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

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ABSTRACT

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: A total of 48 biomarkers, assessing multiple pathophysiological pathways, were measured in 2033 patients included in PROTECT; a trial comparing the effects of rolofylline to placebo in patients with acute heart failure. Patients were classified into four groups according to BMI (<25, 25-30, 30-35 and >35 kg/m^2).

Results: 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 more often women. Median levels of BNP 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 BNP (β=-0.250, $p<0.001$) and RAGE (β=-0.095, $p<0.007$) were inversely correlated to BMI, whereas higher levels of uric acid (β=0.164, $p<0.001$), proADM (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 seven biomarkers and BMI on 180-day mortality.

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

Keywords: Heart failure, obesity, biomarkers, prognosis

INTRODUCTION

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, remodelling, inflammation, renal function or neurohumoral activation.[5] One of the most frequently used biomarkers for the diagnosis and prognosis of HF is (NT-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 obese patients, though 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 (NPR-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, despite the fact NT-proBNP is not degraded through NPR-C. A more likely explanation for the lower levels in obese patients is suggested by Bartels et al. They hypothesize that the expression of BNP in impaired in obese patients due to lipid accumulation, suggesting a link between the fat metabolism and BNP expression.[7] Lower circulating levels have led to the suggestion different cut-off points should be used in obese patients.[8] With the rising prevalence of obesity worldwide, HF in obese patients 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 GDF-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 obese patients with AHF and their behavioural patterns across the BMI spectrum.

METHODS

Study population

The study population consisted of 2033 patients originating from the PROTECT trial (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) which had neutral results.[9-11] 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 in- 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 four different groups based on BMI (weight (kg)/height $(m)^2$). The groups were BMI <25 kg/m^2 (group 1), 25-30 kg/m^2 (group 2), 30-35 kg/m^2 (group 3) and >35 kg/m^2 (group 4) according to the World Health Organization (WHO) groups of BMI. Initially BMI group 1 was separated in <18.5 kg/m2 and 18.5-25 kg/m^2, but there were only 18 patients with a BMI below 18.5 kg/m^2. Therefore, these two groups were merged.

Study procedures

In total 48 biomarkers were evaluated at baseline. A number of markers (Albumin, alanine transaminase (ALT), aspartate transaminase (AST), bicarbonate, blood urea nitrogen (BUN), chloride, creatinine, glucose, hemoglobin, platelet count, potassium, red blood cell (RBC) count, sodium, total cholesterol, triglycerides, uric acid and white blood cell (WBC) count) were determined in ICON Laboratories, Farmingdale, New York. The following 26 biomarkers were assessed by Alere Inc., San Diego, California USA. Using enzyme-linked immunosorbent assays (ELISA) Galectin-3, myeloperoxidase (MPO) and neutrophil gelatinase-associated lipocalin (NGAL) were measured. By using competitive ELISAs on a Luminex® platform angiogenin and C-reactive protein (CRP) were measured. By using sandwich ELISAs on a Luminex® platform D-dimer, endothelial cell-selective

adhesion molecule (ESAM), growth differentiation factor 15 (GDF-15), lymphotoxin beta receptor (LTBR), mesothelin, neuropilin, N-terminal pro C-type natriuretic peptide (NT-proCNP), osteopontin, procalcitonin (PCT), pentraxin-3, periostin, polymeric immunoglobulin receptor (PIGR), pro-adrenomedullin (proADM), prosaposin B (PSAP-B), receptor for advanced glycation endproducts (RAGE), soluble ST-2 (sST-2), syndecan-1, tumor necrosis factor alpha receptor 1 (TNFR-1), Troy, vascular endothelial growth receptor 1 (VEGFR-1) and WAP four-disulphide core domain protein HE4 (WAP-4C) were determined. An extra five biomarkers, Brain Natriuretic Peptide (BNP, Endothelin-1 (ET-1), Interleukin-6 (IL-6), Kidney Injury Molecule (KIM-1) and cardiac Troponin I (cTnI) were assessed by single molecule counting technology by Erenna® Immunoassay System on a microtiter plate by Singulex Inc., Alameda, California USA. Immunoassays to PCT, proADM, Galectin-3 and ST2 were developed by Alere. These research assays have not been standardised to the commercialised 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 *Supplementary table 1*.

Statistical analysis

Normally distributed data are presented as means and standard deviation, 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 was tested using Chi-squared test or Kruskal-Wallis test depending on whether the data was 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 standard deviation. Backward as well as stepwise multivariable analysis was used. The final model with backward analysis consisted of biomarkers, demographics, medical history and prior medication. Proportional hazards survival (Cox PH 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 gender. In Cox PH analysis for biomarker levels, adjustments were made for age, gender 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 below 30 kg/m^2 and above 30 kg/m^2 put out against seven biomarkers.

