, sodium, poorer renal function, and MRA treatment were associated with higher renin and aldosterone. Higher renin was associated with an increased rate of the primary outcome [highest vs. lowest renin tertile: adjusted-HR (95% CI) = 1.47 (1.16–1.86), $P$ = 0.002], whereas higher aldosterone was not [highest vs. lowest aldosterone tertile: adjusted-HR (95% CI) = 1.16 (0.93–1.44), $P$ = 0.19]. Renin and/or aldosterone did not improve the BIOSTAT-CHF prognostic models. The rise in aldosterone with the use of MRAs was observed in EPHESUS and PORTO studies.

**Conclusions** Circulating levels of renin and aldosterone were associated with both the disease severity and use of MRAs. By reflecting both the disease and its treatments, the prognostic discrimination of these biomarkers was poor. Our data suggest that the "point" measurement of renin and aldosterone in HF is of limited clinical utility.

**Keywords** Heart failure; Renin; Aldosterone; Prediction model; Prognosis

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**Correspondence to: João Pedro Ferreira, Centre d'Investigation Clinique 1433 module Plurithématique, CHRU Nancy–Hopitaux de Brabois, Institut Lorrain du Coeur et des Vaisseaux Louis Mathieu, Nancy 54500, France.*
*Email: j.ferreira@chru-nancy.fr*

# Introduction

Activation of the renin–angiotensin–aldosterone system (RAAS) plays a pivotal role in the development and subsequent progression of heart failure (HF); excessive and inappropriate RAAS activation may increase myocardial fibrosis and favour the adverse myocardial remodelling.[1,2] Renin and aldosterone, as markers of RAAS activation, have been associated with poor prognosis in previous studies.[3–7] However, several clinical parameters such as severity of congestion, cardiac and renal function, and HF treatments, e.g. angiotensin converting enzyme inhibitor/angiotensin receptor blocker (ACEi/ARBs), beta blockers, and mineralocorticoid receptor antagonist (MRAs), may also influence the RAAS activation.[1,8,9]

The systems BIOlogy study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF) is a multicenter international European project that assessed the factors associated with under-prescription of life-saving therapies in HF and the respective prognostic implications.[10] The BIOSTAT-CHF study allows for the unique opportunity to explore both the prognostic value and the factors associated with activation of the RAAS, reflected here by the determination of the circulating levels of renin and aldosterone. We also measured renin and aldosterone in a subset of patients of the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) trial[11] and in a cohort of acute HF patients from Porto, Portugal.[12]

The aims of the present study are to investigate (i) the clinical determinants of renin and aldosterone levels, (ii) the association of renin and aldosterone with clinical outcomes, and (iii) the discriminative prognostic value of renin and aldosterone on top of the 'best' clinical model.

# Methods

## Patient population

The description of the BIOSTAT-CHF cohort has been previously published.[10,13] In brief, BIOSTAT-CHF was an investigator-driven multicenter clinical study being consisted of 2,516 patients from 69 centres in 11 European countries with symptoms of HF, which was confirmed by left ventricular ejection fraction $\leq$40% and/or brain natriuretic peptide $>$400 pg/mL or N-terminal pro BNP (NT-proBNP) $>$2,000 pg/mL and treatment of furosemide. From this cohort, we analysed 2,039 patients with available data on renin and aldosterone at baseline. Patients were receiving $<$50% of the target doses of at least one of ACEi/ARBs and beta blockers at the time of inclusion. The first three months of treatment were a treatment optimization phase. During the optimization phase, initiation or uptitration of ACEi/ARB and/or beta blocker was done according to the routine clinical practice of the treating physicians, who were encouraged to follow the European Society of Cardiology guideline.[14]

All patients recruited in BIOSTAT-CHF gave written informed consent to participate in the study. BIOSTAT-CHF was conducted in concordance with the declaration of Helsinki, national ethics, and legal requirements, as well as relevant EU legislation. The study was approved by national and local ethics committees. All patients recruited in BIOSTAT-CHF gave written informed consent to participate in the study.

EPHESUS was designed to assess the effects of eplerenone on morbidity and mortality in patients with a left ventricular ejection fraction (LVEF) $\leq$40% after acute myocardial infarction who had signs and symptoms of HF or diabetes as previously published.[11,15] Among 6,632 patients in the EPHESUS trial, 360 and 366 patients had respectively available renin and aldosterone measurements at screening, 1-month, 3-month, and 6-month visits.

The PORTO study was a prospective, single-center, non-randomized, open-label, and interventional study.[12] Patients presenting with acute HF (AHF) were assigned to either oral spironolactone plus standard AHF care or standard AHF care alone in a Portuguese tertiary hospital. We analysed 97 patients with available renin and aldosterone measurements in the first 24 h and at Day 3 after admission. Spironolactone was administrated after the first sample was collected.

## Biomarkers

Plasma samples were measured at baseline, i.e. when patients with HF visited a medical service in a decompensated state in BIOSTAT-CHF study. Patients could have come at any time during the day, and they have had food before the blood samples had been collected. All patients rested for at least 15 min before collecting the samples. Renin and aldosterone were both measured using a RadioImmunoAssay (Renin: CisBio International; Aldosterone: IBL International) in plasma samples that had previously undergone two freeze/thaw cycles as previously published.[16] For renin, the dynamic range for this assay is 1.0 to 11,160 μIU/mL, and interassay coefficients of variation were 5.0%. The direct renin assay has been demonstrated to yield measurements that have a high correlation with plasma renin activity and high reproducibility.[17–19] The dynamic range for the aldosterone assay is 0.14 to 150 ng/dL, and interassay coefficients of variation were $<$ 7.5%. All the biomarkers were measured either at local hospital site or within the BIOSTAT-CHF central laboratory.

## Statistical analysis

Categorical variables are described as frequencies (percentages), and continuous variables are described as means ±

standard deviation or median (25th and 75th percentiles), depending on the variable distributions. Comparisons of demographic, clinical, and biological parameters among tertiles of renin and aldosterone levels were analysed using chi-squared tests for categorical variables and Kruskal-Wallis test for continuous variables.

Linear regression analyses were performed to assess the associations of clinical variables with renin and aldosterone levels. Clinical variables were entered in the multivariable model with forward selection. Covariates considered to be of potential prognostic impact were age, sex, body mass index, medical history (diabetes mellitus, atrial fibrillation, previous myocardial infarction, prior HF admission, and chronic obstructive pulmonary disease), HF etiologies (ischemic, hypertensive, valvular heart disease, dilated cardiomyopathy, and other), presence of signs and symptoms of congestion (orthopnea, III heart sound, leg edema, and hepatomegaly), systolic blood pressure (SBP), heart rate, LVEF, laboratory findings [haemoglobin, sodium, potassium, blood urea nitrogen, and estimated glomerular filtration rate (eGFR) by the Chronic Kidney Disease Epidemiology Collaboration formula[20]], and treatments (use of ACEi/ARB, beta blockers, and MRA). These variables had a small proportion of missing values ($<$10%), and no multiple imputation was performed.

