Associations of Body Mass Index With Laboratory and Biomarkers in Patients With Acute Heart Failure

Koen W. Streng, MD; Jozine M. ter Maaten, MD, PhD; John G. Cleland, MD; Christopher M. O'Connor, MD; Beth A. Davison, PhD; Marco Metra, MD; Michael M. Givertz, MD; John R. Teerlink, MD; Piotr Ponikowski, MD, PhD; Daniel M. Bloomfield, MD; Howard C. Dittrich, MD; Hans L. Hillege, MD, PhD; Dirk J. van Veldhuisen, MD, PhD; Adriaan A. Voors, MD, PhD; Peter van der Meer, MD, PhD

Background—Plasma concentrations of natriuretic peptides decline with obesity in patients with heart failure. Whether this is true for other biomarkers is unknown. We investigated a wide range of biomarker profiles in acute heart failure across the body mass index (BMI) spectrum.

Methods and Results—A total of 48 biomarkers, assessing multiple pathophysiological pathways, were measured in 2033 patients included in PROTECT (Placebo-Controlled Randomized Study of the Selective A1 Adenosine Receptor Antagonist Rolofylline for Patients Hospitalized With Acute Decompensated Heart Failure and Volume Overload to Assess Treatment Effect on Congestion and Renal Function), a trial comparing the effects of rolofylline to placebo in patients with acute heart failure. Patients were classified into 4 groups according to BMI (<25, 25–30, 30–35, and >35 kg/m^2). Of 2003 patients with known weight and height, mean age was 70±12 years and 67% were men. Patients with a higher BMI (>35 kg/m^2) had higher blood pressures, were younger, and were more often women. Median levels of brain natriuretic peptide were 550 pg/mL in patients with a BMI <25 kg/m^2 and 319 pg/mL in patients with a BMI >35 kg/m^2 (P<0.001). Multivariable regression revealed that brain natriuretic peptide (β=−0.250; P<0.001) and receptor for advanced glycation endproducts (β=−0.095; P<0.007) were inversely correlated to BMI, whereas higher levels of uric acid (β=0.164; P<0.001), proadrenomedullin (β=0.171; P<0.001), creatinine (β=0.118; P=0.003), sodium (β=0.101; P=0.006), and bicarbonate (β=0.094; P=0.009) were associated with higher BMI. No significant interaction was seen between these 7 biomarkers and BMI on 180-day mortality.

Conclusions—The plasma concentrations of several biomarkers are either positively or negatively influenced by BMI. These findings suggest that these markers should be interpreted with caution in patients with obesity. Although concentrations differ, their prognostic value for mortality up to 180 days did not differ.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00354458.

(*Circ Heart Fail*. 2017;10:e003350. DOI: 10.1161/CIRCHEARTFAILURE.116.003350.)

Key Words: biomarkers ■ heart failure ■ mortality ■ obesity ■ prognosis

Biomarkers play an important role in the diagnosis and management of heart failure (HF).[1–4] There are a variety of biomarkers available for HF, reflecting several biological processes such as oxidative stress, myocardial stretch or injury, remodeling, inflammation, renal function, or neurohumoral activation.[5] One of the most frequently used biomarkers for the diagnosis and prognosis of HF is (N-terminal-pro) brain natriuretic peptide (BNP), of which levels show a positive association with left ventricle systolic dysfunction and mortality. Serum levels of BNP are known to be lower in patients with obesity although the underlying severity of HF does not differ. BNP is cleared by type C clearance receptors. Adipose tissue is known to contain more natriuretic peptide clearance receptors-C, which possibly leads to more degradation of circulating BNP.[6] However, obesity is also related with lower circulating levels of NT-proBNP, precursor of BNP,

See Clinical Perspective

Received June 8, 2016; accepted December 6, 2016.

From the Department of Cardiology, University Medical Center Groningen, University of Groningen, The Netherlands (K.W.S., J.M.t.M., H.L.H., D.J.v.V., A.A.V., P.v.d.M.); National Heart and Lung Institute, Royal Brompton and Harefield Hospitals, Imperial College, London, United Kingdom (J.G.C.); Inova Heart and Vascular Institute, Falls Church, VA (C.M.O'C.); Momentum Research, Durham, NC (B.A.D.); University of Brescia, Italy (M.M.); Brigham and Women's Hospital, Boston, MA (M.M.G.); University of California at San Francisco and San Francisco Veterans Affairs Medical Center (J.R.T.); Medical University, Clinical Military Hospital, Wroclaw, Poland (P.P.); Merck Research Laboratories, Rahway, NJ (D.M.B.); and University of Iowa Carver College of Medicine Cardiovascular Research Center (H.C.D.).

The Data Supplement is available at http://circheartfailure.ahajournals.org/lookup/suppl/doi:10.1161/CIRCHEARTFAILURE.116.003350/-/DC1.

Correspondence to P. van der Meer, MD, PhD, Department of Cardiology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands. E-mail p.van.der.meer@umcg.nl

despite the fact NT-proBNP is not degraded through natriuretic peptide clearance receptors-C. A more likely explanation for the lower levels in patients with obesity is suggested by Bartels et al.[7] They hypothesize that the expression of BNP is impaired in patients with obesity because of lipid accumulation, suggesting a link between the fat metabolism and BNP expression. Lower circulating levels have led to the suggestion that different cutoff points should be used in patients with obesity.[8] With the rising prevalence of obesity worldwide, HF in patients with obesity is a growing problem. In contrast to BNP, to date, it is unknown how other (cardiac) biomarkers behave across the BMI spectrum. Little is known about a variety of clinical used markers such as troponin or more novel marker for HF such as galectin-3 or growth differentiation factor 15. The association between BMI and these markers could influence their interpretation in patients with a higher BMI in contrast to patients with a lower BMI.

Therefore, we aimed to study biomarker levels in patients with obesity with acute HF and their behavioral patterns across the BMI spectrum.

Methods

Study Population

The study population consisted of 2033 patients originating from the PROTECT trial (Placebo-Controlled Randomized Study of the Selective A1 Adenosine Receptor Antagonist Rolofylline for Patients Hospitalized With Acute Decompensated Heart Failure and Volume Overload to Assess Treatment Effect on Congestion and Renal Function), which had neutral results.[9–11] The local ethics committee at each participating center approved the trial, and all patients provided written informed consent. Key inclusion criteria were dyspnea at rest or at minimal exertion, BNP level ≥500 pg/mL or NT-proBNP ≥2000 pg/mL, and a creatinine clearance between 20 and 80 mL/min. Other inclusion and exclusion criteria are outlined in the design paper. A total of 48 biomarkers were determined and fully available in 1266 patients. Patients included in the PROTECT trial with weight and height measurements available were included in the analysis. In total, 2003 patients had weight and height available at day 1, and 1742 patients had known weight and height at day 4.

The patients with known height and weight at admission were separated in 4 different groups based on BMI (weight [kg]/height [m]^2). The groups were BMI <25 kg/m^2 (group 1), 25 to 30 kg/m^2 (group 2), 30 to 35 kg/m^2 (group 3), and >35 kg/m^2 (group 4) according to the World Health Organization groups of BMI. Initially BMI group 1 was separated in <18.5 kg/m^2 and 18.5 to 25 kg/m^2, but there were only 18 patients with a BMI <18.5 kg/m^2. Therefore, these 2 groups were merged.

Study Procedures

In total, 48 biomarkers were evaluated at baseline. Many markers (albumin, alanine transaminase, aspartate transaminase, bicarbonate, blood urea nitrogen, chloride, creatinine, glucose, hemoglobin, platelet count, potassium, red blood cell count, sodium, total cholesterol, triglycerides, uric acid, and white blood cell count) were determined in ICON Laboratories, Farmingdale, NY. The following 26 biomarkers were assessed by Alere Inc., San Diego, CA. Using ELISA galectin-3, myeloperoxidase, and neutrophil gelatinase–associated lipocalin (NGAL) were measured. By using competitive ELISAs on a Luminex platform angiogenin and C-reactive protein were measured. By using sandwich ELISAs on a Luminex platform D-dimer, endothelial cell-selective adhesion molecule, growth differentiation factor 15, lymphotoxin beta receptor, mesothelin, neuropilin, N-terminal pro–C-type natriuretic peptide, osteopontin, procalcitonin, pentraxin-3, periostin, polymeric immunoglobulin receptor, proadrenomedullin, prosaposin B, receptor for advanced glycation endproducts (RAGE), soluble ST-2, syndecan-1, tumor necrosis factor-α receptor 1, Troy, vascular endothelial growth receptor 1, and WAP 4-disulphide core domain protein HE4 were determined. An extra 5 biomarkers, BNP (endothelin-1, interleukin-6, kidney injury molecule, and cardiac troponin I were assessed by single-molecule counting technology by Erenna Immunoassay System on a microtiter plate by Singulex Inc., Alameda, CA. Immunoassays to procalcitonin, proadrenomedullin, galectin-3, and ST2 were developed by Alere. These research assays have not been standardized to the commercialized assays used in research or in clinical use and the extent to which each Alere assay correlates with the commercial assay is not fully characterized. Additional information about the assays are presented in Table I in the Data Supplement.

