**Supplemental Material**

**Assessment of Proximal Tubular Function by Tubular Maximum Phosphate Reabsorption Capacity in Heart Failure**

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**Supplementary Method 1. Measurement of lithium by inductively coupled plasma-mass spectrometer**

**Principle of measurement**

After dilution the sample is sprayed in an argon torch of at least 6000°C. This destructs the matrix and leads the to be measured elements to the mass spectrometer for measurement. A calibration curve is used to determine quantities of the measured elements.

**Serum/EDTA plasma**

**Necessary reagents and other materials**

- Water = ultra pure

- Triton X-100, Sigma-Aldrich Pcode 101371896

- Titriplex III, Merck art.: 1.08418.1000

- Nitric acid 65 % p.a. Merck art. 456

- Lithium standard 1000 mg/L Merck art. nr.: 70223

- Yttrium standard 1000 mg/L Merck art. nr.: 18909

- Nitric acid 1%: dilute 100 ml nitric acid 65% with water to 10 liters

- Washing agent: dilute 50 μl concentrate solution for heavy metals to 100 ml with water.

- Concentrate solution for heavy metals: weigh 10 grams Triton X-10 and dissolve under slight heating in water, and let it cool off. Add 10 grams Titriplex III and add water until 100ml.

**Calibrator stock solution:**

The Lithium standard contains 1000 mg/L lithium.

**Internal standard solution:**

Dilute 0,1 ml Yttrium with nitric acid 1% to 1,0 liter.

**Preparation of calibrators:**

- Pipet 100 μL Lithium standard and add until 100,0 mL with nitric acid 1% (V1= 1000 μg/L).

- Dilute 250 μL solution V1 with nitric acid 1% to 50 mL (V2= 5 μg/L).

- Prepare the calibrators according to the scheme below.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Calibrator | 0  | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
| mL V2 | 0  | 0.05  | 0.10  | 0.20  | 0.40  | 0.60  | 0.80  | 1.00  |
| mL nitric acid 1% | 1.00  | 0.95  | 0.90  | 0.80  | 00.60  | 0.40  | 0.20  | 0.00  |
| Concentration µg/L | 0.00  | 0.25  | 0.50  | 1.00  | 2.00  | 3.00  | 4.00  | 5.00  |

**Preparation of control samples:**

- Pipet 100 μL Lithium standard and add until 100 mL with nitric acid 1% (V1= 1000 μg/L).

- Dilute 250 μL solution V1 with nitric acid 1% tot 50 mL (V2= 5 μg/L).

- Prepare the controls according to the scheme below.

|  |  |  |  |
| --- | --- | --- | --- |
| Control | Low  | Med  | High  |
| mL V2 | 0.15  | 0.50  | 0.90  |
| mL nitric acid 1% | 0.85  | 0.50  | 0.10  |
| Concentration µg/L | 0.75  | 2.50  | 4.50  |

**Machine**

iCAP TQ SOP 50240 ICP mass spectrometer ( iCAP TQ Thermo Scientific)

**Volume**

At least 2 ml blood or 1 ml serum or plasma.

**Treatment of sample at laboratory**

**Centrifuge**

Centrifuge the sample at ± 2000 rcf. at room temperature. Transport the serum into a clean, labelled tube.

**Execution**

- Mix the sample.

- Centrifuge 5 min. at ± 2000 rcf.

- Pipet from all calibrators, controls and patients 200 μL in a polypropylene tube of 10 mL and add 2.0 ml internal standard solution.

- Mix and measure.

- Measure each time after ± 20 samples a blank and control or end the measurement series with a blank or control.

**Results**

**Calculation**

The software of ICP-MS calculates from the calibration data the quantities by using linear regression.

Values smaller than 0.5 µg/L are being subtracted with the value of the blank.

**Control and registration**

If the measured values are approved, the results are exported in an Excel format.

**Limit of Quantitation**

0.25 μg/L

**Measurement is applicable to ranges of:**

0.25 - 5.0 μg/L

**Urine**

**Necessary reagents and other materials**

- Water = ultra pure

- Nitric acid 65 % p.a. Merck art. 456

- Lithium standard 1000 mg/L Merck art. nr.: 70223

- Yttrium standard 1000 mg/L Merck art. nr.: 18909

- Washing agent (nitric acid 1%): dilute 100 ml nitric acid 65% with water to 10 liter

- Diluting agent: dilute 0,1 ml Yttrium with washing agent to 1 liter

**Calibrator stock solution**

The Lithium standard contains 1000 mg/L lithium

**Internal standard solution**

The internal standard Yttrium is dissolved in the diluting agent.

**Preparation of calibrators**

- Pipet 100 μL Lithium standard and add with nitric acid 1% to 100,0 ml (V1= 1000 μg/L)

- Dilute 2.5 mL solution V1 with nitric acid 1% to 50 mL (V2= 50 μg/L)

- Prepare the calibrators according to the scheme below.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Calibrator | 0  | 1  | 2  | 3  | 4  | 5  | 6  |
| mL V2 | 0  | 0.10  | 0.20  | 0.40  | 0.60  | 0.80  | 1.00  |
| mL nitric acid | 1.00  | 0.90  | 0.80  | 0.60  | 0.40  | 0.20  | 0.00  |
| Concentration µg/L | 0.00  | 5.00  | 10.0  | 20.0  | 30.0  | 40.0  | 50.0  |

**Preparation of control samples**

- Pipet 100 μL Lithium standard and add with nitric acid 1% to 100 mL (V1= 1000 μg/L)

- Dilute 2.5 mL solution V1 with nitric acid 1% to 50 mL (V2= 50 μg/L)

- Prepare the controls according to the scheme below.

|  |  |  |  |
| --- | --- | --- | --- |
| Control | Low  | Med  | High  |
| mL V2 | 0.15  | 0.50  | 0.90  |
| mL nitric acid 1% | 0.85  | 0.50  | 0.10  |
| Concentration µg/L | 7.50  | 25.0  | 45.0  |

**Machine**

iCAP TQ SOP 50240 ICP massa spectrometer ( iCAP TQ Thermo Scientific)

**Volume**

At least 1 mL urine.