To assess the changes in renin and aldosterone levels after the initiation of MRAs, repeated measures analysis of covariance models were fit in terms of treatment group in the EPHESUS and PORTO studies (eplerenone in the EPHESUS substudy and spironolactone in PORTO study). Changes at each time point were adjusted for baseline values and compared between treatment groups.

The primary outcome was the composite of hospitalization for HF or all-cause mortality. Secondary outcomes were all-cause mortality and cardiovascular mortality. Survival probabilities were estimated using the Kaplan–Meier method. The covariates used for adjustment were chosen from demographic (age and sex), clinical (prior HF admission, use of beta blockers, and SBP), and laboratory (NT-proBNP, blood urea nitrogen, haemoglobin, high-density lipoprotein cholesterol, eGFR, and sodium) parameters as previously published.[21] All parameters used to build the BIOSTAT-CHF risk models are depicted herein (https://biostat-chf.shinyapps.io/calc/). Interactions between renin and aldosterone on clinical outcomes were assessed using both continuous and categorical variables. Curvilinear associations between log-transformed baseline renin, aldosterone levels, and outcome were tested using Cox models with unadjusted and adjusted for the BIOSTAT-CHF risk models in a restricted cubic spline with five knots. The added value of baseline renin and aldosterone levels on the BIOSTAT-CHF risk model was assessed by means of the increased c-index.

All analyses were performed using R version 3.4.0 (R Development Core Team, Vienna, Austria). A two-sided *P* value $<$0.05 was considered statistically significant.

# Results

## Baseline characteristics according to renin and aldosterone levels

Among the 2,039 patients included in BIOSTAT-CHF study, 73% were male patients, mean age was 69 ± 12 years, and mean LVEF was 31 ± 11% (*Table 1*). In the total cohort, median renin and aldosterone levels were 85.3 (IQR 28–247) μIU/mL and 9.4 (IQR 4.4–19.8) ng/dL, respectively. The correlation between renin and aldosterone was weak (Spearman Rho = 0.28).

Patients with higher renin and aldosterone levels were younger, more often male patients, had more often a prior HF admission, lower SBP, lower LVEF, poorer renal function, were less likely to receive target doses of ACEi/ARB, and were more often prescribed MRAs (*Table 1*).

## Clinical determinants of renin and aldosterone levels

In the linear regression models, lower SBP, eGFR, sodium, prior HF admission, no use of beta blocker, and MRA use were the factors that were associated with both higher levels of both renin and aldosterone (*Table 2*). Higher renin levels alone were associated with higher body mass index, previous myocardial infarction, chronic obstructive pulmonary disease, and dilated cardiomyopathy. Higher aldosterone levels alone were associated with no use of ACEi/ARB. The clinical variables associated with the highest tertiles of renin and aldosterone are shown in Supporting Information, *Table S1*. The associations of renin and aldosterone levels by the different doses of ACEi/ARBs or MRAs are depicted in *Table S2*. Renin and aldosterone levels were higher with the use of MRAs and lower with increasing doses of ACEi/ARBs.

## Effects of mineralocorticoid receptor antagonists on renin and on aldosterone levels in EPHESUS and PORTO cohort studies

In the EPHESUS substudy, eplerenone increased aldosterone levels, and patients receiving eplerenone had higher renin levels compared with placebo (*Figure S1*). Both changes persisted thereafter. In addition, we observed that both renin and aldosterone levels tended to increase after the initiation of spironolactone in PORTO study (*Figure S1*).

## Survival analysis

During a median follow up of 21 months, the primary outcome occurred more frequently in patients with higher renin

**Table 1** Patients' characteristics according to renin and aldosterone levels (tertiles) in BIOlogy study to Tailored Treatment in Chronic Heart Failure study