A two-sided p-value <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 NYHA class III (n=965). The mean LVEF in the total cohort was 32±13%. In patients with a BMI above 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, obese patients were less likely to have ischemic heart disease or myocardial infarction compared to patients in lower BMI groups. Patients with a higher BMI were younger, less frequently male, and had higher systolic and diastolic blood pressures and higher heart rate.

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 25-30 kg/m^2 and patients with a BMI between 30-35 kg/m^2. 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, CRP and IL-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 *(Supplementary table 2)*. 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 (*Supplementary table 3).*

Correlates for BMI

In univariable and multivariable linear regression analyses clinical correlates for BMI are assessed and 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 (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 *Supplementary table 4*.

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), proADM (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 seven biomarkers using weight at day 4, which did not significantly alter our findings (*Supplementary table 5*).

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 (HR) 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 (HR 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/m^2 (80%), and the best survival is seen in the group with a BMI 30-35 kg/m^2 (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/m^2, Forest plots were drafted (*Figure 2)*. Within these plots seven biomarkers associated

with BMI were separated into a BMI above or below 30 kg/m^2. 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 AHF, we show several biomarkers to be either positively (proADM, 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.

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 in impaired in obese patients due to lipid accumulation, suggesting a link between the fat metabolism and BNP expression. This could be due to 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 heart failure and a high BMI in our study. The negative correlation between BMI and BNP is not only found in patients with HF, but also in healthy patients.[12] Our data confirms a negative relation between BMI and BNP which influences the clinical interpretation of circulating BNP levels. Out of the seven markers stated to be associated with BMI, BNP seems to be most strongly correlated with BMI. Christensen et al found in patients with chronic HF that only NP and adiponectin were associated with BMI.[13] However they reviewed seven biomarkers in contrast to our 48 biomarkers, and in patients with chronic HF while our database consists of patients with AHF.

Non-cardiac 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 obese patients. Uric acid is the product of the purine metabolism. Purines are mainly found in red meat or shellfish. One of the possible reasons obese patients might have higher circulating levels of uric acid is due to 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 risk factors.[17] Of note, we observed more hypertension and diabetes in our obese patients, 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 AHF, which is linked to the use of diuretics. Depending on the choice of diuretics, diuretics 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. Due to chronic hypoventilation in obese patients bicarbonate raises in reaction to hypercapnia.[20]

Another biomarker strongly associated with a high BMI in our study is proADM, 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] ProADM is often raised in obese patients because adipose tissue contains receptor activity modifying proteins which together form the adrenomedullin receptor. The increased number of receptors is thought to protect against complications of co morbidities in obesity, like diabetes and hypertension, through vasodilatation.[24]

Furthermore several renal biomarkers were evaluated, including plasma KIM-1 and NGAL which are both markers of tubular damage.[25] Both plasma KIM-1 and plasma NGAL are higher in

higher BMI groups. These higher levels of plasma KIM-1 and plasma NGAL suggests 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 obese patients is worse compared to non-obese groups. Obese patients are more often affected by a variety of co-morbidities, such as diabetes and higher blood pressures. These factors could explain the decreased renal function in obese patients.
RAGE, Receptor for advanced glycation endproducts, is expressed in the heart in cardiomyocytes, fibroblasts and inflammatory cells and is released following cardiomyocyte injury. Serum levels of this receptor could therefore reflect the degree of heart failure.[26] However, the predictive value of RAGE is not yet fully established.[27,28] While 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, 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.[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 gender and age, there is a trend towards 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 25-35 kg/m² 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 proADM are strong predictors for (all-cause) mortality in HF patients.[23,34] To evaluate their prognostic value on mortality up to 180 days, graphs were drafted to plot the seven 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.

Study limitations

The main limitation of our study is its retrospective design. A second limitation in our study is the absence of underweight patients. From 2003 patients with known BMI, only 18 people were underweight. In order 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 non-commercial immunoassays of PCT, proADM, 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.

CONCLUSION

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 obese patients. Though concentrations may differ in obese patients, the prognostic value for mortality up to 180 days did not differ per biomarker in patients with a higher BMI.

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Conflict of interest: J.C. was on the Steering Committee for the PROTECT trial, served on the advisory board for MSD, and received payments for both. C.O'C. is a consultant to Merck. P.P. has received honoraria from Merck. E.D. is an employee of Momentum Research Inc., which was contracted to perform work on the project by Merck. M.M has received honoraria and reimbursements from NovaCardia, sponsors of the study, and Merck. M.G. has received institutional research support and served on a scientific Advisory Board for Merck. J.T. has received research funds and consulting fees from Merck. D.B. is an employee of Merck. H.D. served as a consultant to Merck. A.V. has

received speaker and consultancy fees from Merck. All other authors have reported that they have no conflict of interest to declare.