Statistical Analysis

Normally distributed data are presented as means and SD, skewed data as medians and 25th to 75th percentiles, and categorical variables as percentages and frequencies. Intergroup differences between variables were tested using one-way ANOVA for normally distributed data; skewed data were tested using χ^2 test or Kruskal–Wallis test depending on whether the data were continuous or nominal. With multivariable fractional polynomials best fit for each variable was estimated.

To assess predictors of a higher BMI, multivariable linear regression models were constructed. A natural logarithmic transformation of BMI was used (Log BMI). Variables that might correlate with each other were alternated in multivariable analysis. Before entering the variables in the model, variables were standardized by dividing them by their SD. Backward and stepwise multivariable analyses were used. The final model with backward analysis consisted of biomarkers, demographics, medical history, and previous medication. Proportional hazards survival (Cox proportional hazard analysis) was used to estimate the effect of BMI on mortality up to 180 days and the effect of biomarker levels on mortality up to 180 days. In multivariable models to estimate the effect of BMI, adjustments were made for age and sex. In Cox proportional hazard analysis for biomarker levels, adjustments were made for age, sex, and Log BMI.

Kaplan–Meier curves were assessed to estimate the effect of BMI on mortality up to 180 days. Differences in survival rates between the different BMI groups were tested using the log-rank test (Mantel–Cox test). Forest plots were drafted to evaluate the predictive value and hazard ratio of mortality up to 180 days between a BMI <30 kg/m^2 and >30 kg/m^2 put out against 7 biomarkers. A 2-sided P <0.05 was considered statistically significant.

All analyses were performed using IBM SPSS Statistics version 22 and R: a Language and Environment for Statistical Computing, version 3.0.2. (R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline Characteristics

Baseline characteristics for all 2003 patients were divided according to BMI groups. Baseline characteristics are shown in Table 1. Mean age for the total cohort is 70±12 years, with predominantly male patients (67%). Almost half of the patients had New York Heart Association class III (n=965). The mean left ventricular ejection fraction in the total cohort was 32±13%. In patients with a BMI >35 kg/m^2, 89% (n=254) had a history of hypertension and 62% (n=178) had a history of diabetes mellitus. Despite these risk factors, patients with obesity were less likely to have ischemic heart disease or myocardial infarction when compared with patients in lower BMI groups. Patients with a higher BMI were younger, were less frequently men, and had higher systolic and diastolic blood pressures and higher heart rate.

Table 1. Baseline Characteristics

BMI Groups, kg/m²	<25	25–30	30–35	>35	*P* Value
n	591	715	410	287	
Demographics					
Sex (% male)	397 (67)	509 (71)	271 (66)	169 (59)	0.002
Age, y	71±13	72±11	70±11	64±11	<0.001
LVEF, %	32±13	32±13	34±13	33±14	0.27
Systolic blood pressure, mm Hg	121±18	124±17	127±16	128±18	<0.001
Diastolic blood pressure, mm Hg	72±12	73±11	75±12	76±13	<0.001
Heart rate, beats/min	80±15	79±15	80±16	83±16	0.006
Rolofylline administration, %	387 (65.5)	481 (67.3)	275 (67.1)	191 (66.6)	0.92
Medical history, %					
Hypertension	421 (71.2)	560 (78.3)	354 (86.3)	254 (88.5)	<0.001
Diabetes mellitus	165 (27.9)	322 (45.0)	243 (59.3)	178 (62.0)	<0.001
Hyperlipidemia	280 (47.4)	370 (51.7)	230 (56.1)	154 (53.7)	0.045
Ischemic heart disease	390 (66.0)	516 (72.2)	316 (77.1)	170 (59.2)	<0.001
Myocardial infarction	291 (49.2)	380 (53.1)	212 (51.7)	103 (35.9)	<0.001
NYHA class					0.043
1	5 (0.8)	9 (1.3)	4 (1.0)	1 (0.3)	
2	94 (15.9)	118 (16.5)	60 (14.6)	46 (16.0)	
3	292 (49.4)	352 (49.2)	192 (46.8)	129 (44.9)	
4	162 (27.4)	193 (27.0)	138 (33.7)	103 (35.9)	

Values are given as means±SD, median (25th to 75th percentiles) or percentage and frequency. BMI indicates body mass index; LVEF, left ventricular ejection fraction; and NYHA, New York Heart Association.

Biomarkers at Baseline

All biomarkers at baseline are shown in Table 2. A higher BMI is associated with a lower BNP and a higher galectin-3 (P<0.001). Glucose levels are higher in patients with a BMI between 25 and 30 kg/m² and patients with a BMI between 30 and 35 kg/m². The same applies to creatinine (P<0.001), plasma NGAL (P<0.001), uric acid (P<0.001), and sodium (P<0.001). Widely used markers such as troponin-I, C-reactive protein, and interleukin-6 do not differ. Because BMI is determined by weight, patients with more edema could have had a higher BMI. The same statistics were performed with weight on day 4, in a more recompensated state. Data did not significantly differ in outcome (Table II in the Data Supplement). To check for informed censoring, a baseline table was drafted based on all biomarkers available or not all biomarkers available. Data did not substantially differ (Table III in the Data Supplement).

Correlates for BMI

In univariable and multivariable linear regression analyses, clinical correlates for BMI are assessed and are shown in Table 3. A lower age (β=−0.035; P<0.001), a higher diastolic blood pressure (β=0.023; P=0.001), a medical history of diabetes mellitus (β=0.104; P<0.001), and hypertension (β=0.085; P=0.001) are associated with a higher BMI. Univariable regression analyses is shown in Table IV in the Data Supplement. BNP (β=−0.051; P<0.001) and RAGE (β=−0.020; P<0.007) are inversely correlated to BMI. Uric acid (β=0.032; P<0.001), proadrenomedullin (β=0.034; P<0.001), creatinine (β=0.023; P=0.003), sodium (β=0.021; P=0.006), and bicarbonate (β=0.020; P=0.009) are positively correlated with BMI in a multivariable model. Statistics were also performed on these 7 biomarkers using weight at day 4, which did not significantly alter our findings (Table V in the Data Supplement).

BMI and Mortality Up To 180 Days

Cox proportional hazard regression models for BMI predicting mortality up to 180 days are presented in Table 4. In univariable analysis, a higher BMI is associated with lower mortality rates (hazard ratio 0.53; P=0.019). However, in a multivariable model after adjustment for sex and age, there is no longer a significant association between BMI and mortality up to 180 days (hazard ratio 0.69; P=0.21). Figure 1 shows the Kaplan–Meier curve for survival up to 180 days. Whereas the lowest survival rates are in the group with a BMI <25 kg/m² (80%), and the best survival is seen in the group with a BMI 30 to 35 kg/m² (86.1%); there is no significant difference between the groups (P=0.087).

To evaluate the predictive value of biomarkers in relation to mortality for a BMI above and below 30 kg/m², Forest plots were drafted (Figure 2). Within these plots, 7 biomarkers associated with BMI were separated into a BMI above or below 30

