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Biomarker-Guided Versus Guideline-Based Treatment of Patients With Heart Failure

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Biomarker-Guided Versus Evidence-Based Treatment In Heart Failure: results from BIOSTAT-CHF

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ABSTRACT

Background: Heart failure guidelines recommend up-titration of ACE-inhibitors/ARBs, beta-blockers and MRA's to doses used in randomized clinical trials, but these recommended doses are often not reached. Up-titration might however not be necessary in all patients. We aimed to establish the role of blood biomarkers to determine which patients should or should not be up-titrated.

Methods: Clinical outcomes of 2516 patients with worsening heart failure from BIOSTAT-CHF were compared between 3 theoretical treatment scenarios: A) all patients are up-titrated to >50% of recommended doses; B) patients are up-titrated according to a biomarker-based treatment-selection model; C) no patient is up-titrated to >50% of recommended doses. We conducted multivariable Cox regression using 161 biomarkers and their interaction with treatment, weighted for treatment-indication bias to estimate the expected number of deaths and/or heart-failure hospitalizations at 24 months for all three scenarios.

Results: Estimated death/hospitalization rates in 1802 patients with available (bio)markers were 16%, 16%, and 26% respectively in ACE-inhibitor/ARB up-titration scenario A, B and C. Similar rates for beta-blocker and MRA up-titration scenarios A, B, and C were 23%, 19%, and 24%, and 12%, 11% and 24 %, respectively. If up-titration was successful in all patients, an estimated 9.8, 1.3 and 12.3 events per 100 treated patients could be prevented at 24 months by ACE-inhibitor/ARB, beta-blocker and MRA therapy. Similar numbers were 9.9, 4.7 and 13.1 if up-titration treatment decision was based on a biomarker-based treatment-selection model.

Conclusion: Up-titrating patients with heart failure based on biomarker values might have resulted in fewer deaths and/or hospitalizations compared to a hypothetical scenario in which all patients were successfully up-titrated.

Keywords: treatment-decision, biomarkers, Ace-inhibitor/ACE, beta-blocker, MRA

Abbreviations:
ACE-inhibitors: Angiotensin-converting-enzyme inhibitors
ARB: Angiotensin Receptor Blocker
AF: Atrial fibrillation
BMI: Body mass index
BNP: Brain natriuretic peptide
BUN: Blood urea nitrogen
DBP: Diastolic blood pressure
CRP: C-reactive protein
CRT: Cardiac resynchronization therapy
ESC: European Society of Cardiology
FGF23: Fibroblast Growth Factor 23
GFR: Glomerular filtration rate
HDL: High-density lipoprotein
HF: Heart failure
HFrEF: Heart failure with reduced ejection fraction
ICD: Implantable cardioverter-defibrillator
IGFBP-2: Insulin-like growth factor binding protein-2
IQR: Interquartile range
LDL: Low-density lipoprotein

LVEF: Left ventricular ejection fraction
MRA: MBNP: N-terminal prohormone of BNP
NYHA: New York Heart Association
SD: Standard deviation

Condensed Abstract: Heart-failure guidelines recommend up-titration of ACE-inhibitors/ARBs, beta-blockers and MRA to doses used in RCTs. These doses are often not reached, and might not benefit all patients. We determined the probability of mortality and/or heart-failure hospitalization based on a biomarker-profile using 3 theoretical treatment scenarios: A) all patients are up-titrated to >50% recommended doses; B) patients are up-titrated according to a biomarker-based treatment-selection model; C) no patient is up-titrated to >50% recommended doses. Up-titrating patients with heart-failure based on biomarker values might have resulted in fewer deaths and/or hospitalizations compared to a scenario in which all patients were successfully up-titrated.