**Centrifuge**

Centrifuge the sample at ± 2000 rcf. at room temperature. Optional to transfer Breng de urine eventueel over in een schone gelabelde buis.

**Execution**

- Mix the sample.

- Centrifuge 5 minutes at ± 2000 rcf.

- Pipet of all calibrators, controls, and patients samples of 50 μL in a polypropylene tube of 10 mL and add 2 mL of diluting agent.

- Mix and measure the solutions.

- Measure each time after ± 20 samples a blank and control or end the measurement series with a blank or control.

**Results**

**Calculation**

The software of ICP-MS calculates from the calibration data the quantities by using linear regression.

Values smaller than 0.5 µg/L are being subtracted with the value of the blank.

**Control and registration**

If the measured values are approved, the results are exported in an Excel format.

**Limit of Quantitation**

5 μg/L

**Measurement is applicable to ranges of:**

5-50 μg/L

**Regarding the analysis**

The regression coefficients of the calibration line in water and urine are identical and independent from creatinine.

The method is linear until 50 μg/L.

**Supplemental Table 1. Baseline characteristics of study subset and patients without TmP/GFR measurements in BIOSTAT-CHF**

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | Study subsetN = 2,085 | Patients without TmP/GFR measurementsN = 431 | P-value |
| *Clinical characteristics* |
| Age (years) | 69 ± 12 | 69 ± 12 | 0.453 |
| Sex (male), *n* (%) | 1526 (73) | 319 (74) | 0.715 |
| BMI (kg/m2) | 27 (24 – 31) | 27 (24 – 31) | 0.318 |
| NYHA classification (III/IV), *n* (%) | 1250 (60) | 272 (63) | 0.405 |
| Systolic blood pressure (mmHg) | 125 ± 21 | 125 ± 22 | 0.988 |
| Diastolic blood pressure (mmHg) | 75 ± 13 | 75 ± 13 | 0.582 |
| Heart rate (bpm) | 76 (67 – 90) | 76 (66 – 90) | 0.574 |
| Presence of atrial fibrillation/flutter, *n* (%) | 693 (33) | 127 (29) | 0.160 |
| LVEF (%) | 30 (25 – 37) | 30 (25 – 35) | 0.305 |
| Diabetes mellitus, *n* (%) | 675 (32) | 144 (33) | 0.695 |
| Smoking (past or current), *n* (%) | 1311 (63) | 262 (61) | 0.501 |
| *Primary heart failure etiology, n (%)* |
| Ischemic heart disease | 928 (45) | 197 (46) | 0.633 |
| Hypertension | 225 (11) | 31 (7) | 0.032 |
| Cardiomyopathy | 519 (25) | 112 (26) | 0.643 |
| *Medication, n (%)* |
| ACE inhibitor/angiotensin receptor blocker | 1498 (72) | 322 (75) | 0.221 |
| Beta-blocker | 1724 (83) | 368 (85) | 0.164 |
| Loop diuretics | 2075 (99) | 428 (99) | 1 |
| Loop diuretic dose (mg furosemide equivalent) | 40 (40 – 100) | 40 (40 – 120) | 0.326 |
| Aldosterone antagonist | 1096 (53) | 242 (56) | 0.176 |
| *Laboratory values* |
| Hemoglobin (g/dL) | 13.3 (11.9 – 14.5) | 13.4 (11.9 – 14.5) | 0.916 |
| Sodium (mEq/L) | 140 (137 – 142) | 139 (137 – 141) | 0.002 |
| Potassium (mEq/L) | 4.2 (3.9 – 4.6) | 4.2 (3.9 – 4.5) | 0.381 |
| Phosphate (mg/dL) | 2.6 (2.1 – 3.2) | 2.9 (2.4 – 3.4) | <0.001 |
| NT-proBNP (ng/L) | 2728 (1214 – 5932) | 2544 (917 – 4704) | 0.003 |
| ASAT (U/L) | 25 (19 – 35) | 26 (20 – 36) | 0.466 |
| ALAT (U/L) | 25 (17 – 37) | 26 (17 – 41) | 0.251 |
| *Kidney function* |
| Creatinine (mg/dL) | 1.16 (0.95 – 1.47) | 1.16 (0.95 – 1.49) | 0.962 |
| eGFR (mL/min/1.73m2) | 60 ± 23 | 60 ± 23 | 0.948 |
| Plasma NGAL (ng/mL) | 60 (38 – 97) | 62 (38 – 99) | 0.479 |
| Urea (mg/dL) | 68 (46 – 109) | 71 (48 – 112) | 0.337 |
| Urinary creatinine (mg/dL) | 59 (31 – 107) | 57 (29 – 115) | 0.856 |
| Urinary KIM-1(ng/gCr) | 1899 (918 – 3613) | 1625 (751 – 3175) | 0.014 |
| Urinary NGAL (μg/gCr) | 32 (16 – 80) | 28 (14 – 61) | 0.114 |
| UACR (mg/gCr) | 24 (7 – 103) | 18 (6 – 81) | 0.097 |
| FENa (%) | 0.99 (0.44 – 2.22) | 1.41 (0.49 – 3.48) | 0.083 |
| FEUrea (%) | 29 (19 – 41) | 36 (20 – 46) | 0.078 |
| FEPhosphate (%) | 21 (13 – 35) | NA | NA |
| *Neurohormonal activation* |
| Aldosterone (pg/mL) | 94 (44 – 195) | 95 (44 – 204) | 0.619 |
| Renin (µUI/mL) | 89 (29 – 253) | 90 (30 – 305) | 0.736 |
| Aldosterone-renin ratio (ng/dL/ng/mL) | 181 (50 – 515) | 133 (33 – 408) | 0.213 |
| *Biomarkers* |
| FGF23 (RU/mL) | 221 (118 – 591) | 200 (113 – 474) | 0.211 |
| Bio-ADM (pg/mL) | 33 (22 – 54) | 36 (25 – 53) | 0.135 |
| PENK (pmol/L) | 86 (64 – 120) | 87 (60 – 127) | 0.847 |
| Pro-ADM (ng/mL) | 0.50 (0.32 – 0.82) | 0.51 (0.31 – 0.75) | 0.473 |
| Galectin-3 (ng/mL) | 21 (15 – 30) | 22 (16 – 32) | 0.223 |
| GDF-15 (ng/L) | 2734 (1730 – 5613) | 2540 (1581 – 4060) | 0.054 |
| IL-6 (pg/mL) | 5.2 (2.8 – 10.4) | 5.1 (2.9 – 8.7) | 0.305 |
| *Event rates, n (%)* |
| Mortality | 559 (27) | 109 (25) | 0.555 |
| HF hospitalization | 535 (26) | 87 (20) | 0.019 |
| Combined end point | 878 (42) | 155 (36) | 0.021 |