Table 2. Biomarkers at Baseline

BMI Groups, kg/m^2	<25	25–30	30–35	>35	*P* Value
n	591	715	410	287	
Biomarkers					
Albumin, g/dL	3.84±0.45	3.85±0.43	3.85±0.44	3.83±0.40	0.95
Alt, g/dL	21.0 (15–35)	21.0 (15–32)	20.0 (15–29)	21.5 (15–31)	0.063
Angiogenin, ng/mL	1806.2 (1212–2605)	1866.7 (1226–2936)	1860.4 (1322–2760)	1936.8 (1241–2886)	0.19
Ast, U/L	26 (20–36)	25 (20–33)	24 (18–31)	24 (19–33)	0.004
Bicarbonate, mEq/L	24.0±3.9	23.7±3.6	23.9±3.9	24.8±3.9	0.002
Blood urea nitrogen, mg/dL	28.0 (21–39)	30.0 (23–41)	31.5 (23–43)	28.0 (21–41)	0.001
BNP, pg/mL	549.7 (286–934)	450.2 (270–789)	421.6 (224–780)	319.0 (195–550)	<0.001
Chloride, mEq/L	100.4±5.0	101.0±5.0	101.3±4.9	100.5±4.7	0.014
Cholesterol total, mg/dL	143 (119–171)	140 (115–174)	142 (115–174)	137 (114–169)	0.39
Creatinine, mg/dL	1.30 (1.08–1.60)	1.40 (1.20–1.80)	1.50 (1.20–1.90)	1.30 (1.10–1.80)	<0.001
CRP, mg/mL	13.3 (7.0–26.5)	13.6 (6.6–26.9)	14.1 (7.6–29.8)	15.1 (9.3–26.8)	0.088
D-Dimer, ng/mL	172.2 (90.5–371.5)	160.3 (90.6–381.8)	148.2 (90.6–283.5)	165.1 (90.6–305.9)	0.20
Endothelin 1, pg/mL	6.6 (4.6–9.1)	6.9 (5.1–9.2)	7.1 (5.2–9.5)	6.9 (4.9–9.3)	0.19
ESAM, ng/mL	61.3 (56.0–68.6)	62.2 (56.6–70.1)	61.6 (56.1–70.2)	61.9 (55.7–68.6)	0.74
Galectin-3, ng/mL	33.6 (25.4–45.2)	36.8 (28.0–49.4)	37.6 (28.9–49.7)	38.1 (28.4–53.2)	<0.001
GDF-15, nL/mL	4.6 (3.1–6.3)	4.4 (3.1–6.3)	4.7 (3.1–6.3)	4.5 (3.0–6.3)	0.85
Glucose, mg/dL	121.0 (99–151)	130.0 (103–166)	133.0 (103–171)	128.0 (103–173)	<0.001
Hemoglobin, g/dL	12.8±2.02	12.7±1.97	12.6±1.97	12.7±1.96	0.19
Interleukin-6, pg/mL	10.8 (6.1–18.6)	11.1 (6.6–21.1)	11.6 (6.8–21.0)	11.7 (6.9–22.1)	0.18
KIM-1, pg/mL	247.6 (161.3–426.9)	301.8 (194.7–477.4)	320.1 (189.9–552.1)	333.6 (208.2–532.8)	<0.001
LTBR, ng/mL	0.38 (0.26–0.53)	0.42 (0.28–0.60)	0.42 (0.28–0.60)	0.43 (0.29–0.61)	0.004
Mesothelin, ng/mL	86.9 (74.5–100.1)	87.1 (74.8–101.6)	86.4 (73.3–102.1)	85.6 (75.1–97.4)	0.76
Myeloperoxidase, nL/mL	33.0 (17.2–68.0)	37.2 (20.1–75.8)	34.0 (17.9–67.6)	29.9 (16.9–66.4)	0.25
Neuropilin, ng/mL	13.0 (8.3–18.1)	12.0 (7.8–17.3)	12.1 (8.2–17.2)	12.9 (8.7–17.5)	0.29
NGAL, ng/mL	72.8 (48.2–112.4)	87.6 (54.4–146.0)	86.0 (57.5–148.3)	83.9 (52.9–138.1)	<0.001
NT-pro-CNP, ng/mL	0.040 (0.029–0.059)	0.044 (0.030–0.060)	0.042 (0.031–0.059)	0.039 (0.026–0.061)	0.18
Osteopontin, ng/mL	115.6 (80.2–177.1)	111.5 (75.0–168.6)	109.2 (78.5–152.8)	109.4 (79.2–161.3)	0.21
Pentraxin-3, ng/mL	4.9 (3.1–7.5)	4.3 (2.8–6.8)	4.2 (2.9–6.9)	3.8 (2.5–6.3)	0.001
Periostin, ng/mL	5.9 (3.3–9.6)	5.3 (3.0–8.9)	5.4 (3.2–8.7)	5.5 (3.2–8.2)	0.20
PIGR, ng/mL	389.7 (263.2–601.6)	403.9 (266.9–706.8)	406.6 (264.4–655.0)	355.6 (240.4–653.3)	0.31
Potassium, mEq/L	4.24±0.60	4.32±0.58	4.29±0.56	4.28±0.64	0.16
Pro-ADM, nL/mL	2.4 (1.4–4.4)	2.8 (1.6–4.9)	2.9 (1.6–4.8)	3.4 (1.9–5.5)	0.002
Procalcitonin, nL/mL	0.020 (0.010–0.048)	0.021 (0.010–0.050)	0.024 (0.014–0.046)	0.021 (0.011–0.055)	0.27
Platelet count, *10^9/L	218.5 (167.0–278.0)	216.0 (168.5–274.0)	212.5 (178.3–262.8)	221.0 (179.0–267.0)	0.75
PSAB-B, ng/mL	40.3 (30.0–55.6)	38.5 (28.6–53.5)	36.9 (27.8–51.8)	36.5 (25.9–49.7)	0.003
RAGE, ng/mL	5.0 (3.6–7.0)	5.1 (3.7–6.8)	5.2 (3.7–6.8)	4.8 (3.5–5.9)	0.042
RBC, *10^{12}/L	4.25±0.65	4.22±0.64	4.23±0.66	4.33±0.68	0.12
Sodium, mEq/L	138.8±4.1	139.3±4.2	139.9±4.1	139.7±3.8	<0.001
ST-2, ng/mL	3.7 (1.2–8.5)	3.3 (0.96–7.9)	3.2 (0.93–7.4)	3.9 (0.93–7.1)	0.33
Syndecan-1, ng/mL	8.3 (6.9–9.9)	8.3 (6.9–10.2)	8.5 (7.0–10.4)	8.4 (7.2–10.1)	0.43

(*Continued*)

Table 2. Continued

BMI Groups, kg/m²	<25	25–30	30–35	>35	*P* Value
TNF-R1a, ng/mL	2.9 (2.1–4.4)	3.3 (2.4–4.7)	3.3 (2.3–4.8)	3.3 (2.2–4.8)	0.008
Triglycerides, mg/dL	82.0 (59–112)	87.5 (64–122)	95.0 (68–132)	99.0 (73–134)	<0.001
Troponin I, pg/mL	11.0 (5.6–23.5)	10.7 (5.7–24.0)	10.7 (5.6–21.0)	10.1 (5.4–22.8)	0.70
Troy, ng/mL	0.08 (0.06–0.12)	0.10 (0.07–0.13)	0.09 (0.07–0.13)	0.09 (0.06–0.13)	<0.001
Uric acid, mg/dL	8.56±2.65	9.18±2.58	9.18±2.42	9.24±2.58	<0.001
VEGFR, ng/mL	0.41 (0.25–0.58)	0.36 (0.24–0.58)	0.38 (0.24–0.56)	0.41 (0.27–0.66)	0.068
WAP4C, ng/mL	26.6 (14.6–51.8)	28.8 (14.5–53.2)	27.8 (15.2–48.9)	23.3 (11.6–50.4)	0.16
WBC, *10⁹/L	7.2 (5.9–8.9)	7.6 (6.0–9.3)	7.6 (6.1–9.5)	7.6 (6.4–9.2)	0.058

Values are given as means±SD, median (25th to 75th percentiles) or percentage and frequency.

Alt indicates alanine transaminase; Ast, aspartate transaminase, BMI, body mass index; BNP, brain natriuretic peptide; CRP, C-reactive protein; ESAM, endothelial cell-selective adhesion molecule; GDF, growth differentiation factor 15; KIM-1, kidney injury molecule 1; LTBR, lymphotoxin β receptor; NGAL, neutrophil gelatinase–associated lipocalin; NT-pro-CNP, N-terminal pro C-type natriuretic peptide; PIGR, polymeric immunoglobulin receptor; pro-ADM, proadrenomedullin; PSAB-B, prosaposin B; RAGE, receptor for advanced glycation endproducts; RBC, red blood cell count; VEGFR, vascular endothelial growth receptor 1; WAP4C, WAP four-disulphide core domain protein HE4; and WBC, white blood cell count.

kg/m². There is no significant interaction between BMI and any of the biomarkers.

Discussion

In a wide spectrum of biomarkers, measured in a large group of patients with acute HF, we show several biomarkers to be either positively (proadrenomedullin, uric acid, creatinine, sodium, and bicarbonate) or negatively (BNP and RAGE) correlated with BMI. The prognostic value of the biomarkers for mortality up to 180 days was similar in patients with lower and higher BMI.

Table 3. Multivariable Predictors of BMI

Variable	β	95% CI	*t* Value	*P* Value
BNP, pg/mL	−0.051	−0.07 to 0.04	−6.95	<0.001
History of DM	0.096	0.07 to 0.12	6.74	<0.001
Age, y	−0.034	−0.05 to 0.02	−4.91	<0.001
Pro-ADM, nL/mL	0.034	0.02 to 0.05	4.40	<0.001
Uric acid, mg/dL	0.032	0.02 to 0.05	4.34	<0.001
History of hypertension	0.061	0.03 to 0.094	3.59	<0.001
Systolic blood pressure, mm Hg	0.025	0.01 to 0.04	3.28	0.001
History of depression	0.076	0.03 to 0.12	3.20	0.001
Creatinine, mg/dL	0.023	0.01 to 0.04	2.98	0.003
Sodium, mEq/L	0.021	0.01 to 0.04	2.78	0.006
RAGE, ng/mL	−0.020	−0.04 to 0.01	−2.69	0.007
Bicarbonate, mEq/L	0.020	0.01 to 0.03	2.63	0.009
Heart rate, beats/min	0.014	0.00 to 0.03	1.92	0.055

All univariable significant variables ($P<0.1$) where entered in a multivariable backward model. Only one measurement of blood pressure (systolic/diastolic) and renal function (creatinine, creatinine clearance, and neutrophil gelatinase–associated lipocalin) was entered because of collinearity.

Adjusted R^2 = 0.276. BMI indicates body mass index; BNP, brain natriuretic peptide; CI, confidence interval; DM, diabetes mellitus; pro-ADM, proadrenomedullin; and RAGE, receptor for advanced glycation endproducts.

Cardiac Biomarkers and Obesity

Previous studies have already showed that a higher BMI is associated with lower serum BNP levels, but despite these findings, there is still no consensus about the underlying mechanism. A possible hypothesis is thought to be that the expression of BNP is impaired in patients with obesity because of lipid accumulation, suggesting a link between the fat metabolism and BNP expression. This could be because of the fact that triglyceride accumulation in the heart could lead to cellular stress and apoptosis. BNP induces lipolysis in adipocytes and might reduce the release of free fatty acids and its adverse effects.[7] Circulating levels of BNP were also strongly negatively related to patients with acute HF and a high BMI in our study. The negative correlation between BMI and BNP is found in not only patients with HF but also healthy patients.[12] Our data confirm a negative relation between BMI and BNP, which influences the clinical interpretation of circulating BNP levels. Out of the 7 markers stated to be associated with BMI, BNP seems to be most strongly correlated with BMI. Christensen et al[13] found in patients with chronic HF that only NP and adiponectin were associated with BMI. However, they reviewed 7 biomarkers in contrast to our 48 biomarkers, and in patients with chronic HF while our database consists of patients with acute HF.