| | | Renin levels | | | | Aldosterone levels | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Global ($n$ = 2039) | Low, 0–40 μIU/mL ($n$ = 684) | Intermediate,41–170 μIU/mL ($n$ = 679) | High, >171 μIU/mL ($n$ = 675) | $P$ value | Low, 0–5 ng/dL ($n$ = 681) | Intermediate, 6–14 ng/dL ($n$ = 690) | High, >15 ng/dL ($n$ = 668) | $P$ value |
| Age, years | 68.5 ± 12.1 | 69.2 ± 12.2 | 68.7 ± 12.5 | 67.4 ± 11.5 | 0.005 | 69.7 ± 12.1 | 68.6 ± 11.9 | 67.0 ± 12.1 | <0.001 |
| Male, $n$ (%) | 1,481 (72.6%) | 468 (68.4%) | 477 (70.3%) | 536 (79.3%) | <0.001 | 481 (70.6%) | 492 (71.3%) | 508 (76.0%) | 0.052 |
| Body mass index, kg/m$^2$ | 27.8 ± 5.5 | 27.5 ± 5.5 | 27.6 ± 5.2 | 28.3 ± 5.6 | 0.07 | 27.5 ± 5.5 | 27.9 ± 5.5 | 28.0 ± 5.4 | 0.15 |
| Medical history | | | | | | | | | |
| Hypertension, $n$ (%) | 1,259 (61.7%) | 472 (69.0%) | 419 (61.7%) | 368 (54.4%) | <0.001 | 426 (62.6%) | 458 (66.4%) | 375 (56.1%) | <0.001 |
| Diabetes mellitus, $n$ (%) | 656 (32.2%) | 207 (30.3%) | 216 (31.8%) | 233 (34.5%) | 0.24 | 230 (33.8%) | 224 (32.5%) | 202 (30.2%) | 0.37 |
| Atrial fibrillation, $n$ (%) | 932 (45.7%) | 316 (46.2%) | 300 (44.2%) | 316 (46.7%) | 0.61 | 305 (44.8%) | 325 (47.1%) | 302 (45.2%) | 0.66 |
| Myocardial infarction, $n$ (%) | 750 (36.8%) | 205 (30.0%) | 243 (35.8%) | 302 (44.7%) | <0.001 | 260 (38.2%) | 242 (35.1%) | 248 (37.1%) | 0.48 |
| COPD, $n$ (%) | 346 (17.0%) | 95 (13.9%) | 114 (16.8%) | 137 (20.3%) | 0.007 | 137 (20.1%) | 99 (14.3%) | 110 (16.5%) | 0.02 |
| Prior HF hospitalization, $n$ (%) | 649 (31.8%) | 182 (26.6%) | 220 (32.4%) | 247 (36.5%) | <0.001 | 177 (26.0%) | 235 (34.1%) | 237 (35.5%) | <0.001 |
| HF aetiology | | | | | <0.001 | | | | 0.004 |
| Ischemic heart disease, $n$ (%) | 881 (44.1%) | 249 (37.1%) | 295 (44.5%) | 337 (50.9%) | | 301 (45.5%) | 286 (42.1%) | 294 (44.8%) | |
| Hypertensive heart disease, $n$ (%) | 204 (10.2%) | 111 (16.5%) | 60 (9.0%) | 33 (5.0%) | | 76 (11.5%) | 74 (10.9%) | 54 (8.2%) | |
| Valvular heart disease, $n$ (%) | 150 (7.5%) | 50 (7.5%) | 53 (8.0%) | 47 (7.1%) | | 50 (7.6%) | 50 (7.4%) | 50 (7.6%) | |
| Dilated cardiomyopathy, $n$ (%) | 458 (22.9%) | 148 (22.1%) | 143 (21.6%) | 167 (25.2%) | | 116 (17.5%) | 171 (25.2%) | 171 (26.1%) | |
| Other, $n$ (%) | 303 (15.2%) | 113 (16.8%) | 112 (16.9%) | 78 (11.8%) | | 118 (17.9%) | 98 (14.4%) | 87 (13.3%) | |
| Clinical profile | | | | | | | | | |
| NYHA III + IV, $n$ (%) | 1,234 (62.3%) | 397 (59.7%) | 387 (58.7%) | 450 (68.4%) | <0.001 | 450 (68.4%) | 403 (60.4%) | 381 (58.0%) | <0.001 |
| Orthopnea, $n$ (%) | 715 (35.1%) | 233 (34.1%) | 221 (32.6%) | 261 (38.8%) | 0.045 | 250 (36.8%) | 242 (35.1%) | 223 (33.4%) | 0.43 |
| Leg edema, $n$ (%) | 1711 (84.0%) | 573 (83.8%) | 573 (84.4%) | 565 (83.7%) | 0.93 | 576 (84.7%) | 585 (84.8%) | 550 (82.3%) | 0.38 |
| Systolic BP, mmHg | 124.6 ± 21.8 | 133.2 ± 22.2 | 123.9 ± 19.6 | 116.6 ± 20.2 | <0.001 | 127.4 ± 22.6 | 126.5 ± 21.9 | 119.8 ± 19.9 | <0.001 |
| Heart rate, bpm | 80.1 ± 19.7 | 82.1 ± 21.6 | 79.1 ± 19.0 | 78.9 ± 18.2 | 0.03 | 81.5 ± 21.7 | 79.8 ± 19.1 | 78.9 ± 18.0 | 0.44 |
| LVEF, % | 31.1 ± 10.8 | 32.7 ± 10.6 | 31.4 ± 11.5 | 29.0 ± 9.8 | <0.001 | 32.8 ± 11.4 | 30.6 ± 10.3 | 29.8 ± 10.4 | <0.001 |
| LVEF <40%, $n$ (%) | 1623 (88.7%) | 539 (85.6%) | 535 (88.1%) | 549 (92.7%) | <0.001 | 509 (84.6%) | 569 (90.3%) | 545 (91.3%) | <0.001 |
| Medication | | | | | | | | | |
| ACEi/ARB, $n$ (%) | 1467 (71.9%) | 497 (72.7%) | 476 (70.1%) | 494 (73.1%) | 0.42 | 514 (75.5%) | 518 (75.1%) | 435 (65.1%) | <0.001 |
| ACEi/ARB target dose, $n$ (%) | 259 (12.7%) | 110 (16.1%) | 80 (11.8%) | 69 (10.2%) | 0.003 | 96 (14.1%) | 99 (14.3%) | 64 (9.6%) | 0.02 |
| Beta blocker, $n$ (%) | 1694 (83.1%) | 572 (83.6%) | 568 (83.7%) | 554 (82.0%) | 0.63 | 566 (83.1%) | 584 (84.6%) | 544 (81.4%) | 0.29 |

(*Continues*)


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**Table 1** (continued)

| | | Renin levels | | | | Aldosterone levels | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Global ($n$ = 2039) | Low, 0–40 μIU/mL ($n$ = 684) | Intermediate,41–170 μIU/mL ($n$ = 679) | High, >171 μIU/mL ($n$ = 675) | $P$ value | Low, 0–5 ng/dL ($n$ = 681) | Intermediate, 6–14 ng/dL ($n$ = 690) | High, >15 ng/dL ($n$ = 668) | $P$ value |
| Beta blocker target dose, $n$ (%) | 117 (5.7%) | 44 (6.4%) | 39 (5.7%) | 34 (5.0%) | 0.54 | 39 (5.7%) | 48 (7.0%) | 30 (4.5%) | 0.15 |
| MRA, $n$ (%) | 1076 (52.8%) | 320 (46.8%) | 340 (50.1%) | 416 (61.5%) | <0.001 | 334 (49.0%) | 330 (47.8%) | 412 (61.7%) | <0.001 |
| Loop diuretics dose, mg | 40.0 (20.0–80.0) | 40.0 (20.0–80.0) | 40.0 (20.0–80.0) | 40.0 (20.0–100.0) | 0.03 | 40.0 (20.0–80.0) | 40.0 (20.0–75.0) | 40.0 (25.0–100.0) | 0.02 |
| Laboratory | | | | | | | | | |
| Haemoglobin, g/dL | 13.2 ± 1.9 | 13.3 ± 1.8 | 13.2 ± 2.0 | 13.1 ± 1.9 | 0.09 | 12.7 ± 2.0 | 13.4 ± 1.8 | 13.4 ± 1.9 | <0.001 |
| Blood urea nitrogen, mg/dL | 41.4 ± 33.1 | 34.7 ± 30.7 | 39.7 ± 29.9 | 50.1 ± 36.4 | <0.001 | 40.7 ± 32.2 | 41.4 ± 35.6 | 42.1 ± 31.1 | 0.13 |
| eGFR, mL/min/L.73m$^2$ | 62.0 ± 24.3 | 66.2 ± 24.1 | 61.7 ± 25.9 | 58.1 ± 22.0 | <0.001 | 63.3 ± 24.8 | 62.4 ± 23.2 | 60.3 ± 24.9 | 0.03 |
| Sodium, mmol/L | 139.2 ± 4.0 | 140.5 ± 3.6 | 139.6 ± 3.6 | 137.5 ± 4.2 | <0.001 | 139.4 ± 3.9 | 139.7 ± 3.9 | 138.5 ± 4.2 | <0.001 |
| Potassium, mmol/L | 4.3 ± 0.6 | 4.2 ± 0.5 | 4.3 ± 0.6 | 4.3 ± 0.6 | 0.19 | 4.2 ± 0.6 | 4.3 ± 0.6 | 4.3 ± 0.6 | 0.003 |
| BNP, pg/mL | 773 (424–1,353) | 786 (457–1,186) | 687 (320–1,353) | 793 (451–1,485) | 0.89 | 1,009 (598–1,457) | 590 (283–923) | 892 (338–1,678) | <0.001 |
| Renin, μIU/mL | 83.9 (27.4–246.1) | 17.6 (10.4–27.8) | 84.6 (60.8–117.2) | 386.3 (246.6–1535.4) | <0.001 | 54.9 (19.7–163.6) | 71.7 (24.8–203.9) | 154.3 (55.7–415.5) | <0.001 |
| Aldosterone, ng/dL | 9.3 (4.3–19.3) | 6.6 (3.4–12.7) | 9.4 (4.4–18.0) | 13.8 (5.8–29.9) | <0.001 | 3.0 (1.8–4.3) | 9.4 (7.3–11.9) | 27.2 (19.6–44.2) | <0.001 |

ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; BNP, brain natriuretic peptide; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; HF, heart failure; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association. Values are Mean ± standard deviation, $n$ (%) or median (25th to 75th percentile).