\*Only 275 measurements available

Normally distributed continuous variables are presented as mean ± standard deviation and non-normally distributed continuous variables as median (interquartile range). Abbreviations: ACE, angiotensin-converting enzyme; ALAT, alanine transaminase; ASAT, aspartate aminotransferase; Bio-ADM, bioactive adrenomedullin; BMI, body mass index; eGFR, estimated glomerular filtration rate; FE, fractional excretion; FGF23, fibroblast growth factor 23; gCR, gram of urinary creatinine; GDF‐15, growth differentiation factor 15; IL-6, interleukin 6; KIM-1, kidney injury molecule-1; LVEF, left ventricular ejection fraction; NGAL, Neutrophil Gelatinase-Associated Lipocalin; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association; pro-ADM, proadrenomedullin; TmP/GFR, tubular maximum phosphate reabsorption capacity; UACR, urine albumin-to-creatinine ratio

**Supplemental Table 2. Baseline characteristics according to low/high TmP/GFR and presence of CKD\***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Variable | CKD + Low TmP/GFR, *n* = 635 | CKD + High TmP/GFR, *n* = 443 | P-value | No CKD + Low TmP/GFR, *n* = 408 | No CKD + High TmP/GFR, *n* = 599 | P-value |
| *Clinical characteristics* |
| Age (years) | 74 ± 9 | 73 ± 10 | **0.007** | 65 ± 11 | 63 ± 12 | **<0.001** |
| Sex (male), *n* (%) | 458 (72) | 292 (66) | **0.035** | 316 (77) | 460 (82) | 0.868 |
| BMI (kg/m2) | 27 (24 – 30) | 27 (24 – 30) | 0.646 | 28 (24 – 32) | 27 (24 – 31) | 0.116 |
| NYHA classification (III/IV), *n* (%) | 415 (65) | 292 (66) | 0.562 | 228 (56) | 315 (53) | 0.068 |
| Systolic blood pressure (mmHg) | 124 ± 22 | 124 ± 22 | 0.933 | 128 ± 21 | 124 ± 23 | **0.005** |
| Diastolic blood pressure (mmHg) | 72 ± 13 | 74 ± 14 | 0.058 | 77 ± 12 | 76 ± 14 | 0.250 |
| Heart rate (bpm) | 75 (65 – 85) | 76 (67 – 90) | **0.010** | 77 (68 – 90) | 79 (70 – 91) | 0.389 |
| Presence of atrial fibrillation/flutter, *n* (%) | 229 (36) | 165 (37) | 0.722 | 131 (32) | 168 (28) | 0.178 |
| LVEF (%) | 30 (25 – 38) | 30 (25 – 38) | 0.640 | 30 (25 – 35) | 30 (25 – 35) | 0.566 |
| Diabetes mellitus, *n* (%) | 236 (37) | 162 (37) | 0.892 | 118 (29) | 159 (27) | 0.449 |
| Smoking (past or current), *n* (%) | 397 (63) | 251 (57) | 0.053 | 263 (64) | 400 (67) | **0.004** |
| *Primary heart failure etiology, n (%)* |
| Ischemic heart disease | 345 (54) | 200 (45) | **0.019** | 166 (41) | 217 (36) | 0.602 |
| Hypertension | 57 (9) | 62 (14) | 0.074 | 54 (13) | 52 (7) | **0.012** |
| Cardiomyopathy | 107 (17) | 95 (21) | 0.315 | 116 (28) | 201 (34) | 0.099 |
| *Medication, n (%)* |
| ACE inhibitor/angiotensin receptor blocker | 420 (66) | 302 (68) | 0.528 | 308 (75) | 468 (78) | 0.367 |
| Beta-blocker | 521 (82) | 357 (81) | 0.598 | 339 (83) | 507 (85) | 0.567 |
| Loop diuretics | 633 (99) | 441 (99) | 1 | 407 (99) | 594 (99) | 0.437 |
| Loop diuretic dose (furosemide equivalent) | 80 (40 – 125) | 40 (40 – 120) | **<0.001** | 40 (40 – 80) | 40 (40 – 80) | 0.328 |
| Aldosterone antagonist | 288 (45) | 219 (49) | 0.208 | 241 (59) | 348 (58) | 0.809 |
| *Laboratory values* |