Noncardiac Biomarkers and Obesity

One of the biomarkers in our study which is strongly correlated to a high BMI is uric acid. Recent studies provided a couple of reasons why uric acid is elevated in patients with obesity. Uric acid is the product of the purine metabolism. Purines are mainly found in red meat or shellfish. One of the possible reasons patients with obesity might have higher circulating levels of uric acid is because of a higher intake of purines.[14] Furthermore, adipose tissue is known to secrete uric acid. Obesity creates more mRNA expression and activity of the xanthine oxidoreductase, which converts xanthine into uric acid, resulting in increased levels of uric acid.[15,16] High uric acid levels are known to play a role in the development of metabolic syndrome, a clustering of abdominal obesity, insulin resistance, dyslipidemia, and elevated blood pressures, all cardiovascular

Table 4. Cox Proportional Hazard Survival Regression Analysis for the Prediction of Mortality Up To 180 Days

180-Day Mortality	Hazard Ratio (95% CI)	*P* Value
Per log BMI	0.526 (0.307–0.899)	0.019
Adjusted for sex	0.533 (0.311–0.914)	0.022
Adjusted for sex and age	0.691 (0.390–1.224)	0.21
Adjusted for sex and BNP	0.638 (0.341–1.194)	0.16

BMI indicates body mass index; BNP, brain natriuretic peptide; and CI, confidence interval.

risk factors.[17] Of note, we observed more hypertension and diabetes mellitus in our patients with obesity although less ischemic heart disease and myocardial infarction.

Higher levels of serum bicarbonate are also correlated with a higher BMI. Bicarbonate is more often raised in patients with acute HF, which is linked to the use of diuretics. Depending on the choice of diuretics, they often give electrolyte and acid disorders. Changes in potassium, sodium, uric acid, and bicarbonate are not uncommon.[18] Furthermore, studies have shown that bicarbonate is associated with worsening renal function, more HF events, and higher mortality.[18,19] A possible explanation for the correlation between a higher BMI and bicarbonate might be that a higher serum bicarbonate is associated with obesity hypoventilation syndrome. Because of chronic hypoventilation in patients with obesity, bicarbonate raises in reaction to hypercapnia.[20]

Another biomarker strongly associated with a high BMI in our study is proadrenomedullin, a precursor for adrenomedullin. Adrenomedullin is a vasodilator peptide, synthesized by a variety of tissues, for example, heart, lungs, and kidney. Most important function of adrenomedullin in cardiovascular diseases seems to be its effects against oxidative stress.[21] This biomarker has recently been described as strong predictor for all-cause mortality.[22,23] Proadrenomedullin is often raised in patients with obesity because adipose tissue contains receptor activity modifying proteins that together form the adrenomedullin receptor. The increased number of receptors is thought to protect against complications of comorbidities in obesity, like diabetes mellitus and hypertension, through vasodilatation.[24] Furthermore, several renal biomarkers were evaluated, including plasma kidney injury molecule-1 and NGAL, which are both markers of tubular damage.[25] Both plasma kidney injury molecule-1 and plasma NGAL are higher in higher BMI groups. These higher levels of plasma kidney injury molecule-1 and plasma NGAL suggest tubular damage in patients with a higher BMI. Despite the higher creatinine clearance found in this study, these findings suggest that the renal function in patients with obesity is worse compared with nonobese groups. Patients with obesity are more often affected by a variety of comorbidities, such as diabetes mellitus and higher blood pressures. These factors could explain the decreased renal function in patients with obesity.

RAGE is expressed in the heart in cardiomyocytes, fibroblasts, and inflammatory cells and is released after cardiomyocyte injury. Serum levels of this receptor could therefore reflect the degree of HF.[26] However, the predictive value of RAGE is not yet fully established.[27,28] Although vascular cells express RAGE, this contributes to soluble forms of RAGE. These soluble forms of RAGE have been shown to be lower in patients with metabolic syndrome. One of the possible explanations is that circulating RAGE may function as a decoy or a natural inhibitor to bind to the membrane RAGE receptor,

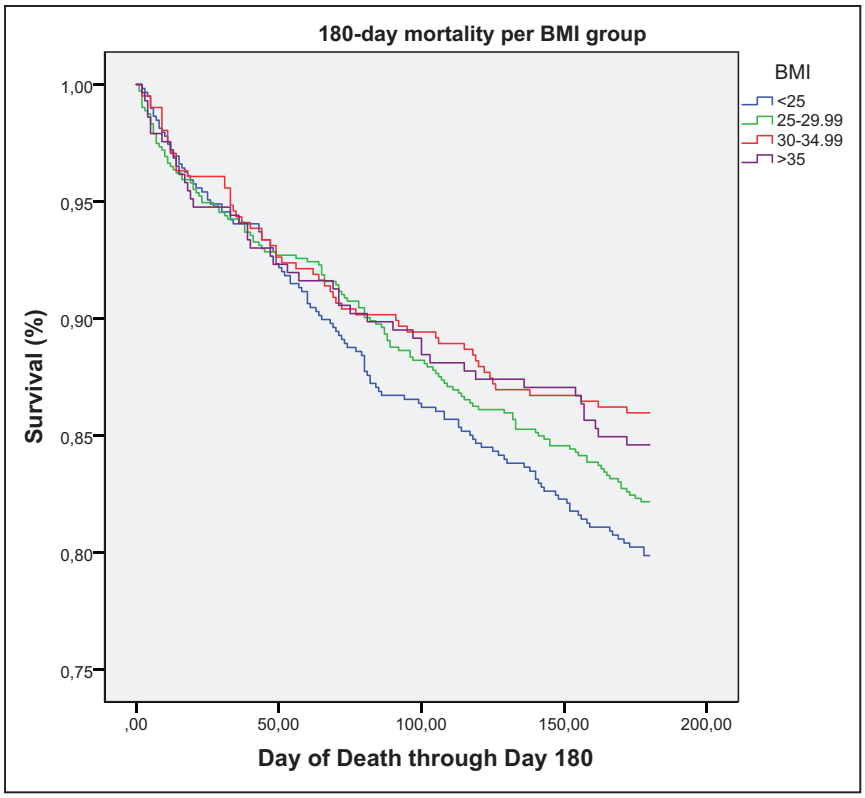

Figure 1. Kaplan–Meier survival analysis by different body mass index (BMI) groups.

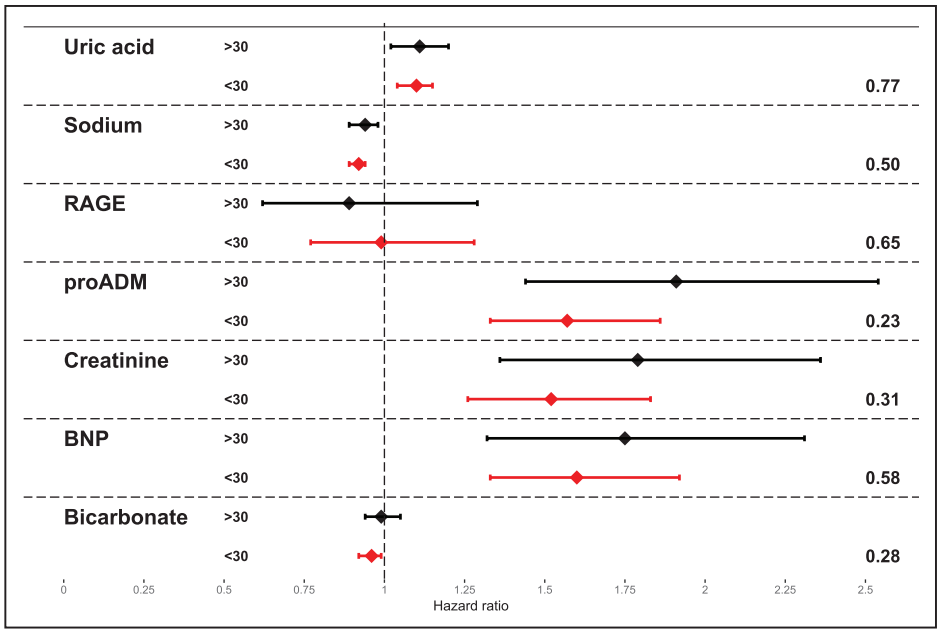

Figure 2. Biomarkers separated by body mass index (BMI) on mortality up to 180 days. Hazard ratio for mortality up to 180 days plotted for 7 biomarkers separated by BMI below and above 30 kg/m^2. On the right *P* value for interaction. No significant interaction is seen, concluding that a biomarker can have a predictive value on mortality up to 180 d, which remains the same in a BMI above and below 30 kg/m^2. BNP indicates brain natriuretic peptide; pro-ADM, proadrenomedullin; and RAGE, receptor for advanced glycation endproducts.

and thus prevent AGEs to bind to the receptor and exert any biological actions. This way RAGE might play an important role in the development of (complications associated with) diabetes mellitus.[29,30]

Obesity and Mortality

In this study, we showed that a higher BMI is associated with a lower mortality risk, in accordance to recent studies linking (pre)obesity to significant lower mortality rates in acute HF.[31–33] However after correction for sex and age, there is a trend toward the obesity paradox, but there is no longer a significant correlation between BMI and survival rates. Still, there is a trend visible: a BMI between 25 and 35 kg/m^2 is more favorable than a normal weight. To ensure our measurement using BMI on day 1 was not overestimated by decompensation, BMI on day 4 was also used which possibly shows a more recompensated state. This did not give any significant alternative outcome. BNP and proadrenomedullin are strong predictors for (all-cause) mortality in patients with HF.[23,34] To evaluate their prognostic value on mortality up to 180 days, graphs were drafted to plot the 7 biomarkers found to be associated with BMI separated by a BMI below and above 30 kg/m^2. These hazard ratios were plotted along with a *P*-value for interaction. There is no significant interaction between any of these biomarkers and BMI. Thus, can be concluded that levels of these biomarkers may differ in patients with a higher BMI and might need to be interpreted differently; however, their prognostic value on mortality up to 180 days does not differ.