**Table 2** Multivariable model for the associations of clinical profiles with renin and aldosterone levels in BIOlogy study to Tailored Treatment in Chronic Heart Failure study

| Variable | Renin | | | Aldosterone | | |
|---|---|---|---|---|---|---|
| | $\beta$ | 95% CI | $R^2$ = 0.26 *P* value | $\beta$ | 95% CI | $R^2$ = 0.10 *P* value |
| (Constant) | 21.69 | 19.28 to 24.09 | <0.001 | 5.79 | 3.54 to 8.04 | <0.001 |
| Age, years (per 5 years) | | | | −0.04 | −0.07 to −0.01 | 0.004 |
| Male | 0.21 | 0.05 to 0.36 | 0.010 | | | |
| Body mass index, kg/m$^2$ (per 5 kg/m$^2$) | 0.14 | 0.07 to 0.20 | <0.001 | | | |
| Medical history | | | | | | |
| Myocardial infarction | 0.33 | 0.14 to 0.53 | 0.001 | | | |
| Diabetes | | | | −0.14 | −0.27 to −0.03 | 0.018 |
| Prior HF hospitalization | 0.21 | 0.07 to 0.36 | 0.005 | 0.26 | 0.13 to 0.39 | <0.001 |
| Chronic obstructive pulmonary disease | 0.28 | 0.10 to 0.46 | 0.003 | | | |
| HF etiologies* | | | | | | |
| Other | | (reference) | | | | |
| Ischemic heart disease | 0.24 | −0.003 to 0.48 | 0.053 | | | |
| Hypertensive heart disease | −0.13 | −0.41 to 0.15 | 0.37 | | | |
| Valvular heart disease | 0.27 | −0.03 to 0.57 | 0.08 | | | |
| Dilated cardiomyopathy | 0.31 | 0.08 to 0.54 | 0.007 | | | |
| Physical examination | | | | | | |
| III heart sound | | | | 0.24 | 0.03–0.44 | 0.025 |
| Systolic BP, mmHg (per 10 mmHg) | −0.20 | −0.23 to −0.16 | <0.001 | −0.06 | −0.09 to −0.04 | <0.001 |
| Laboratory | | | | | | |
| Haemoglobin, g/dL | | | | 0.14 | 0.10 to 0.17 | <0.001 |
| eGFR, mL/min/1.73 m$^2$ (per 5 mL/min/1.73 m$^2$) | −0.04 | −0.06 to −0.03 | <0.001 | −0.03 | −0.05 to −0.02 | <0.001 |
| Sodium, mmol/L | −0.11 | −0.13 to −0.09 | <0.001 | −0.03 | −0.04 to −0.01 | 0.001 |
| Medication | | | | | | |
| ACEi/ARB | | | | −0.27 | −0.41 to −0.13 | <0.001 |
| Beta blocker | −0.33 | −0.51 to −0.14 | <0.001 | −0.21 | −0.37 to −0.04 | 0.014 |
| MRA | 0.33 | 0.19 to 0.53 | 0.001 | 0.23 | 0.11 to 0.36 | <0.001 |

Renin and aldosterone levels were expressed by natural logarithm transformation.
*Other aetiology was considered as the reference group among HF etiologies.
ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; CI, confidence interval; eGFR, estimated glomerular filtration rate; HF, heart failure; MRA, mineralocorticoid receptor antagonist.

levels (per each tertile increase; *Figure 1*). Similar results were found in patients without MRA prescription (*Figure S2*). Compared with the lowest tertile, the highest renin tertile was associated with an increased rate of the primary outcome [adjusted-HR (95% CI) = 1.48 (1.25–1.76), $P < 0.001$] (*Table 3*). Concordantly, highest renin levels were associated with increased rates of all-cause mortality and cardiovascular mortality (*Table S3*). The association of renin levels with the primary outcome after adjustment for the BIOSTAT-CHF risk models using restricted cubic spline regression analysis is shown in *Figure 2*. A log-normalized renin above 6.55 (= 700μIU/mL) was associated with a higher incidence of the primary outcome.

Higher aldosterone levels were not associated with the primary outcome [adjusted-HR (95% CI) = 1.09 (0.92–1.28), *P* = 0.32] (*Table 3*). Similar results were found for all-cause and cardiovascular mortality (Supporting Information, *Figure S3*).

There was no interaction between renin and aldosterone on the primary outcome (*P* value > 0.1). As a sensitivity analysis, the associations of renin and aldosterone levels with the primary outcome in ambulatory and hospitalized patients are shown in *Table S4*. Furthermore, survival analyses for the primary outcome across European regions are also presented in *Table S5*.

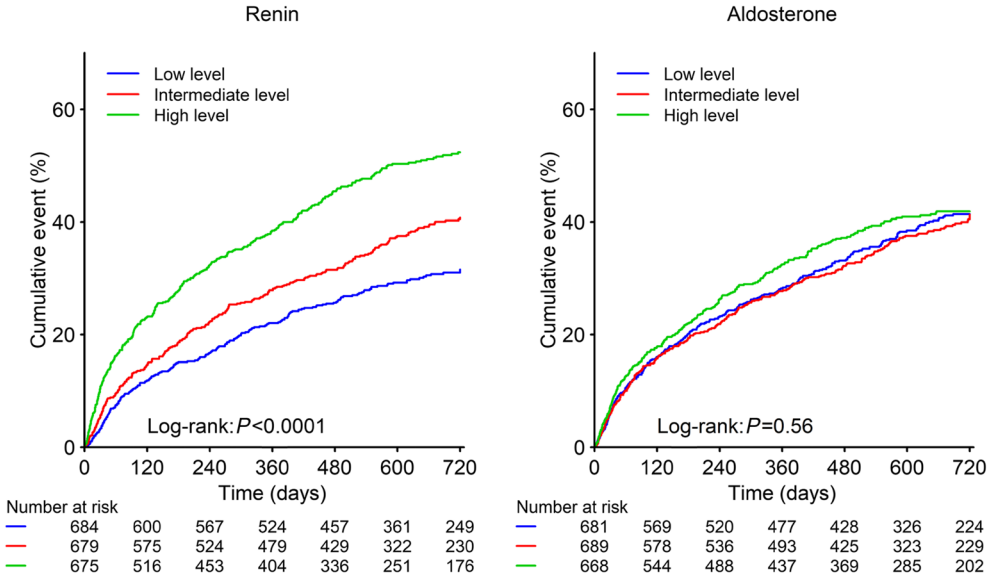
## Renin and aldosterone on top of the BIOSTAT-CHF risk model

Renin and aldosterone levels did not improve risk stratification on top of the BIOSTAT-CHF risk model [for renin: increased c-index (95% CI) = 0.28 (−0.33–0.87), *P* = 0.37; for aldosterone; 0.03 (−0.05–0.102), *P* = 0.51] (*Table 4*). The discriminative value of renin and aldosterone across baseline HF treatment strata is shown in *Table S6*. A consistent absence of discriminative value of renin and aldosterone was observed across treatment strata.