| Hemoglobin (g/dL) | 12.5 (11.1 – 13.7) | 13.1 (11.7 – 14.4) | **<0.001** | 13.5 (12.1 – 14.5) | 14.4 (12.8 – 15.1) | **<0.001** |
| Sodium (mEq/L) | 140 (137 – 142) | 139 (136 – 142) | 0.376 | 140 (138 – 142) | 140 (137 – 141) | **0.049** |
| Potassium (mEq/L) | 4.2 (3.9 – 4.7) | 4.3 (3.9 – 4.7) | 0.248 | 4.2 (3.9 – 4.5) | 4.2 (3.9 – 4.6) | 0.108 |
| Phosphate (mg/dL) | 6.8 (5.6 – 8.0) | 10.2 (9.0 – 11.6)  | **<0.001** | 6.4 (5.3 – 7.3) | 9.6 (8.3 – 11.1) | **<0.001** |
| NT-proBNP (ng/L) | 3992 (1877 – 8728) | 3617 (1702 – 7305) | 0.060 | 1982 (882 – 3999) | 1928 (901 – 4436) | 0.626 |
| ASAT (U/L) | 25 (18 – 35) | 25 (19 – 36) | 0.346 | 25 (20 – 34) | 26 (20 – 36) | 0.284 |
| ALAT (U/L) | 21 (15 – 32) | 23 (16 – 34) | **0.022** | 26 (18 – 38) | 29 (20 – 44) | **0.006** |
| *Kidney function* |
| Creatinine (mg/dL) | 1.54 (1.30 – 1.91) | 1.38 (1.21 – 1.63) | **<0.001** | 0.96 (0.83 – 1.10) | 0.94 (0.80 – 1.07) | 0.069 |
| eGFR (mL/min/1.73m2) | 40 ± 12 | 46 ± 11 | **<0.001** | 78 ± 13 | 81 ± 14 | **<0.001** |
| Plasma NGAL (ng/mL) | 83 (53 – 125) | 74 (46 – 118) | **0.027** | 44 (27 – 67) | 47 (32 – 70) | 0.119 |
| Urea (mg/dL) | 89 (61 – 142) | 81 (55 – 133) | **0.027** | 49 (37 – 76) | 52 (36 – 85) | 0.680 |
| Urinary creatinine (mg/dL) | 48 (28 – 79) | 53 (29 – 104) | **0.005** | 72 (37 – 118) | 71 (33 – 123) | 0.892 |
| Urinary KIM-1(ng/gCr) | 2239 (1136 – 4068) | 2099 (1109 – 4260) | 0.891 | 1729 (751 – 3198) | 1621 (738 – 2921) | 0.397 |
| Urinary NGAL (μg/gCr) | 44 (19 – 115) | 34 (16 – 81) | **0.007** | 28 (12 – 57) | 28 (14 – 56) | 0.922 |
| UACR (mg/gCr) | 44 (10 – 167) | 28 (9 – 112) | **0.015** | 17 (6 – 83) | 16 (6 – 53) | 0.355 |
| FENa (%) | 1.55 (0.71 – 3.39)  | 1.21 (0.49 – 2.50) | **<0.001** | 0.65 (0.34 – 1.31) | 0.66 (0.31 – 1.43) | 0.908 |
| FEUrea (%) | 34 (23 – 45) | 26 (17 – 38) | **<0.001** | 29 (20 – 41) | 27 (17 – 39) | **0.027** |
| FEPhosphate (%) | 41 (29 – 56) | 18 (12 – 25) | **<0.001** | 25 (17 – 33) | 11 (7 – 17) | **<0.001** |
| *Neurohormonal activation* |
| Aldosterone (pg/mL) | 96 (42 – 209) | 103 (49 – 227) | 0.183 | 79 (36 – 161) | 94 (47 – 196) | **0.008** |
| Renin (µUI/mL) | 106 (36 – 299) | 106 (31 – 287) | 0.475 | 67 (21 – 194) | 79 (27 – 238) | 0.061 |
| Aldosterone-renin ratio (ng/dL/ng/mL) | 145 (39 – 443) | 179 (50 – 539) | 0.054 | 218 (53 – 559) | 195 (67 – 548) | 0.805 |
| *Biomarkers* |
| FGF23 (RU/mL) | 387 (175 – 1002) | 296 (148 – 702) | **0.005** | 154 (95 – 359) | 154 (97 – 306) | 0.966 |
| Bio-ADM (pg/mL) | 42 (28 – 68) | 36 (24 – 57) | **<0.001** | 28 (20 – 45) | 27 (19 – 42) | 0.565 |
| PENK (pmol/L) | 118 (89 – 163) | 107 (82 – 140) | **0.001** | 68 (55 – 87) | 68 (53 – 84) | 0.560 |
| Pro-ADM (ng/mL) | 0.73 (0.46 – 1.14) | 0.62 (0.40 – 0.93) | **<0.001** | 0.38 (0.24 – 0.59) | 0.38 (0.25 – 0.59) | 0.985 |
| Galectin-3 (ng/mL) | 26 (19 – 36) | 24 (18 – 33) | **0.030** | 17 (13 – 23) | 18 (13 – 24) | 0.466 |
| GDF-15 (ng/L) | 3706 (2266 – 6276) | 3626 (2241 – 6024) | 0.574 | 2072 (1396 – 3218) | 2057 (1320 – 3395) | 0.861 |
| IL-6 (pg/mL) | 6.2 (3.4 – 12.3) | 5.4 (3.2 – 11.3) | 0.861 | 4.5 (2.4 – 9.7) | 4.5 (2.5 – 8.4) | 0.588 |