Limitations

The main limitation of our study is its retrospective design. The second limitation in our study is the absence of underweight patients. From 2003 patients with known BMI, only 18 people were underweight. To provide more or less even groups, patients with underweight were merged with normal weight patients. Furthermore, there are predominantly male patients in our cohort. Another limitation concerns the noncommercial immunoassays of procalcitonin, proadrenomedullin, galectin-3, and ST2. These research assays have not been standardized to the commercialized assays used in research or in clinical use and the correlation of each Alere assay to the commercial assay is not fully characterized.

Conclusions

The plasma concentrations of 7 out of 48 biomarkers were either positively or negatively influenced by BMI. These findings suggest that these markers should be interpreted with caution in patients with obesity. Although concentrations may differ in patients with obesity, the prognostic value for mortality up to 180 days did not differ per biomarker in patients with a higher BMI.

Sources of Funding

The PROTECT trial was supported by NovaCardia, a subsidiary of Merck.

Disclosures

Dr Cleland was on the Steering Committee for the PROTECT trial, served on the advisory board for Merck and received payments for both. Dr O'Connor is a consultant to Merck. Dr Ponikowski has received honoraria from Merck. Dr Davison is an employee of Momentum Research Inc., which was contracted to perform work on the project by Merck. Dr Metra has received honoraria and reimbursements from NovaCardia, sponsors of the study, and Merck. Dr Givertz has received institutional research support and served on a scientific advisory board for Merck. Dr Teerlink has received research funds and consulting fees from Merck. Dr Bloomfield is an employee of Merck. Dr Dittrich served as a consultant to Merck. Dr

Voors has received speaker and consultancy fees from Merck. The other authors report no conflicts..

References

1. Demissei BG, Valente MA, Cleland JG, O'Connor CM, Metra M, Ponikowski P, Teerlink JR, Cotter G, Davison B, Givertz MM, Bloomfield DM, Dittrich H, van der Meer P, van Veldhuisen DJ, Hillege HL, Voors AA. Optimizing clinical use of biomarkers in high-risk acute heart failure patients. *Eur J Heart Fail*. 2016;18:269–280. doi: 10.1002/ejhf.443.
2. ter Maaten JM, Valente MA, Metra M, Bruno N, O'Connor CM, Ponikowski P, Teerlink JR, Cotter G, Davison B, Cleland JG, Givertz MM, Bloomfield DM, Dittrich HC, van Veldhuisen DJ, Hillege HL, Damman K, Voors AA. A combined clinical and biomarker approach to predict diuretic response in acute heart failure. *Clin Res Cardiol*. 2016;105:145–153. doi: 10.1007/s00392-015-0896-2.
3. O'Connor CM, Fiuzat M, Lombardi C, Fujita K, Jia G, Davison BA, Cleland J, Bloomfield D, Dittrich HC, Delucca P, Givertz MM, Mansoor G, Ponikowski P, Teerlink JR, Voors AA, Massie BM, Cotter G, Metra M. Impact of serial troponin release on outcomes in patients with acute heart failure: analysis from the PROTECT pilot study. *Circ Heart Fail*. 2011;4:724–732. doi: 10.1161/CIRCHEARTFAILURE.111.961581.
4. Brouwers FP, van Gilst WH, Damman K, van den Berg MP, Gansevoort RT, Bakker SJ, Hillege HL, van Veldhuisen DJ, van der Harst P, de Boer RA. Clinical risk stratification optimizes value of biomarkers to predict new-onset heart failure in a community-based cohort. *Circ Heart Fail*. 2014;7:723–731. doi: 10.1161/CIRCHEARTFAILURE.114.001185.
5. Braunwald E. Biomarkers in heart failure. *N Engl J Med*. 2008;358:2148–2159. doi: 10.1056/NEJMra0800239.
6. Madamanchi C, Alhosaini H, Sumida A, Runge MS. Obesity and natriuretic peptides, BNP and NT-proBNP: mechanisms and diagnostic implications for heart failure. *Int J Cardiol*. 2014;176:611–617. doi: 10.1016/j.ijcard.2014.08.007.
7. Bartels ED, Nielsen JM, Bisgaard LS, Goetze JP, Nielsen LB. Decreased expression of natriuretic peptides associated with lipid accumulation in cardiac ventricle of obese mice. *Endocrinology*. 2010; 151: 5218–5225.
8. Daniels LB, Clopton P, Bhalla V, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, Omland T, Storrow AB, Abraham WT, Wu AH, Steg PG, Westheim A, Knudsen CW, Perez A, Kazanegra R, Herrmann HC, McCullough PA, Maisel AS. How obesity affects the cut-points for B-type natriuretic peptide in the diagnosis of acute heart failure. Results from the Breathing Not Properly Multinational Study. *Am Heart J*. 2006;151:999–1005. doi: 10.1016/j.ahj.2005.10.011.
9. Cotter G, Dittrich HC, Weatherley BD, Bloomfield DM, O'Connor CM, Metra M, Massie BM; Protect Steering Committee, Investigators, and Coordinators. The PROTECT pilot study: a randomized, placebo-controlled, dose-finding study of the adenosine A1 receptor antagonist rolofylline in patients with acute heart failure and renal impairment. *J Card Fail*. 2008;14:631–640. doi: 10.1016/j.cardfail.2008.08.010.
10. Weatherley BD, Cotter G, Dittrich HC, DeLucca P, Mansoor GA, Bloomfield DM, Ponikowski P, O'Connor CM, Metra M, Massie BM; PROTECT Steering Committee, Investigators, and Coordinators. Design and rationale of the PROTECT study: a placebo-controlled randomized study of the selective A1 adenosine receptor antagonist rolofylline for patients hospitalized with acute decompensated heart failure and volume overload to assess treatment effect on congestion and renal function. *J Card Fail*. 2010;16:25–35. doi: 10.1016/j.cardfail.2009.10.025.
11. Massie BM, O'Connor CM, Metra M, Ponikowski P, Teerlink JR, Cotter G, Weatherley BD, Cleland JG, Givertz MM, Voors A, DeLucca P, Mansoor GA, Salerno CM, Bloomfield DM, Dittrich HC; PROTECT Investigators and Committees. Rolofylline, an adenosine A1-receptor antagonist, in acute heart failure. *N Engl J Med*. 2010;363:1419–1428. doi: 10.1056/NEJMoa0912613.
12. Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Wilson PW, Vasan RS. Impact of obesity on plasma natriuretic peptide levels. *Circulation*. 2004;109:594–600. doi: 10.1161/01.CIR.0000112582.16683.EA.
13. Christensen HM, Schou M, Goetze JP, Faber J, Frystyk J, Flyvbjerg A, Kistorp C. Body mass index in chronic heart failure: association with biomarkers of neurohormonal activation, inflammation and endothelial dysfunction. *BMC Cardiovasc Disord*. 2013;13:80. doi: 10.1186/1471-2261-13-80.
14. Torralba KD, De Jesus E, Rachabattula S. The interplay between diet, urate transporters and the risk for gout and hyperuricemia: current and future directions. *Int J Rheum Dis*. 2012;15:499–506. doi: 10.1111/1756-185X.12010.
15. Tsushima Y, Nishizawa H, Tochino Y, Nakatsuji H, Sekimoto R, Nagao H, Shirakura T, Kato K, Imaizumi K, Takahashi H, Tamura M, Maeda N, Funahashi T, Shimomura I. Uric acid secretion from adipose tissue and its increase in obesity. *J Biol Chem*. 2013;288:27138–27149. doi: 10.1074/jbc.M113.485094.
16. Lima WG, Martins-Santos ME, Chaves VE. Uric acid as a modulator of glucose and lipid metabolism. *Biochimie*. 2015;116:17–23. doi: 10.1016/j.biochi.2015.06.025.
17. Tian Y, Chen K, Xie Z, Fang Y, Wang H, Nie Y, Hu D, Mu Y. The association between serum uric acid levels, metabolic syndrome and cardiovascular disease in middle aged and elderly Chinese: results from the DYSlipidemia International Study. *BMC Cardiovasc Disord*. 2015;15:66. doi: 10.1186/s12872-015-0059-4.
18. Cooper LB, Mentz RJ, Gallup D, Lala A, DeVore AD, Vader JM, AbouEzzeddine OF, Bart BA, Anstrom KJ, Hernandez AF, Felker GM. Serum bicarbonate in acute heart failure: relationship to treatment strategies and clinical outcomes. *J Card Fail*. 2016;22:738–742. doi: 10.1016/j.cardfail.2016.01.007.
19. Dobre M, Yang W, Pan Q, Appel L, Bellovich K, Chen J, Feldman H, Fischer MJ, Ham LL, Hostetter T, Jaar BG, Kallem RR, Rosas SE, Scialla JJ, Wolf M, Rahman M, and the CRIC SI. Persistent high serum bicarbonate and the risk of heart failure in patients with chronic kidney disease (CKD): a report from the Chronic Renal Insufficiency Cohort (CRIC) Study. *J Am Heart Assoc*. 2015;4:e001599.
20. Bingol Z, Pıhtılı A, Cagatay P, Okumus G, Kıyan E. Clinical predictors of obesity hypoventilation syndrome in obese subjects with obstructive sleep apnea. *Respir Care*. 2015;60:666–672. doi: 10.4187/respcare.03733.
21. Ogura S, Shimosawa T. Oxidative stress and organ damages. *Curr Hypertens Rep*. 2014;16:452. doi: 10.1007/s11906-014-0452-x.
22. Eggers KM, Venge P, Lindahl B, Lind L. Associations of mid-regional pro-adrenomedullin levels to cardiovascular and metabolic abnormalities, and mortality in an elderly population from the community. *Int J Cardiol*. 2013;168:3537–3542. doi: 10.1016/j.ijcard.2013.05.005.
23. Klip IT, Voors AA, Anker SD, Hillege HL, Struck J, Squire I, van Veldhuisen DJ, Dickstein K; OPTIMAAL Investigators. Prognostic value of mid-regional pro-adrenomedullin in patients with heart failure after an acute myocardial infarction. *Heart*. 2011;97:892–898. doi: 10.1136/hrt.2010.210948.
24. Li Y, Jiang C, Wang X, Zhang Y, Shibahara S, Takahashi K. Adrenomedullin is a novel adipokine: adrenomedullin in adipocytes and adipose tissues. *Peptides*. 2007;28:1129–1143. doi: 10.1016/j.peptides.2007.03.005.
25. Sabbisetti VS, Waikar SS, Antoine DJ, Smiles A, Wang C, Ravisankar A, Ito K, Sharma S, Ramadesikan S, Lee M, Briskin R, De Jager PL, Ngo TT, Radlinski M, Dear JW, Park KB, Betensky R, Krolewski AS, Bonventre JV. Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. *J Am Soc Nephrol*. 2014;25:2177–2186. doi: 10.1681/ASN.2013070758.
26. Ramasamy R, Schmidt AM. Receptor for advanced glycation end products (RAGE) and implications for the pathophysiology of heart failure. *Curr Heart Fail Rep*. 2012;9:107–116. doi: 10.1007/s11897-012-0089-5.
27. Willemsen S, Hartog JW, van Veldhuisen DJ, van der Meer P, Roze JF, Jaarsma T, Schalkwijk C, van der Horst IC, Hillege HL, Voors AA. The role of advanced glycation end-products and their receptor on outcome in heart failure patients with preserved and reduced ejection fraction. *Am Heart J*. 2012;164:742–749.e3. doi: 10.1016/j.ahj.2012.07.027.
28. Li W, Katzmarzyk PT, Horswell R, Zhang Y, Wang Y, Johnson J, Hu G. Body mass index and heart failure among patients with type 2 diabetes mellitus. *Circ Heart Fail*. 2015;8:455–463. doi: 10.1161/CIRCHEARTFAILURE.114.001837.
29. Momma H, Niu K, Kobayashi Y, Huang C, Chujo M, Otomo A, Tadaura H, Miyata T, Nagatomi R. Higher serum soluble receptor for advanced glycation end product levels and lower prevalence of metabolic syndrome among Japanese adult men: a cross-sectional study. *Diabetol Metab Syndr*. 2014;6:33. doi: 10.1186/1758-5996-6-33.
30. Norata GD, Garlaschelli K, Grigore L, Tibolla G, Raselli S, Redaelli L, Buccianti G, Catapano AL. Circulating soluble receptor for advanced glycation end products is inversely associated with body mass index and waist/hip ratio in the general population. *Nutr Metab Cardiovasc Dis*. 2009;19:129–134. doi: 10.1016/j.numecd.2008.03.004.
31. Lainscak M, von Haehling S, Doehner W, Anker SD. The obesity paradox in chronic disease: facts and numbers. *J Cachexia Sarcopenia Muscle*. 2012;3:1–4. doi: 10.1007/s13539-012-0059-5.