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.

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.

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.

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.

5. Braunwald E. Biomarkers in heart failure. *N Engl J Med.* 2008; 358: 2148-2159.

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.

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.

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.

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.

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.

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.

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: 80-2261-13-80.

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.

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.

16. Lima WG, Martins-Santos ME, Chaves VE. Uric acid as a modulator of glucose and lipid metabolism. *Biochimie.* 2015; 116: 17-23.

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: 66-015-0059-4.

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.

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. *Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease.* 2015; 4: e001599.

20. Bingol Z, Pihtili A, Cagatay P, Okumus G, Kiyan E. Clinical predictors of obesity hypoventilation syndrome in obese subjects with obstructive sleep apnea. *Respir Care.* 2015; 60: 666-672.

21. Ogura S, Shimosawa T. Oxidative stress and organ damages. *Curr Hypertens Rep.* 2014; 16: 452-014-0452-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.

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.

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.

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.

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.

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.

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. *Circulation.Heart failure.* 2015; 8: 455-463.

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: 33-5996-6-33.

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.

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.

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.

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.

34. Lourenco P, Ribeiro A, Pintalhao M, Silva S, Bettencourt P. Predictors of Six-Month Mortality in BNP-Matched Acute Heart Failure Patients. *Am J Cardiol.* 2015; 116: 744-748.

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 (years)		71 ±13	72 ±11	70 ±11	64 ±11	<0.001
LVEF (%)		32 ±13	32 ±13	34 ±13	33 ±14	0.27
Systolic Blood Pressure (mmHg)		121 ±18	124 ±17	127 ±16	128 ±18	<0.001
Diastolic Blood Pressure (mmHg)		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 ± standard deviation, median (25th to 75th percentiles) or percentage and frequency

LVEF = left ventricular ejection fraction, NYHA = New York Heart Association

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 (14.8-29)	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 (18.5-32.5)	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

BMI groups (kg/m^2)	<25	25-30	30-35	>35	*P value*
N =	591	715	410	287	
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 (103-165.5)	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
Interleuking 6 (pg/ml)	10.8 (6.1-18.6)	11.1 (6.6-21.1)	11.6 (6.8-21)	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)	86.0 (57.5-148.3)	83.9 (52.9-138.1)	<0.001
NTpro-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

BMI groups (kg/m^2)	<25	25-30	30-35	>35	*P value*
N =	591	715	410	287	
proADM (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
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 (*10^9/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 ± 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, NGAL = neutrophil gelatinase-associated lipocalin, NTpro-CNP = N-terminal pro C-type natriuretic peptide, 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

*Table 3; Multivariable predictors of BMI**

Variable	β	95% CI	t-value	p-value
BNP (pg/ml)	-0.051	-0.07--0.04	-6.95	<0.001
History of DM	0.096	0.07-0.12	6.74	<0.001
Age (years)	-0.034	-0.05--0.02	-4.91	<0.001
proADM (nl/ml)	0.034	0.02-0.05	4.40	<0.001
Uric acid (mg/dL)	0.032	0.02-0.05	4.34	<0.001
History of hypertension	0.061	0.03-0.094	3.59	<0.001
Systolic blood pressure (mmHg)	0.025	0.01-0.04	3.28	0.001
History of depression	0.076	0.03-0.12	3.20	0.001
Creatinine (mg/dL)	0.023	0.01-0.04	2.98	0.003
Sodium (mEq/L)	0.021	0.01-0.04	2.78	0.006
RAGE (ng/ml)	-0.020	-0.04-0.01	-2.69	0.007
Bicarbonate (mEq/L)	0.020	0.01-0.03	2.63	0.009
Heart rate (beats/min)	0.014	0.00-0.03	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, NGAL) was entered because of collinearity.

Adjusted $R^2 = 0.276$

Table 4; Cox PH 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 gender**	0.533 (0.311-0.914)	0.022
- **Adjusted for gender and age**	0.691 (0.390-1.224)	0.21
- **Adjusted for gender and BNP**	0.638 (0.341-1.194)	0.16

Figure legends:

Figure 1; Kaplan-Meier survival analysis by different BMI groups

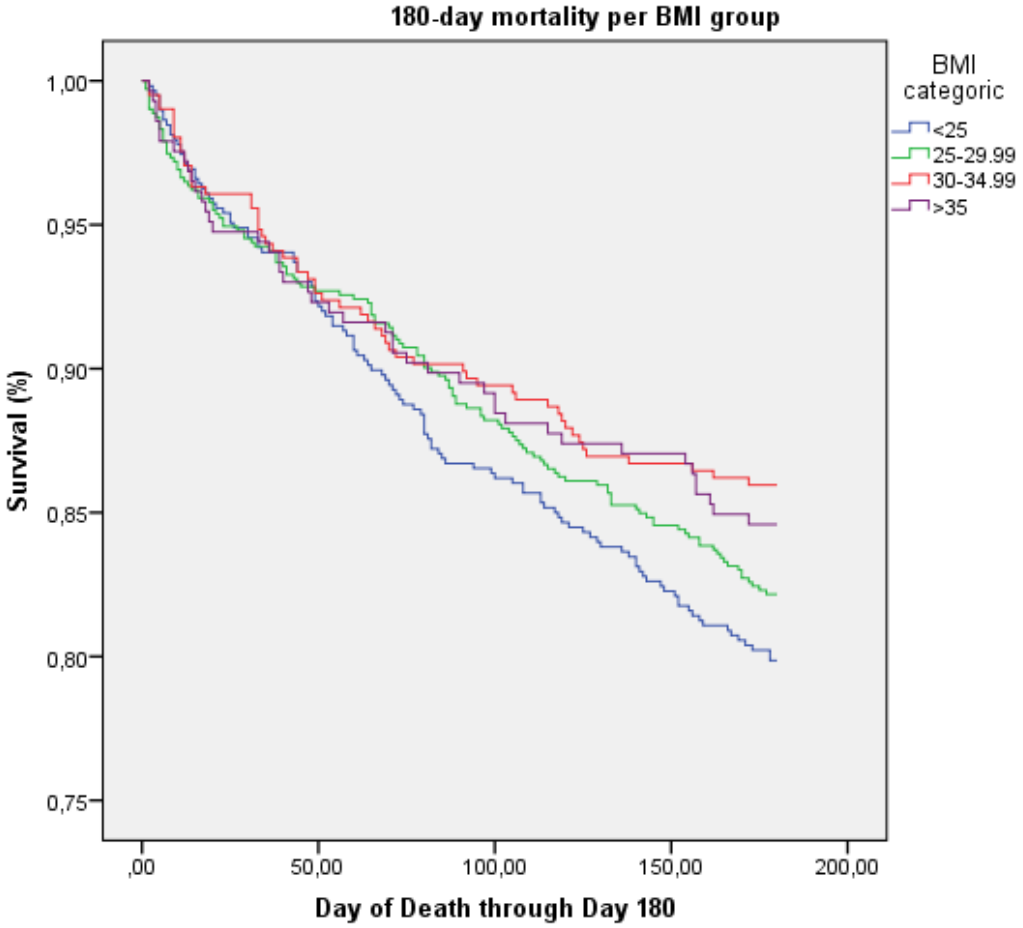
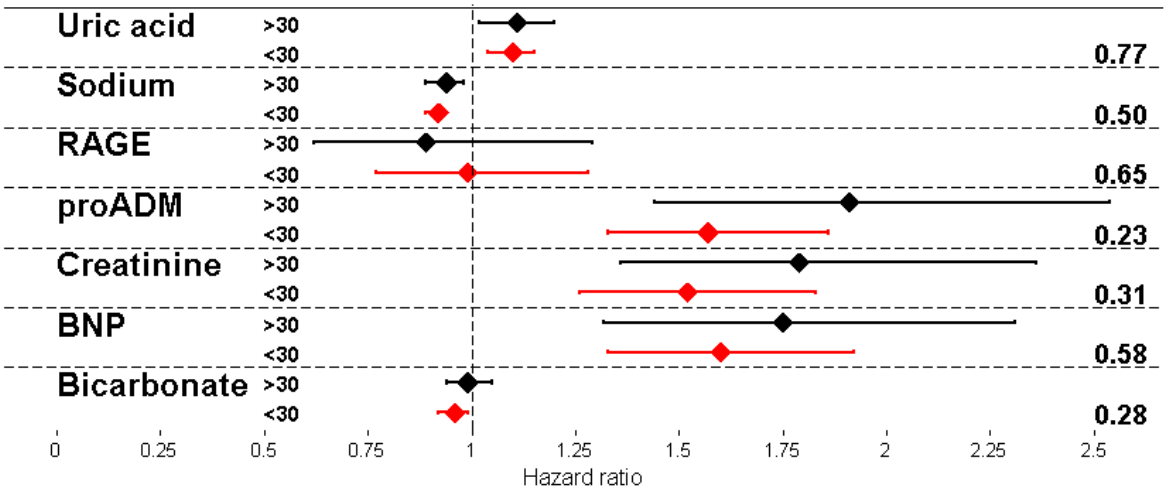


Figure 2; Biomarkers seperated by BMI on mortalityup to 180 days



Hazard ratio for mortality up to 180 days plotted for seven biomarkers seperated by BMI below and above 30 kg/m2. 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 days which remains the same in a BMI above and below 30 kg/m2.