\*Defined as eGFR < 60 mL/min/1.73m2

Normally distributed continuous variables are presented as mean ± standard deviation and non-normally distributed continuous variables as median (interquartile range). Abbreviations: ACE, angiotensin-converting enzyme; ALAT, alanine transaminase; ASAT, aspartate aminotransferase; Bio-ADM, bioactive adrenomedullin; BMI, body mass index; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FE, fractional excretion; FGF23, fibroblast growth factor 23; gCR, gram of urinary creatinine; GDF‐15, growth differentiation factor 15; IL-6, interleukin 6; KIM-1, kidney injury molecule-1; LVEF, left ventricular ejection fraction; NGAL, Neutrophil Gelatinase-Associated Lipocalin; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association; pro-ADM, proadrenomedullin; TmP/GFR, tubular maximum phosphate reabsorption capacity; UACR, urine albumin-to-creatinine ratio

**Supplemental Table 3. Baseline characteristics according to low\*, normal, and high TmP/GFR**

|  |
| --- |
| TmP/GFR (mmol/L) |
| Variable | Low, *n* = 13920.53 (0.38 – 0.67)[min: -0.38, max: 0.80] | Normal, *n* = 6000.95 (0.87 – 1.08)[min: 0.80, max: 1.35] | High, *n* = 931.57 (1.46 – 1.76)[min: 1.36, max: 6.81] | **P for trend** |
| *Clinical characteristics* |
| Age (years) | 70.3 ± 11.4 | 66.8 ± 12.6 | 62.9 ± 12.4 | **<0.001** |
| Sex (male), *n* (%) | 1041 (75) | 419 (69) | 66 (71) | **0.035** |
| BMI (kg/m2) | 27 (24 – 30) | 27 (24 – 31) | 26 (24 – 30) | 0.258 |
| NYHA classification (III/IV), *n* (%) | 875 (63) | 327 (55) | 48 (52) | **<0.001** |
| Systolic blood pressure (mmHg) | 125 ± 21 | 124 ± 24 | 122 ± 21 | 0.424 |
| Diastolic blood pressure (mmHg) | 75 ± 13 | 75 ± 15 | 75 ± 13 | 0.352 |
| Heart rate (bpm) | 75 (66 – 88) | 79 (70 – 92) | 78 (68 – 90) | **<0.001** |
| Presence of atrial fibrillation/flutter, *n* (%) | 480 (34) | 189 (32) | 24 (26) | **0.047** |
| LVEF (%) | 30 (25 – 37) | 30 (25 – 35) | 30 (25 – 38) | 0.346 |
| Diabetes mellitus, *n* (%) | 461 (36) | 185 (31) | 29 (31) | 0.346 |
| Smoking (past or current), *n* (%) | 867 (67) | 387 (65) | 57 (61) | 0.581 |
| *Primary HF etiology, n (%)* |
| Ischemic heart disease | 655 (47) | 240 (40) | 33 (35) | **<0.001** |
| Hypertension | 151 (11) | 70 (12) | 4 (4) | 0.396 |
| Cardiomyopathy | 329 (24) | 155 (26) | 35 (38) | **0.009** |
| *Medication, n (%)* |
| ACE inhibitor/angiotensin receptor blocker | 983 (71) | 453 (76) | 62 (67) | 0.293 |
| Beta-blocker | 1153 (89) | 497 (83) | 74 (80) | 0.618 |
| Loop diuretics | 1387 (99) | 596 (99) | 92 (99) | 0.214 |
| Loop diuretic dose (furosemide equivalent) | 40 (40 – 120) | 40 (40 – 80) | 40 (40 – 80) | **<0.001** |
| Aldosterone antagonist | 717 (55) | 323 (54) | 56 (60) | 0.093 |
| *Laboratory values* |
| Hemoglobin (g/dL) | 13.0 ± 1.9 | 13.6 ± 1.8 | 13.7 ± 1.9 | **<0.001** |
| Sodium (mEq/L) | 140 (137 – 142) | 140 (137 – 141) | 139 (136 – 141) | **0.045** |
| Potassium (mEq/L) | 4.2 (3.9 – 4.6) | 4.2 (3.9 – 4.6) | 4.3 (4.1 – 4.7) | **0.024** |
| Phosphate (mg/dL) | 2.3 (1.9 – 2.7) | 3.3 (3.0 – 3.7) | 4.4 (3.9 – 5.0) | **<0.001** |
| NT-proBNP (ng/L) | 2851 (1262 – 6061) | 2584 (1105 – 5879) | 2347 (1153 – 4759) | 0.064 |
| ASAT (U/L) | 25 (19 – 34) | 26 (20 – 36) | 28 (21 – 41) | **0.042** |
| ALAT (U/L) | 23 (16 – 35) | 27 (18 – 38) | 32 (23 – 54) | **<0.001** |
| *Kidney function* |
| Creatinine (mg/dL) | 1.21 (1.00 – 1.59) | 1.10 (0.90 – 1.31) | 1.00 (0.84 – 1.13) | **<0.001** |