32. Fonarow GC, Srikanthan P, Costanzo MR, Cintron GB, Lopatin M; ADHERE Scientific Advisory Committee and Investigators. An obesity paradox in acute heart failure: analysis of body mass index and inhospital mortality for 108,927 patients in the Acute Decompensated Heart Failure National Registry. *Am Heart J*. 2007;153:74–81. doi: 10.1016/j.ahj.2006.09.007.
33. Shah R, Gayat E, Januzzi JL Jr, Sato N, Cohen-Solal A, diSomma S, Fairman E, Harjola VP, Ishihara S, Lassus J, Maggioni A, Metra M, Mueller C, Mueller T, Parenica J, Pascual-Figal D, Peacock WF, Spinar J, van Kimmenade R, Mebazaa A; GREAT (Global Research on Acute Conditions Team) Network. Body mass index and mortality in acutely decompensated heart failure across the world: a global obesity paradox. *J Am Coll Cardiol*. 2014;63:778–785. doi: 10.1016/j.jacc.2013.09.072.
34. Lourenço P, Ribeiro A, Pintalhão M, Silva S, Bettencourt P. Predictors of Six-Month Mortality in BNP-Matched Acute Heart Failure Patients. *Am J Cardiol*. 2015;116:744–748. doi: 10.1016/j.amjcard.2015.05.046.

CLINICAL PERSPECTIVE

In the diagnosis and prognosis of heart failure biomarkers are commonly used. As known, one of the most frequently used markers is (N-terminal-pro) brain natriuretic peptide. Serum levels of brain natriuretic peptide are known to be lower in patients with obesity, troubling diagnosis in these patients. We have assessed 48 biomarkers in 2003 patients enrolled in the PROTECT trial (Placebo-Controlled Randomized Study of the Selective Adenosine Receptor Antagonist Rolofylline for Patients Hospitalized With Acute Decompensated Heart Failure and Volume Overload to Assess Treatment Effect on Congestion and Renal Function). The plasma concentrations of brain natriuretic peptide, proadrenomedullin, uric acid, creatinine, sodium, receptor for advanced glycation endproducts, and bicarbonate were either positively or negatively influenced by body mass index. These findings suggest that these markers should be interpreted with caution in patients with obesity. Although the concentrations of these biomarkers may differ in patients with obesity, their prognostic value for 180-day mortality did not differ for these biomarkers in patients with a higher body mass index.

Circulation
Heart Failure

Associations of Body Mass Index With Laboratory and Biomarkers in Patients With Acute Heart Failure

Koen W. Streng, Jozine M. ter Maaten, John G. Cleland, Christopher M. O'Connor, Beth A. Davison, Marco Metra, Michael M. Givertz, John R. Teerlink, Piotr Ponikowski, Daniel M. Bloomfield, Howard C. Dittrich, Hans L. Hillege, Dirk J. van Veldhuisen, Adriaan A. Voors and Peter van der Meer

Circ Heart Fail. 2017;10:
doi: 10.1161/CIRCHEARTFAILURE.116.003350
Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1941-3289. Online ISSN: 1941-3297

SUPPLEMENTAL MATERIAL

Supplementary table 1; Assay details with assay range per biomarker

Biomarker	Lower cut-off	Upper cut-off	Inter assay coefficient of variation (%)	%Below detection limit	% Above detection limit	% In Range
Angiogenin	39.990	28185.32	5	0	0	100
CRP	41.500	63763.55	5	0	7	93
D-dimer	90.571	46104.57	14	32	0	68
ESAM-1	18.767	109.65	18	0	1	99
ET-1	0.5	250	7	0	0	100
Galectin-3	0.508	86.22	5	0	4	96
GDF-15	0.156	6.31	8	0	28	72
IL-6	0.10	0.88	13	0	0	100
KIM-1	2	1000		0	0	100
LTBR	0.003	18.08	10	0	0	100
Mesothelin	36.423	265.88	10	1	0	98
MPO	1.947	308.61	10	0	4	96
Neuropilin	0.506	269.19	13	0	0	100
NGAL	0.524	1462.00	17	0	0	100
NT-ProCNP	0.001	4.18	8	0	0	100
Osteopontin	6.421	716.85	36	0	1	99
PCT	0.002	1.70	8	0	1	99
Pentraxin-3	0.031	65.41	7	0	0	100
Periostin	0.173	177.31	8	1	0	99
PIGR	12.519	1074.06	6	0	12	88
proADM	0.027	10.20	5	1	5	93
PSAP-B	4.623	131.98	17	0	1	99
RAGE	0.022	30.77	8	0	0	100
sST-2	0.928	260.37	9	44	0	56
Syndecan-1	0.445	29.76	7	0	0	100
TNFR-1	0.028	27.35	7	0	0	100
cTnI	0.20	1000	10	0	1.4	98.6