## Aldosterone-to-renin ratio

The median aldosterone-to-renin ratio (ARR) was 0.11 (IQR 0.03-0.31) (ng/dL)/(μIU/mL). Patients with lower ARR (driven by higher renin levels) were more often male patients, had more often cardiovascular comorbidities, lower SBP, LVEF, sodium concentrations, and poorer renal function, and were more often prescribed ACEi/ARBs (*Table S7*). Compared with the highest tertile, the lowest ARR was associated with an increased rate of the primary outcome [adjusted-HR (95% CI) = 1.31 (1.11–1.55), *P* = 0.002] (*Table S8*) but did not improve

**Figure 1** Survival curves for the primary outcome according to renin and aldosterone levels in BIOlogy study to Tailored Treatment in Chronic Heart Failure study.




risk stratification on top of the BIOSTAT-CHF risk model [increased c-index (95% CI) = 0.15 (−0.25–0.55), $P$ = 0.47].

## Discussion

In patients with symptomatic HF, we assessed the clinical determinants and prognostic implications of baseline renin and aldosterone levels. Our main findings are as follows: (1) higher baseline renin and aldosterone levels were associated with HF severity, worse symptoms, poorer renal function and were influenced by treatment with ACEi/ARBs, beta blockers, and MRAs; (ii) higher renin but not aldosterone was independently associated with poor prognosis; (iii) renin and aldosterone levels did not improve risk stratification on top of the 'best' BIOSTAT-CHF prognostic models; and (iv) initiation of MRAs was associated with increased levels of renin and aldosterone in the EPHESUS substudy and in an AHF cohort (PORTO).

### Clinical determinants of renin–angiotensin–aldosterone system activation

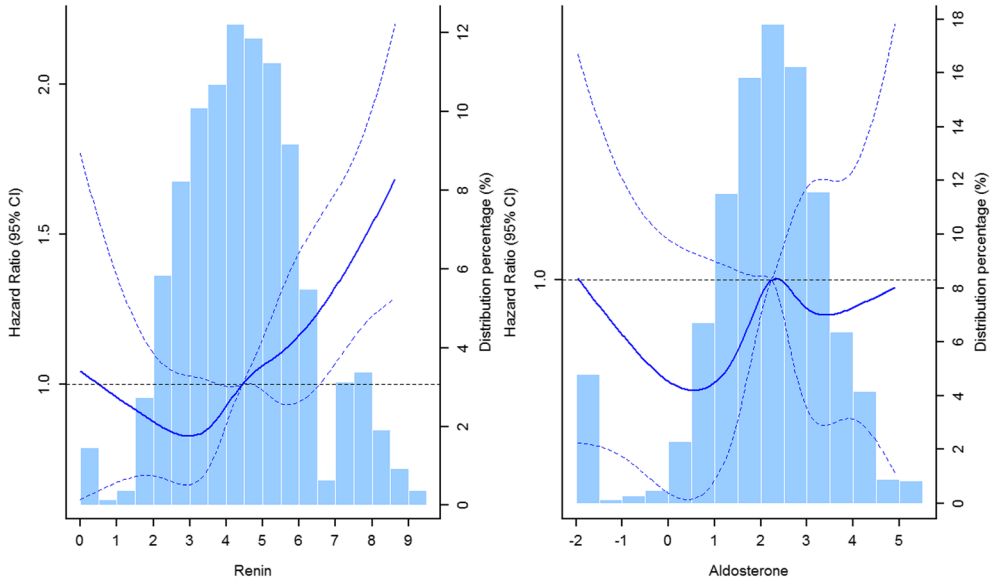
In response to a decrease in baroreceptor stretch, a rise in renin levels ultimately result in sodium and water retention by triggering sequential activation of peptides in the RAAS cascade such as angiotensin II and aldosterone.[22–24] Angiotensin

**Table 3** Cox proportional hazards models of renin and aldosterone levels for the primary outcome in BIOlogy study to Tailored Treatment in Chronic Heart Failure study

| | | | Univariable model | | Multivariable model | |
|---|---|---|---|---|---|---|
| | | | HR (95 % CI) | $P$ value | HR (95% CI) | $P$ value |
| Renin | Continuous | | 1.20 (1.16–1.25) | <0.001 | 1.11 (1.06–1.15) | <0.001 |
| | Tertiles | Low | (reference) | | (reference) | |
| | | Intermediate | 1.34 (1.12–1.61) | 0.001 | 1.17 (0.98–1.41) | 0.08 |
| | | High | 2.01 (1.70–2.38) | <0.001 | 1.48 (1.25–1.76) | <0.001 |
| Aldosterone | Continuous | | 1.01 (0.96–1.07) | 0.58 | 1.02 (0.97–1.07) | 0.53 |
| | Tertiles | Low | (reference) | | (reference) | |
| | | Intermediate | 0.96 (0.82–1.13) | 0.65 | 1.10 (0.93–1.29) | 0.28 |
| | | High | 1.05 (0.89–1.24) | 0.55 | 1.09 (0.92–1.28) | 0.32 |
| | Interaction between renin and aldosterone | Continuous | | 0.06 | | 0.14 |
| | | Categorical | | 0.08 | | 0.13 |

Renin and aldosterone levels as continuous variables were expressed by natural logarithm transformation.
CI, confidence interval; HR, hazard ratio.

**Figure 2** Restricted cubic spline regression for the associations of renin or aldosterone with the primary outcome in BIOlogy study to Tailored Treatment in Chronic Heart Failure study.