| eGFR (mL/min/1.73m2) | 57 ± 22 | 66 ± 21 | 74 ± 23 | **<0.001** |
| Plasma NGAL (ng/mL) | 63 (38 – 102) | 57 (36 – 90) | 62 (40 – 87) | **0.044** |
| Urea (mg/dL) | 71 (47 – 114) | 64 (43 – 99) | 61 (42 – 88) | **<0.001** |
| Urinary creatinine (mg/dL) | 58 (31 – 102) | 63 (31 – 114) | 63 (28 – 113) | 0.086 |
| Urinary KIM-1(ng/gCr) | 1920 (946 – 3632) | 1829 (847 – 3481) | 1649 (731 – 3821) | 0.161 |
| Urinary NGAL (μg/gCr) | 32 (16 – 85) | 31 (15 – 70) | 30 (17 – 66) | 0.057 |
| UACR (mg/gCr) | 27 (8 – 118) | 22 (7 – 83) | 16 (6 – 56) | **0.009** |
| FENa (%) | 1.05 (0.49 – 2.31) | 0.88 (0.38 – 2.02) | 0.66 (0.31 – 1.68) | **<0.001** |
| FEUrea (%) | 31 (21 – 42) | 26 (17 – 39) | 22 (15 – 36) | **<0.001** |
| FEPhosphate (%) | 28 (19 – 43) | 13 (9 – 18) | 6 (3 – 8) | **<0.001** |
| *Neurohormonal activation* |
| Aldosterone (pg/mL) | 89 (40 – 184) | 99 (49 – 197) | 147 (73 – 263) | **<0.001** |
| Renin (µUI/mL) | 86 (28 – 243) | 91 (29 – 289) | 143 (35 – 299) | 0.230 |
| Aldosterone-renin ratio (ng/dL/ng/mL) | 175 (46 – 496) | 201 (58 – 535) | 173 (68 – 615) | 0.140 |
| *Biomarkers* |
| FGF23 (RU/mL) | 237 (122 – 642) | 200 (111 – 494) | 173 (115 – 359) | **<0.001** |
| Bio-ADM (pg/mL) | 35 (23 – 56) | 31 (21 – 50) | 29 (21 – 46) | **0.001** |
| PENK (pmol/L) | 90 (66 – 124) | 82 (60 – 110) | 77 (64 – 96) | **<0.001** |
| Pro-ADM (ng/mL) | 0.53 (0.34 – 0.87) | 0.45 (0.29 – 0.73) | 0.41 (0.26 – 0.80) | **<0.001** |
| Galectin-3 (ng/mL) | 22 (16 – 30) | 21 (15 – 28) | 21 (15 – 32) | **0.044** |
| GDF-15 (ng/L) | 2840 (1786 – 4958) | 2534 (1593 – 4192) | 2934 (1567 – 4188) | **0.002** |
| IL-6 (pg/mL) | 5.3 (2.8 – 10.4) | 5.1 (2.8 – 10.5) | 4.9 (2.7 – 9.0) | 0.260 |

\*Defined as <0.80 mmol/L, incidence of low TmP/GFR: 1392 (67%)

Normally distributed continuous variables are presented as mean ± standard deviation and non-normally distributed continuous variables as median (interquartile range). Abbreviations: ACE, angiotensin-converting enzyme; ALAT, alanine transaminase; ASAT, aspartate aminotransferase; Bio-ADM, bioactive adrenomedullin; BMI, body mass index; eGFR, estimated glomerular filtration rate; FE, fractional excretion; FGF23, fibroblast growth factor 23; gCR, gram of urinary creatinine; GDF‐15, growth differentiation factor 15; IL-6, interleukin 6; KIM-1, kidney injury molecule-1; LVEF, left ventricular ejection fraction; NGAL, Neutrophil Gelatinase-Associated Lipocalin; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association; pro-ADM, proadrenomedullin; TmP/GFR, tubular maximum phosphate reabsorption capacity; UACR, urine albumin-to-creatinine ratio

**Supplemental Table 4. Multivariable regression analysis for log TmP/GFR with phosphate included**

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | Standardised Beta | *T* | P-value |
| Serum phosphate | 0.800 | 78.148 | **<0.001** |
| Log FEUrea | -0.346 | -18.532 | **<0.001** |
| Log urea | -0.347 | -15.992 | **<0.001** |
| Log creatinine | -0.108 | -6.722 | **<0.001** |
| Log urinary creatinine | -0.104 | -5.923 | **<0.001** |
| Hemoglobin | 0.060 | 5.543 | **<0.001** |
| Log FGF23 | -0.058 | -4.760 | **<0.001** |
| Log loop diuretic dose\* | -0.037 | -3.518 | **<0.001** |
| Log urinary osteopontin | -0.039 | -2.480 | **0.006** |
| Log BNP | 0.024 | 2.065 | **0.046** |

Complete case analysis N = 1,672, R2 = 0.832.