Troy	0.003	3.62	10	0	0	100
VEGFR-1	0.028	31.27	8	0	0	100
WAP-4C	0.907	110.87	8	1	6	93

Supplementary table 2; Baseline characteristics and biomarkers with BMI calculated based on weight at day 4

BMI groups based on weight day 4 (kg/m2)		**<25**	**25-30**	**30-35**	**>35**	***P value***
N =		631	610	305	196	
Demographics						
Sex (% Male)		439 (70)	421 (69)	200 (66)	110 (56)	0.003
Age (years)		71 ±13	71 ±10	69 ±10	63 ±12	<0.001
LVEF (%)		32 ±13	32 ±13	33 ±13	33 ±14	0.52
Systolic Blood Pressure (mmHg)		122 ±18	125 ±17	126 ±17	129 ±19	<0.001
Diastolic Blood Pressure (mmHg)		73 ±11	74 ±12	75 ±12	76 ±13	0.001
Heart Rate (beats/min)		80 ±15	81 ±16	80 ±16	84 ±16	0.016
Rolofylline administration (%)		418 (66.2)	408 (66.9)	208 (68.2)	131 (66.8)	0.95
Medical History						
Hypertension (%)		454 (71.9)	492 (80.7)	269 (88.2)	179 (91.3)	<0.001
Diabetes Mellitus (%)		183 (29.0)	298 (48.9)	183 (60.0)	125 (63.8)	<0.001
Hyperlipidemia (%)		282 (44.7)	309 (50.7)	173 (56.7)	106 (54.1)	0.003
Ischemic Heart Disease (%)		424 (67.2)	445 (73.0)	229 (75.1)	121 (61.7)	0.002
Myocardial Infarction (%)		319 (50.6)	320 (52.5)	156 (51.1)	70 (35.7)	<0.001
NYHA Class						0.17
	1	3 (0.5)	10 (1.6)	1 (0.3)	1 (0.5)	
	2	98 (15.5)	98 (16.1)	44 (14.4)	30 (15.3)	
	3	306 (48.5)	293 (48.0)	147 (48.2)	83 (42.3)	
	4	185 (29.3)	179 (29.3)	105 (34.4)	75 (38.3)	
Biomarkers						
Albumin (g/dL)		3.83 ±0.46	3.84 ±0.43	3.86 ±0.43	3.84 ±0.40	0.81

BMI groups based on weight day 4 (kg/m2)	<25	25-30	30-35	>35	*P value*
N =	631	610	305	196	
Alt (g/dL)	21.0 (15-35)	21.0 (15-30)	20 (15-30)	20.0 (14-33)	0.14
Angiogenin (ng/ml)	1792.6 (1201-2707)	1934.2 (1293-2931)	1892.1 (1299-2827)	1982.9 (1287-2868)	0.12
Ast (U/L)	26 (20-36)	24 (19-32)	24 (19-31)	23 (18-33.0)	0.005
Bicarbonate (mEq/L)	23.9 ±3.9	23.7 ±3.5	24.0 ±3.9	24.9 ±3.8	0.004
Blood urea nitrogen (mg/dL)	28.0 (21-40)	30.0 (22-41)	32.0 (23-44)	27.0 (21-39)	0.001
BNP (pg/ml)	549.7 (288-950)	467.4 (275-801)	416.8 (214-696)	302.0 (176-525)	<0.001
Chloride (mEq/L)	100.7 ±5.0	101.2 ±5.1	101.3 ±4.7	100.6 ±4.7	0.091
Cholesterol total (mg/dL)	141 (119-174)	142 (116-174)	140.5 (110-167)	136 (115-169)	0.24
Creatinine (mg/dL)	1.30 (1.10-1.70)	1.40 (1.20-1.80)	1.50 (1.20-1.90)	1.30 (1.10-1.70)	<0.001
CRP (ng/ml)	13155 (6581-26461)	13675 (6691-26756)	13704 (7509-27114)	15114 (8840-28840)	0.12
D-Dimer (ng/ml)	166.7 (90.6-376.3)	156.4 (90.6-355.8)	156.2 (90.6-278.3)	159.4 (90.6-306.1)	0.40
Endothelin 1 (pg/ml)	6.5 (4.6-9.0)	7.0 (5.2-9.3)	7.1 (5.2-9.5)	6.8 (4.9-9.1)	0.123
ESAM (ng/ml)	61.2 (55.7-68.6)	62.4 (56.6-69.9)	61.2 (55.4-69.5)	61.6 (57.1-69.2)	0.48
Galectin-3 (ng/ml)	34.0 (25.7-46.1)	36.2 (28.1-48.6)	37.6 (28.1-48.4)	37.3 (28.1-52.7)	0.003
GDF-15 (nl/ml)	4.4 (3.1-6.3)	4.4 (3.1-6.3)	4.6 (3.1-6.3)	4.3 (3.0-6.3)	0.89
Glucose (mg/dL)	123.0 (99-151)	130.0 (103-168)	137.0 (104.3-177)	125.0 (103.5-168.8)	<0.001
Hemoglobin (g/dL)	12.9 ±1.99	12.7 ±2.04	12.6 ±1.92	12.6 ±1.91	0.32
Interleuking 6 (pg/ml)	10.3 (6.0-20.0)	11.4 (6.7-20.3)	11.5 (6.4-23.2)	11.5 (7.4-22.1)	0.14
KIM-1 (pg/ml)	258.1	311.1	304.1	327.1	0.001

BMI groups based on weight day 4 (kg/m2)	<25	25-30	30-35	>35	*P value*
N =	631	610	305	196	
	(165.8-430.3)	(197.2-501.2)	(186.2-517.0)	(199.2-528.1)	
LTBR (ng/ml)	0.38 (0.26-0.55)	0.42 (0.28-0.60)	0.41 (0.28-0.57)	0.42 (0.30-0.61)	0.017
Mesothelin (ng/ml)	86.5 (73.4-100.5)	88.3 (75.0-101.4)	85.0 (72.6-98.3)	84.8 (74.2-98.2	0.23
Myeloperoxidase (nl/ml)	33.0 (17.7-67.5)	38.2 (19.8-78.3)	31.8 (18.7-63.5)	29.3 (16.8-66.3)	0.26
Neuropilin (ng/ml)	12.9 (8.3-17.9)	12.0 (7.8-17.1)	11.9 (8.2-16.7)	12.9 (9.0-17.5)	0.24
NGAL (ng/ml)	75.8 (48.4-118.3)	86.2 (55.4-141.7)	86.2 (57.5-146.2)	84.0 (49.7-131.6)	0.002
NTpro-CNP (ng/ml)	0.042 (0.030-0.060)	0.042 (0.029-0.060)	0.042 (0.030-0.059)	0.039 (0.026-0.063)	0.51
Osteopontin (ng/ml)	115.1 (76.3-168.7)	111.5 (77.3-164.9)	106.4 (78.7-150.3)	105.8 (79.2-157.0)	0.42
Pentraxin-3 (ng/ml)	4.7 (3.0-7.3)	4.4 (2.9-6.9)	4.0 (2.6-6.5)	3.5 (2.5-6.2)	<0.001
Periostin (ng/ml)	5.8 (3.3-9.6)	5.2 (3.1-8.9)	5.4 (3.2-8.6)	5.7 (3.3-8.4)	0.19
PIGR (ng/ml)	388.5 (259.1-631.4)	401.9 (262.3-637.9)	406.6 (253.2-630.6)	328.7 (240.0-682.4)	0.61
Potassium (mEq/L)	4.27 ±0.59	4.32 ±0.59	4.29 ±0.58	4.31 ±0.63	0.55
proADM (nl/ml)	2.6 (1.4-4.7)	2.9 (1.5-4.8)	2.9 (1.6-5.1)	3.4 (1.8-5.4)	0.079
Procalcitonin (nl/ml)	0.020 (0.010-0.046)	0.021 (0.011-0.043)	0.024 (0.013-0.049)	0.021 (0.011-0.055)	0.17
Platelet count (*10^9/L)	218.0 (166.8-275.0)	215.0 (176-275.0)	214.0 (175.5-254.3)	221.0 (180.0-261.0)	0.77
PSAB-B (ng/ml)	39.6 (29.6-54.5)	37.9 (28.3-52.8)	36.0 (26.1-50.1)	35.2 (25.9-49.7)	0.016
RAGE (ng/ml)	5.0 (3.6-6.9)	5.1 (3.7-6.9)	5.0 (3.6-6.5)	4.7 (3.5-5.8)	0.10
RBC (*10^12/L)	4.25 ±0.65	4.26 ±0.67	4.24 ±0.65	4.33 ±0.67	0.45