II and aldosterone may also play a crucial role in promoting kidney damage by regulating inflammation and reparative processes that follow the tissue fibrosis.[25–27] These mechanisms may explain our observations that high renin and aldosterone levels were associated with lower SBP, lower sodium levels, and poorer renal function.[3–5,8,28] In addition, previous studies have shown that renin was overexpressed in visceral and perivascular adipose tissue in an obese population, which may explain the association between renin and body mass index in the present study.[29,30] A prior HF admission and specific HF etiologies, e.g. patients with an ischemic aetiology or dilated cardiomyopathy (particularly the latter), may partly contribute to progression of ventricular remodelling,[31–33] resulting in a higher degree of (excessive) RAAS activation.[1,34]

Circulating levels of renin and aldosterone are also influenced by HF treatment. Renin is upregulated in response to activation of the sympathetic nervous system,[35] hence explaining the association between beta blocker treatment and lower renin and aldosterone levels.[36] Moreover, the current analysis also showed the association of higher doses of ACEi/ARB with lower aldosterone, suggesting that despite the aldosterone 'escape' phenomenon,[37,38] a chronic decrease of aldosterone levels in patients taking ACEi/ARB therapy may occur. On the other hand, increased renin and aldosterone levels in patients treated with MRAs are consistent with previous reports.[4,5,39,40] This is likely related to an increase in angiotensin II via feedback mechanisms of the RAAS cascade or by direct regulation of aldosterone synthase by MRA treatment.[28,41,42] Indeed, the present analysis showed continuous increases in renin and aldosterone after administration with MRAs.

## Association of renin and aldosterone with outcomes

Median baseline levels of renin (85.3 μIU/mL) and aldosterone (9.4 ng/dL) in this cohort were lower than in other recent reports,[3,5,28,43,44] potentially being influenced by the relative clinical stability and insufficient blockage of RAAS cascade in the current study. We show that renin (but not aldosterone) was associated with the primary outcome (all-cause

**Table 4** Discrimination of renin and aldosterone levels for the primary Outcome in BIOSTAT-CHF study

| | | c-index (95% CI) | *P* value | Increased c-index | *P* value |
|---|---|---|---|---|---|
| Renin model | BIOSTAT-CHF risk model | 76.5 (74.5 to 78.6) | <0.001 | | |
| | + Renin | 76.8 (74.7 to 78.8) | <0.001 | 0.27 (−0.33 to 0.87) | 0.37 |
| Aldosterone model | BIOSTAT-CHF risk model | 76.5 (74.5 to 78.6) | <0.001 | | |
| | + Aldosterone | 76.5 (74.5 to 78.6) | <0.001 | 0.03 (−0.05 to 0.102) | 0.51 |

C-statistic was calculated to compare the discriminatory power to predict primary outcome of baseline renin and aldosterone levels on top of the BIOSTAT-CHF risk model. Renin and aldosterone levels as continuous variables are expressed by natural logarithm transformation. BIOSTAT-CHF, BIOlogy study to Tailored Treatment in Chronic Heart Failure; CI, confidence interval.

mortality and/or HF admission). Studies examining the prognostic value of these biomarkers in the field of HF have yielded conflicting results. In a post hoc analysis of EVEREST, aldosterone levels were assessed in 1,850 placebo-treated patients with AHF and a LVEF≤40%.[3] During a median follow up of 9.9 months (during which 19.0% of patients died), the highest quartile of aldosterone was significantly associated with higher incidence of all-cause mortality. A post hoc analysis of diuretic optimization strategies in acute heart failure and cardiorenal rescue study in acute decompensated heart failure assessed renin and aldosterone at baseline in 427 patients with AHF.[5] Within 60 days, 6% patients died, and 30% were hospitalized. Renin and aldosterone were not associated with the composite outcome of death or HF rehospitalization. A recent report of the Aliskiren Trial on Acute Heart Failure Outcomes (ASTRONAUT) assessed baseline renin in 1,306 patients in both the aliskiren and placebo arms.[4] Here, increasing renin levels were associated with poorer prognosis. In the Valsartan Heart Failure Trial,[45] baseline renin level was associated with a higher incidence of mortality, while aldosterone was not. This is in line with findings of several smaller observational studies.[6,7,46] To the best of our knowledge, our study is first to assess the (lack of) prognostic value of renin and aldosterone on top of a well-calibrated risk model. By demonstrating a lack of discriminatory prognostic improvement, our findings suggest a limited prognostic utility of renin and aldosterone.

### Clinical implications

In the present analysis, we demonstrated that renin and aldosterone levels were mainly associated with the patients' clinical severity (e.g. prior HF admission, lower SBP, sodium concentration, and poorer renal function) and by the used therapies (e.g. ACEi/ARB and MRAs) in consistency with the existing literature. Importantly, these biomarkers do not improve risk prediction on top of an already well-performing clinical risk score. Furthermore, there was a consistent lack of discriminative value of renin and aldosterone levels across HF treatment regimens or different European regions. Consequently, a 'point' measurement of renin and aldosterone levels in patients with decompensated HF should be of clinically limited utility.

## Limitations

Our study has several limitations. This is a post hoc analysis of the BIOSTAT-CHF; hence, the limitations inherent to observational data are present herein, and causality cannot be inferred. By design, BIOSTAT-CHF enrolled patients not on optimal guideline medical therapy. Although this condition is frequent, results may not be generalizable to patients on optimal therapy. All renin and aldosterone samples were frozen and thawed with a same number of freeze/thaw cycles. It may be associated with increased level of renin.[47,48] However, renin level in this study was lower than that in previous reports[18,28,43,44]; the effect of freeze/thaw cycles therefore may be limited.

## Conclusions

Renin and aldosterone activation were associated with both the patients' poor clinical condition (neurohormonal activation) and HF treatments (feedback mechanism). Renin and/or aldosterone did not improve risk stratification. These findings suggest that the 'point' measurement of these biomarkers in patients with HF is of limited utility, both for ascertaining the patients' clinical condition and prognosis (as they may reflect both the disease severity and the use of life-saving therapies).

## Conflict of interest

PR reports personal fees (consulting) from Novartis, Relypsa, AstraZeneca, Grünenthal, Stealth Peptides, Fresenius, Idorsia, Vifor Fresenius Medical Care Renal Pharma, Vifor and CTMA; lecture fees from Bayer and CVRx; cofounder ofCardioRenal. All the other authors have no conflicts of interest to disclose with regards to the present manuscript.

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## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1**. Multivariable Model for the Associations of Clinical Profiles with the Highest Tertile of Renin and Aldosterone Levels in BIOSTAT-CHF study.

**Table S2.** Associations of ACEi/ARB and MRA with Renin and Aldosterone Levels at Baseline in BIOSTAT-CHF study.
**Table S3.** Cox Hazard Models of Renin and Aldosterone Levels for the Clinical Outcomes in BIOSTAT-CHF study.
**Table S4.** Cox Hazard Models of Renin and Aldosterone Levels for the Primary Outcome in Ambulant and Hospitalized Patients in BIOSTAT-CHF study.
**Table S5.** Survival Analyses for the Primary Outcome according to Different European Regions in the BIOSTAT-CHF Study.
**Table S6.** Discrimination of Renin and Aldosterone Levels for the Primary Outcome in BIOSTAT-CHF study across Heart Failure Treatment Regimens.
**Table S7.** Patients' Characteristics according to Aldosterone-to-Renin Ratio (Tertiles).
**Table S8.** Cox Hazard Models of Aldosterone-to-Renin Ratio for the Clinical Outcomes.
**Figure S1.** Changes in Renin and Aldosterone by Mineralocorticoid Receptor Antagonist (Eplerenone and Spironolactone) in EPHESUS and PORTO Studies.
**Figure S2.** Survival Curves for the Primary Outcome according to Renin and Aldosterone Levels in Patients without MRAs Prescription in BIOSTAT-CHF study.
**Figure S3.** Associations of Renin and Aldosterone with Composite Outcome, All-Cause Mortality and Cardiovascular Mortality in BIOSTAT-CHF study.