\*In furosemide equivalent

Abbreviations: BNP, brain natriuretic peptide; FE, fractional excretion; FGF23, fibroblast growth factor 23; TmP/GFR, tubular maximum phosphate reabsorption capacity

**Supplemental Table 5. Cox regression analysis of serum phosphate**

|  |  |
| --- | --- |
|  | Univariable |
| Outcomes | **HR (95% CI)** | **P-value** |
| All-cause mortality | 0.86 (0.65 – 1.13) | 0.273 |
| HF hospitalization | 0.82 (0.62 – 1.09) | 0.164 |
| All-cause mortality or HF hospitalization | 0.87 (0.70 – 1.08) | 0.198 |

Complete case analysis N=2,085.
Abbreviations: CI, confidence interval; HR, hazard ratio; HF, heart failure

**Supplemental Table 6. Cox proportional hazards analysis according to low/high TmP/GFR and presence of CKD predicting all-cause mortality**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Univariable | Adjusted for BIOSTAT risk score\* and serum phosphate |
| Groups | **N of events (%)** | **HR (95% CI)** | **P-value** | **HR (95% CI)** | **P-value** |
| High TmP/GFR + non-CKD | 95 (17) | 1.0 (Reference) | Ref | 1.0 (Reference) | Ref |
| High TmP/GFR + CKD | 134 (24) | 2.00 (1.53 – 2.60) | **<0.001** | 1.09 (0.83 – 1.45) | 0.536 |
| Low TmP/GFR + non-CKD | 77 (14) | 1.21 (0.89 – 1.63) | 0.218 | 1.14 (0.83 – 1.58) | 0.414 |
| Low TmP/GFR + CKD | 253 (45) | 2.98 (2.35 – 3.77) | **<0.001** | 1.44 (1.09 – 1.92) | **0.012** |

\* Variables in BIOSTAT risk score:
All-cause mortality: age, log blood urea nitrogen (BUN), log NT-proBNP, hemoglobin, and beta-blocker use at baseline
HF hospitalization: age, HF hospitalization in previous year, peripheral oedema, systolic blood pressure, and eGFR
Combined end point: age, HF hospitalization in previous year, systolic blood pressure, log NT-proBNP, hemoglobin, high-density lipoprotein, sodium, and beta-blocker use at baseline

Complete case analysis in adjusted analyses N=2,085.

Abbreviations: BIOSTAT, A systems BIOlogy Study to TAilored Treatment in Chronic

Heart Failure; CKD, chronic kidney disease; CI, confidence interval; eGFR, estimated

glomerular filtration rate; HF, heart failure; HR, hazard ratio; NT-proBNP, N-terminal pro

brain natriuretic peptide; TmP/GFR, tubular maximum phosphate reabsorption capacity

**Supplemental Table 7. Cox proportional hazards analysis according to low/high TmP/GFR and presence of CKD predicting the combined end point**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Univariable | Adjusted for BIOSTAT risk score\* and serum phosphate |
| Groups | **N of events (%)** | **HR (95% CI)** | **P-value** | **HR (95% CI)** | **P-value** |
| High TmP/GFR + non-CKD | 179 (20) | 1.0 (Reference) | Ref | 1.0 (Reference) | Ref |
| High TmP/GFR + CKD | 201 (23) | 1.60 (1.31 (1.96) | **<0.001** | 0.87 (0.70 – 1.09) | 0.222 |
| Low TmP/GFR + non-CKD | 126 (14) | 1.01 (0.80 – 1.27) | 0.937 | 0.94 (0.73 – 1.20) | 0.607 |
| Low TmP/GFR + CKD | 372 (42) | 2.49 (1.09 – 2.98) | **<0.001** | 1.17 (0.94 – 1.46) | 0.169 |

\* Variables in BIOSTAT risk score:
All-cause mortality: age, log blood urea nitroge, log NT-proBNP, hemoglobin, and beta-blocker use at baseline
HF hospitalization: age, HF hospitalization in previous year, peripheral oedema, systolic blood pressure, and eGFR
Combined end point: age, HF hospitalization in previous year, systolic blood pressure, log NT-proBNP, hemoglobin, high-density lipoprotein, sodium, and beta-blocker use at baseline

Complete case analysis in adjusted analyses N=2,085.

Abbreviations: BIOSTAT, A systems BIOlogy Study to TAilored Treatment in Chronic

Heart Failure; CKD, chronic kidney disease; CI, confidence interval; eGFR, estimated

glomerular filtration rate; HF, heart failure; HR, hazard ratio; NT-proBNP, N-terminal pro

brain natriuretic peptide; TmP/GFR, tubular maximum phosphate reabsorption capacity