BMI groups based on weight day 4 (kg/m2)	<25	25-30	30-35	>35	*P value*
N =	631	610	305	196	
Sodium (mEq/L)	138.8 ±4.2	139.6 ±4.1	140.0 ±4.1	139.8 ±4.0	<0.001
ST-2 (ng/ml)	3.5 (1.1-7.9)	3.2 (1.0-8.2)	3.1 (0.93-7.5)	3.5 (0.93-6.3)	0.36
Syndecan-1 (ng/ml)	8.2 (6.9-9.9)	8.3 (7.0-10.1)	8.5 (7.0-10.2)	8.4 (7.3-10.2)	0.31
TNF-R1a (ng/ml)	2.9 (2.1-4.5)	3.3 (2.3-4.5)	3.2 (2.3-4.8)	3.3 (2.1-4.7)	0.043
Triglycerides (mg/dL)	83.0 (59-114)	88.0 (64-119)	95.0 (70-137)	98.5 (73-135)	<0.001
Troponin I (pg/ml)	11.6 (6.0-24.7)	10.5 (5.7-21.7)	11.3 (5.6-24.9)	9.9 (5.3-18.5)	0.15
Troy (ng/ml)	0.08 (0.06-0.12)	0.10 (0.07-0.13)	0.09 (0.06-0.13)	0.09 (0.06-0.13)	0.018
Uric acid (mg/dL)	8.65 ±2.72	9.08 ±2.46	9.40 ±2.51	9.23 ±2.50	<0.001
VEGFR (ng/ml)	0.39 (0.25-0.59)	0.37 (0.25-0.57)	0.39 (0.24-0.63)	0.40 (0.26-0.58)	0.48
WAP4C (ng/ml)	25.8 (13.9-52.3)	29.4 (15.2-50.4)	26.2 (14.8-49.0)	21.6 (10.9-46.3)	0.15
WBC (*10^9/L)	7.3 (5.9-9.1)	7.6 (6.1-9.2)	7.6 (6.3-9.6)	7.5 (6.4-9.2)	0.11

Values are given as means ± standard deviation, median (25th to 75th percentiles) or percentage and frequency

Alt = alanine transaminase, Ast = aspartate transaminase, BNP = brain natriuretic peptide, CRP = C-reactive protein, ESAM = endothelial cell-selective adhesion molecule, KIM-1 = kidney injury molecule 1, LTBR = lymphotoxin beta receptor, LVEF = left ventricular ejection fraction, NGAL = neutrophil gelatinase-associated lipocalin, NTpro-CNP = N-terminal pro C-type natriuretic peptide, NYHA = New York Heart Association, PIGR = polymeric immunoglobulin receptor, proADM = pro-adrenomedullin, PSAB-B = prosaposin B, RAGE = receptor for advanced glycation endproducts, RBC = red blood cell count, VEGFR = vascular endothelial growth receptor 1, WAP4C = WAP four-disulphide core domain protein HE4, WBC = white blood cell count

Supplementary table 3; Baseline table based on all biomarkers available

BMI groups (kg/m2)	All biomarkers available	Not all biomarkers available	*P value*
N =	1266	767	
Demographics			
Sex (% Male)	841 (66.4)	524 (68.2)	0.40
Age (years)	71±11	69±12	<0.001
LVEF (%)	32±13	33±13	0.74
Systolic Blood Pressure (mmHg)	125±18	124±17	0.29
Diastolic Blood Pressure (mmHg)	74±12	74±12	0.97
Heart Rate (beats/min)	80±16	80±15	0.72
Body mass index (kg/m^2)	29±6	29±6	0.44
Clinical Profile			
Orthopnea (%)	1204 (96.1)	717 (95.9)	0.80
Rales (%)	1152 (91.1)	673 (88.7)	0.070
Edema (%)	1085 (85.8)	657 (86.3)	0.72
Jugular venous pressure (%)	998 (87.5)	613 (89.2)	0.26
Medical History			
Hypertension (%)	1008 (79.6)	608 (79.2)	0.81
Diabetes Mellitus (%)	587 (46.4)	335 (43.6)	0.24
Hyperlipidemia (%)	655 (51.7)	400 (52.1)	0.89
Smoking (%)	252 (20.0)	168 (21.9)	0.30
Ischemic Heart Disease (%)	894 (70.6)	523 (68.1)	0.23
Myocardial Infarction (%)	624 (49.3)	377 (49.2)	0.90
PCI (%)	341 (26.9)	181 (23.8)	0.094
CABG (%)	294 (23.4)	142 (18.6)	0.012
Peripheral Vascular Disease (%)	144 (11.4)	76 (9.9)	0.30
Atrial Fibrillation (%)	682 (54.2)	422 (55.5)	0.57
NYHA Class			0.28
1	15 (1.2)	3 (0.4)	
2	206 (17.1)	121 (16.8)	
3	624 (51.7)	358 (49.7)	
4	362 (30.0)	237 (32.9)	
ICD therapy (%)	206 (16.3)	119 (15.5)	0.65
Stroke (%)	117 (9.2)	66 (8.6)	0.62
Asthma, bronchitis or COPD (%)	248 (19.6)	154 (20.1)	0.79
Medication			
ACE inhibitor (%)	778 (61.5)	478 (62.6)	0.63
ARB (%)	196 (15.5)	125 (16.4)	0.60
Betablocker (%)	965 (76.3)	582 (76.2)	0.96

Supplementery table 4; Univariable analysis BMI

Variable	β	95% CI	t-value	p-value
Gender	0.023	0.01-0.04	2.44	0.015
Age	-0.035	-0.04--0.03	-7.97	<0.001
Systolic blood pressure	0.027	0.02-0.04	6.01	<0.001
Diastolic blood pressure	0.023	0.01-0.03	5.01	<0.001
Heart rate	0.009	0.00-0.02	2.04	0.041
History of Diabetes Mellitus	0.104	0.09-0.12	11.8	<0.001
History of depression	0.062	0.03-0.10	3.44	0.001
History of hypertension	0.085	0.06-0.11	7.70	<0.001
History of hyper/hypothyroid	-0.021	-0.05-0.01	-1.48	0.14
History of hyperlipidemia	0.032	0.02-0.05	3.60	<0.001
History of smoking	0.009	-0.01-0.03	0.76	0.45
Mitral regurtation	-0.047	-0.07--0.03	-4.93	<0.001
Asthma, bronchitis or COPD	0.027	0.01-0.05	2.42	0.016
Ischemic heart disease	-0.007	-0.03-0.01	-0.74	0.46
Myocardial infarction	-0.027	-0.05--0.10	-3.03	0.002
CABG	-0.011	-0.03-0.01	-1.01	0.31
Peripheral vascular disease	-0.038	-0.07--0.01	-2.67	0.008
LVEF	0.013	0.00-0.03	2.01	0.045
NYHA Class	0.010	-0.00-0.02	1.58	0.12
ALAT	-0.005	-0.01-0.00	-1.02	0.31
ASAT	-0.004	-0.01-0.01	-0.96	0.34
Bicarbonate	0.012	0.00-0.02	2.53	0.011
Blood urea nitrogen	0.012	0.00-0.02	2.61	0.009
BNP	-0.032	-0.04-0.02	-6.41	<0.001
Calculated creatinine clearance	0.016	0.01-0.03	3.51	<0.001
Chloride	0.005	0.00-0.01	1.14	0.25
Cholesterol	-0.01	-0.02-0.00	-2.32	0.020
Creatinine	0.024	0.02-0.03	5.27	<0.001
CRP	0.008	0.00-0.02	1.63	0.10
Galectin-3	0.020	0.01-0.03	4.27	<0.001
Glucose	0.016	0.01-0.03	3.56	<0.001
Hemoglobin	-0.011	-0.02-0.00	-2.33	0.020
KIM-1	0.014	0.00-0.02	2.77	0.006
LTBR	0.012	0.00-0.02	2.53	0.011
NGAL	0.014	0.00-0.03	2.76	0.006
Pentraxin-3	-0.012	-0.02-0.00	-2.54	0.011
proADM	0.016	0.01-0.03	3.34	0.001
PSAB-B	-0.019	-0.03-0.01	-3.97	<0.001
RAGE	-0.011	-0.02-0.00	-2.34	0.019
Sodium	0.018	0.01-0.03	3.94	<0.001

TNF-R1a	0.009	0.00-0.02	1.93	0.054
Triglycerides	0.018	0.01-0.03	3.88	<0.001
Troy	0.010	0.00-0.02	2.15	0.032
Uric acid	0.022	0.01-0.03	4.62	<0.001

COPD = Chronic Obstructive Pulmonary Disease; CABG = Coronary Artery Bypass Graft; LVEF = Left Ventricular Ejection Fraction; NYHA = New York Heart Association; ALAT = Alanine transaminase; ASAT = Aspartate transaminase; BNP = Brain natriuretic peptide; CRP = C-reactive protein; KIM-1 = Kidney injury molecule 1; LTBR = Lymphotoxin beta receptor; NGAL = neutrophil gelatinase-associated lipocalin; PSAB-B = prosaposin B; TNR-R1a = tumor necrosis factor a.

Supplementary table 5; Univariable regression analysis with weight day 4

Variable*	β	95% CI	t-value	p-value
Bicarbonate	0.011	0.00-0.02	2.26	0.024
BNP	-0.036	-0.05--0.03	-6.81	<0.001
Creatinine	0.021	0.01-0.03	4.41	<0.001
proADM	0.015	0.01-0.03	3.05	0.002
RAGE	-0.009	-0.02-0.00	-1.67	0.095
Sodium	0.017	0.01-0.03	3.61	<0.001
Uric acid	0.024	0.01-0.03	4.81	<0.001

**Univariable regression analysis for the seven biomarkers found to be significantly associated with BMI with weight at day 4*