## References


1. Weber KT. Aldosterone in congestive heart failure. *N Engl J Med* 2001; **345**: 1689–1697.
2. Schrier RW, Abraham WT. Hormones and hemodynamics in heart failure. *N Engl J Med* 1999; **341**: 577–585.
3. Girerd N, Pang PS, Swedberg K, Fought A, Kwasny MJ, Subacius H, Konstam MA, Maggioni A, Gheorghiade M, Zannad F, investigators E. Serum aldosterone is associated with mortality and re-hospitalization in patients with reduced ejection fraction hospitalized for acute heart failure: analysis from the EVEREST trial. *Eur J Heart Fail* 2013; **15**: 1228–1235.
4. Vaduganathan M, Cheema B, Cleveland E, Sankar K, Subacius H, Fonarow GC, Solomon SD, Lewis EF, Greene SJ, Maggioni AP, Bohm M, Zannad F, Butler J, Gheorghiade M. Plasma renin activity, response to aliskiren, and clinical outcomes in patients hospitalized for heart failure: the ASTRONAUT trial. *Eur J Heart Fail* 2018; **20**: 677–686.
5. Mentz RJ, Stevens SR, DeVore AD, Lala A, Vader JM, AbouEzzeddine OFF, Khazanie P, Redfield MM, Stevenson LW, O'Connor CM, Goldsmith SR, Bart BA, Anstrom KJ, Hernandez AF, Braunwald E and Felker GM. Decongestion strategies and renin-angiotensin-aldosterone system activation in acute heart failure. *JACC Heart Fail* 2015; **3**: 97-107.
6. Vergaro G, Emdin M, Iervasi A, Zyw L, Gabutti A, Poletti R, Mammini C, Giannoni A, Fontana M, Passino C. Prognostic value of plasma renin activity in heart failure. *Am J Cardiol* 2011; **108**: 246–251.
7. Ueda T, Kawakami R, Nishida T, Onoue K, Soeda T, Okayama S, Takeda Y, Watanabe M, Kawata H, Uemura S, Saito Y. Plasma renin activity is a strong and independent prognostic indicator in patients with acute decompensated heart failure treated with renin-angiotensin system inhibitors. *Circ J* 2015; **79**: 1307–1314.
8. Rossi F, Mascolo A, Mollace V. The pathophysiological role of natriuretic peptide-RAAS cross talk in heart failure. *Int J Cardiol* 2017; **226**: 121–125.
9. McMurray J, Pfeffer MA. New therapeutic options in congestive heart failure: Part I. *Circulation* 2002; **105**: 2099–2106.
10. Voors AA, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Hillege HL, Lang CC, Ter Maaten JM, Ng L, Ponikowski P, Samani NJ, van Veldhuisen DJ, Zannad F, Zwinderman AH, Metra M. A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. *Eur J Heart Fail* 2016; **18**: 716–726.
11. Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurley S, Kleiman J, Gatlin M. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med* 2003; **348**: 1309–1321.
12. Ferreira JP, Santos M, Almeida S, Marques I, Bettencourt P, Carvalho H. Mineralocorticoid receptor antagonism in acutely decompensated chronic heart failure. *Eur J Intern Med* 2014; **25**: 67–72.
13. Ouwerkerk W, Voors AA, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Hillege HL, Lang CC, Ter Maaten JM, Ng LL, Ponikowski P, Samani NJ, van Veldhuisen DJ, Zannad F, Metra M, Zwinderman AH. Determinants and clinical outcome of uptitration of ACE-inhibitors and beta-blockers in patients with heart failure: a prospective European study. *Eur Heart J* 2017; **38**: 1883–1890.
14. Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJ, Ponikowski P, Poole-Wilson PA, Stromberg A, van Veldhuisen DJ, Atar D, Hoes AW, Keren A, Mebazaa A, Nieminen M, Priori SG, Swedberg K. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the task force for the diagnosis and treatment of acute and chronic heart failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur J Heart Fail* 2008; **10**: 933–989.
15. Pitt B, Williams G, Remme W, Martinez F, Lopez-Sendon J, Zannad F, Neaton J, Roniker B, Hurley S, Burns D, Bittman R, Kleiman J. The EPHESUS trial: eplerenone in patients with heart failure due to systolic dysfunction complicating acute myocardial infarction. Eplerenone post-AMI heart failure efficacy and survival study. *Cardiovasc Drugs Ther* 2001; **15**: 79–87.
16. Ter Maaten JM, Voors AA, Damman K, van der Meer P, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Hillege HL, Lang CC, Metra M, Navis G, Ng L, Ouwerkerk W, Ponikowski P, Samani NJ, van Veldhuisen DJ, Zannad F, Zwinderman AH, de Borst MH. Fibroblast growth factor 23 is related to profiles indicating volume overload, poor therapy optimization and prognosis in patients with new-onset and worsening heart failure. *Int J Cardiol* 2018; **253**: 84–90.
17. Morganti A. A comparative study on inter and intralaboratory reproducibility of renin measurement with a conventional enzymatic method and a new chemiluminescent assay of immunoreactive renin. *J Hypertens* 2010; **28**: 1307–1312.
18. Tsutamoto T, Sakai H, Tanaka T, Fujii M, Yamamoto T, Wada A, Ohnishi M, Horie M. Comparison of active renin