**Supplemental Table 8. Baseline characteristics of the EMPA-RESPONSE-AHF cohort stratified by above or below median TmP/GFR**

|  |
| --- |
| TmP/GFR (mmol/L) |
| Variable | Below median, *n* = 390.78 (0.70 – 0.91)[min: 0.38, max: 0.97] | Above median, *n* = 391.13 (1.04 – 1.27)[min: 0.98, max: 2.21] | **P-value** |
| *Clinical characteristics* |
| Age (years) | 76 ± 12 | 73 ± 10 | 0.294 |
| Sex (male), *n* (%) | 27 (69) | 26 (67) | >0.999 |
| BMI (kg/m2) | 28 ± 7 | 28 ± 6 | 0.697 |
| NYHA classification (III/IV), *n* (%) | 38 (97) | 35 (95) | 0.646 |
| Systolic blood pressure (mmHg) | 123 ± 23 | 125 ± 25 | 0.782 |
| Diastolic blood pressure (mmHg) | 70 ± 13 | 78 ± 16 | **0.016** |
| Heart rate (bpm) | 72 (65 – 80) | 86 (71 – 101) | **0.005** |
| Presence of atrial fibrillation/flutter, *n* (%) | 29 (74) | 26 (67) | 0.620 |
| LVEF if known\* (%) | 40 (29 – 50) | 27 (19 – 48) | 0.223 |
| Diabetes mellitus type II, *n* (%) | 12 (31) | 13 (33) | >0.999 |
| Smoking (past or current), *n* (%) | 26 (67) | 29 (74) | 0.620 |
| *Primary heart failure etiology, n (%)* |
| Ischemic heart disease | 10 (26) | 11 (28) | >0.999 |
| Hypertension | 3 (8) | 6 (15) | 0.478 |
| Rhythm disturbances | 5 (13) | 12 (31) | 0.099 |
| *Medication, n (%)* |
| Treatment arm (empagliflozin) | 15 (38) | 24 (62) | 0.070 |
| ACE inhibitor/angiotensin receptor blocker/ARNi | 27 (69) | 24 (62) | 0.747 |
| Beta-blocker | 25 (64) | 27 (69) | 0.683 |
| Loop diuretics | 39 (100) | 39 (100) | >0.999 |
| Loop diuretic dose received until baseline (furosemide equivalent) | 207 ± 314 | 193 ± 139 | 0.810 |
| Aldosterone antagonist | 20 (51) | 15 (38) | 0.417 |
| *Laboratory values* |
| Hemoglobin (g/dL) | 12.3 ± 1.5 | 13.3 ± 1.9 | **0.009** |
| Sodium (mEq/L) | 140 (136 – 142) | 140 (138 – 143) | 0.696 |
| Potassium (mEq/L) | 3.9 (3.5 – 4.3) | 4.1 (3.7 – 4.3) | 0.243 |
| Phosphate (mg/dL) | 3.2 (2.8 – 3.5) | 4.0 (3.7 – 4.5) | **<0.001** |
| NT-proBNP (ng/L) | 6078 (4443 – 9483) | 4079 (3146 - 6964) | **0.011** |
| Creatinine (mg/dL) | 1.29 (1.03 – 1.58) | 1.20 (0.95 – 1.56) | 0.552 |
| eGFR (mL/min/1.73m2) | 52 ± 17 | 55 ± 18 | 0.488 |
| Blood urea nitrogen (mg/dL) | 26 (21 – 36) | 27 (21 – 37) | 0.779 |
| Urinary creatinine (mg/dL) | 33 (17 – 49) | 42 (31 – 71) | **0.014** |
| UACR (mg/gCr) | 71 (36 – 179) | 62 (19 – 185) | 0.374 |
| FENa (%) | 2.49 (1.28 – 5.49) | 1.95 (0.76 – 3.55) | 0.054 |
| FEUrea (%) | 44 (35 – 52) | 32 (23 – 40) | **<0.001** |
| FEPhosphate (%) | 23 (18 – 28) | 16 (9 – 21) | **<0.001** |

\*N = 46

Normally distributed continuous variables are presented as mean ± standard deviation and non-normally distributed continuous variables as median (interquartile range). Abbreviations: ACE, angiotensin converting enzyme; ARNi, angiotensin receptor-neprilysin inhibitor; BMI, body mass index; eGFR, estimated glomerular filtration rate; FE, fractional excretion; gCR, gram of urinary creatinine; LVEF, left ventricular ejection fraction; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association; TmP/GFR, tubular maximum phosphate reabsorption capacity; UACR, urine albumin-to-creatinine ratio

**Supplemental Figure 1. Kaplan-Meier curve for the combined end point for quartiles of TmP/GFR**



Kaplan Meier curves for the combined end point stratified by quartiles of TmP/GFR. The indicators TmPGFR=Q1 to TmPGFR=Q4 represent the first quartile of TmP/GFR to fourth quartile of TmP/GFR, respectively. The thick lines represent the survival curves with their corresponding 95% confidence intervals in a lighter shade.

Abbreviations: TmP/GFR, tubular maximum phosphate reabsorption capacity

**Supplemental Figure 2. Kaplan-Meier curves according to low/high TmP/GFR and presence of CKD predicting the combined end point**



Kaplan Meier curves for the combined end point stratified by TmP/GFR above or below the median and presence versus absence of CKD. “High TmP/GFR” indicates TmP/GFR above the median, whereas “low TmP/GFR” indicates TmP/GFR below the median. The thick lines represent the survival curves with their corresponding 95% confidence intervals in a lighter shade.

Abbreviations: CKD, chronic kidney disease; TmP/GFR, tubular maximum phosphate reabsorption capacity

**Supplemental Figure 3. Correlation plot of TmP/GFR with fractional excretion of lithium**

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Correlation coefficient (Pearson): -0.513
Complete case analysis N=87.