concentration and plasma renin activity as a prognostic predictor in patients with heart failure. *Circ J* 2007; **71**: 915–921.
19. Hartman D, Sagnella GA, Chesters CA, Macgregor GA. Direct renin assay and plasma renin activity assay compared. *Clin Chem* 2004; **50**: 2159–2161.
20. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150**: 604–612.
21. Voors AA, Ouwerkerk W, Zannad F, van Veldhuisen DJ, Samani NJ, Ponikowski P, Ng LL, Metra M, Ter Maaten JM, Lang CC, Hillege HL, van der Harst P, Filippatos G, Dickstein K, Cleland JG, Anker SD, Zwinderman AH. Development and validation of multivariable models to predict mortality and hospitalization in patients with heart failure. *Eur J Heart Fail* 2017; **19**: 627–634.
22. Verbrugge FH, Steels P, Grieten L, Nijst P, Tang WH, Mullens W. Hyponatremia in acute decompensated heart failure: depletion versus dilution. *J Am Coll Cardiol* 2015; **65**: 480–492.
23. Schrier RW. Role of diminished renal function in cardiovascular mortality: marker or pathogenetic factor? *J Am Coll Cardiol* 2006; **47**: 1–8.
24. Schrier RW, De Wardener HE. Tubular reabsorption of sodium ion: influence of factors other than aldosterone and glomerular filtration rate. 2. *N Engl J Med* 1971; **285**: 1292–1303.
25. Brewster UC, Perazella MA. The renin-angiotensin-aldosterone system and the kidney: effects on kidney disease. *Am J Med* 2004; **116**: 263–272.
26. Wolf G. Angiotensin II as a mediator of tubulointerstitial injury. *Nephrol Dial Transplant* 2000; **15**: 61–63.
27. Weber KT, Swamynathan SK, Guntaka RV, Sun Y. Angiotensin II and extracellular matrix homeostasis. *Int J Biochem Cell Biol* 1999; **31**: 395–403.
28. Pavo N, Goliasch G, Wurm R, Novak J, Strunk G, Gyongyosi M, Poglitsch M, Saemann MD, Hulsmann M. Low- and high-renin heart failure phenotypes with clinical implications. *Clin Chem* 2018; **64**: 597–608.
29. Lastra G, Sowers JR. Obesity and cardiovascular disease: role of adipose tissue, inflammation, and the renin-angiotensin-aldosterone system. *Horm Mol Biol Clin Invest* 2013; **15**: 49–57.
30. Engeli S, Negrel R, Sharma AM. Physiology and pathophysiology of the adipose tissue renin-angiotensin system. *Hypertension* 2000; **35**: 1270–1277.
31. Gheorghiade M, De Luca L, Fonarow GC, Filippatos G, Metra M, Francis GS. Pathophysiologic targets in the early phase of acute heart failure syndromes. *Am J Cardiol* 2005; **96**: 11G–17G.
32. Assomull RG, Prasad SK, Lyne J, Smith G, Burman ED, Khan M, Sheppard MN, Poole-Wilson PA, Pennell DJ. Cardiovascular magnetic resonance, fibrosis, and prognosis in dilated cardiomyopathy. *J Am Coll Cardiol* 2006; **48**: 1977–1985.
33. Schuster A, Morton G, Chiribiri A, Perera D, Vanoverschelde JL, Nagel E. Imaging in the management of ischemic cardiomyopathy: special focus on magnetic resonance. *J Am Coll Cardiol* 2012; **59**: 359–370.
34. Hirsch AT, Pinto YM, Schunkert H, Dzau VJ. Potential role of the tissue renin-angiotensin system in the pathophysiology of congestive heart failure. *Am J Cardiol* 1990; **66**: 22D–30D discussion 30D-32D.
35. Berl T, Henrich WL, Erickson AL, Schrier RW. Prostaglandins in the beta-adrenergic and baroreceptor-mediated secretion of renin. *Am J Physiol* 1979; **236**: F472–F477.
36. Buhler FR, Laragh JH, Baer L, Vaughan ED Jr, Brunner HR. Propranolol inhibition of renin secretion. A specific approach to diagnosis and treatment of renin-dependent hypertensive diseases. *N Engl J Med* 1972; **287**: 1209–1214.
37. Zannad F. Angiotensin-converting enzyme inhibitor and spironolactone combination therapy. New objectives in congestive heart failure treatment. *Am J Cardiol* 1993; **71**: 34a–39a.
38. Cleland JG, Dargie HJ, Hodsman GP, Ball SG, Robertson JI, Morton JJ, East BW, Robertson I, Murray GD, Gillen G. Captopril in heart failure. A double blind controlled trial. *Br Heart J* 1984; **52**: 530–535.
39. Ferreira JP, Santos M, Almeida S, Marques I, Bettencourt P, Carvalho HC. High-dose spironolactone changes renin and aldosterone levels in acutely decompensated heart failure. *Cor Vasa* 2014; **56**: 463–470.
40. Rousseau MF, Gurne O, Duprez D, Van Mieghem W, Robert A, Ahn S, Galanti L, Ketelslegers JM. Beneficial neurohormonal profile of spironolactone in severe congestive heart failure: results from the RALES neurohormonal substudy. *J Am Coll Cardiol* 2002; **40**: 1596–1601.
41. Rousseau MF, Konstam MA, Benedict CR, Donckier J, Galanti L, Melin J, Kinan D, Ahn S, Ketelslegers JM, Pouleur H. Progression of left ventricular dysfunction secondary to coronary artery disease, sustained neurohormonal activation and effects of ibopamine therapy during long-term therapy with angiotensin-converting enzyme inhibitor. *Am J Cardiol* 1994; **73**: 488–493.
42. Eudy RJ, Sahasrabudhe V, Sweeney K, Tugnait M, King-Ahmad A, Near K, Loria P, Banker ME, Piotrowski DW, Boustany-Kari CM. The use of plasma aldosterone and urinary sodium to potassium ratio as translatable quantitative biomarkers of mineralocorticoid receptor antagonism. *J Transl Med* 2011; **9**: 180.
43. Kanno Y, Yoshihisa A, Watanabe S, Takiguchi M, Yokokawa T, Sato A, Miura S, Shimizu T, Nakamura Y, Abe S, Sato T, Suzuki S, Oikawa M, Saitoh S, Takeishi Y. Prognostic significance of insomnia in heart failure. *Circ J* 2016; **80**: 1571–1577.
44. Meijers WC, van der Velde AR, Muller Kobold AC, Dijck-Brouwer J, Wu AH, Jaffe A, de Boer RA. Variability of biomarkers in patients with chronic heart failure and healthy controls. *Eur J Heart Fail* 2017; **19**: 357–365.
45. Latini R, Masson S, Anand I, Salio M, Hester A, Judd D, Barlera S, Maggioni AP, Tognoni G, Cohn JN, Val-He FTI. The comparative prognostic value of plasma neurohormones at baseline in patients with heart failure enrolled in Val-HeFT. *Eur Heart J* 2004; **25**: 292–299.
46. Nijst P, Verbrugge FH, Martens P, Bertrand PB, Dupont M, Francis GS, Tang WW, Mullens W. Plasma renin activity in patients with heart failure and reduced ejection fraction on optimal medical therapy. *J Renin Angiotensin Aldosterone Syst* 2017; **18**: 1470320317729919.
47. Kley HK, Rick W. The effect of storage and temperature on the analysis of steroids in plasma and blood. *J Clin Chem Clin Biochem Zeitschrift fur klinische Chemie und klinische Biochem* 1984; **22**: 371–378.
48. Hillebrand JJ, Heijboer AC, Endert E. Effects of repeated freeze-thaw cycles on endocrine parameters in plasma and serum. *Ann Clin Biochem* 2017; **54**: 289–292.