Introduction

Major improvements in pharmaceutical and device heart failure treatment of heart failure have been achieved in the past year. Evidence from large randomized clinical trials demonstrates that that angiotensin-converting-enzyme inhibitors (ACE-inhibitors), beta-blockers and mineralocorticoid receptor antagonists (MRAs) improve clinical outcome in patients with mild to moderate heart failure (1-8). In large randomized clinical trials, treatment doses were up-titrated to pre-specified doses, which have become the guideline-recommended doses. (9-12). Despite these improvements and recommendations, the prognosis of patients with heart failure remains poor (13-16), and in daily clinical practice the majority of patients do not achieve recommended doses (17-19). Although it is expected that most patients that achieve recommended doses will benefit from treatment, selected patients might not benefit from the recommended doses, but will experience side effects of ACE-inhibitors and beta-blocker treatment. A personalized medicine approach where patients who will not benefit from recommended ACE-inhibitor/ARB and beta-blocker heart failure treatment might be selected by biomarkers, and might reduce the number of patients receiving treatment without benefit and improve overall outcome.

In this *in silico* study, we used data from the BIOSTAT-CHF project to identify such treatment-selection markers. We hypothesized that biomarkers measured at baseline in serum/plasma of heart failure patients can identify whether patients benefit from recommended heart failure treatment or not. We developed models to estimate this benefit using 161 established and novel biomarkers, including standard biochemical blood-parameters. We compared three theoretical treatment scenarios: A) all patients are up-titrated to >50% of recommended doses according to the ESC guidelines (9-11); B) patients will be up-titrated by a biomarker-based treatment-selection model; C) no patient is treated at >50% of recommended dose.

Methods

Patients

BIOSTAT-CHF is a multicenter prospective study of 2516 patients from 69 centers in 11 European countries (20). Included patients were aged >18 years with symptoms of new-onset or worsening heart failure, confirmed either by a left ventricular ejection fraction (LVEF) of ≤40% or B-type Natriuretic Peptide (BNP) and/or (N-terminal pro) B-type natriuretic peptide (NT-proBNP) plasma levels >400 pg/ml or >2,000pg/ml, respectively. At inclusion, patients were treated with either oral or intravenous furosemide ≥40 mg/day or equivalent at the time of inclusion, and were not previously treated with evidence based therapies (ACE-inhibitor/ARB and beta-blocker) or were receiving ≤ 50% of the target doses of these drugs at the time of inclusion and had an anticipated initiation or up-titration of ACE-inhibitor/ARB and/or beta-blocker therapy by the treating physician. IRB approval was obtained in all countries.

Evidence-based heart failure treatment

Patients were treated according to evidence based ESC heart failure guidelines available at time of inclusion (9-11). These recommend up-titrating patients to recommended doses of ACE-inhibitors/ARBs and beta-blockers, unless not tolerated or contra-indicated (9-11). In BIOSTAT-CHF, sub-optimally treated patients were included, and physicians were encouraged to up-titrate patients to recommended treatment doses within 3 months after inclusion.

We recently published data from BIOSTAT-CHF showing that up-titrating patients to at least 50% of recommended ACE-inhibitor/ARB and beta-blocker doses results in comparable survival and/or heart failure related hospitalization reduction compared with patients that reached ≥100% of recommended doses (21). We therefore considered patients successfully up-titrated when >50% of recommended dose was achieved after 3 months of up-titration. Inversely, we

defined non-responders as patients who did not achieve more that 50% recommended treatment dose. All analyses were separately performed for ACE-inhibitors/ARBs and beta-blockers. In addition to ACE-inhibitor/ARB and beta-blocker treatment, we also looked at MRA guideline recommended treatment. Here we defined successful treatment as patients who achieved ≥50% of recommended treatment, and non-responding patient when <50% of recommended treatment dose was achieved. MRA treatment data was available at 9 months after inclusion.

Disease outcome

Median follow-up of the BIOSTAT-CHF project was 21 months with an interquartile range of 15-27 months. Primary patient outcome in BIOSTAT-CHF was the first occurrence of all-cause mortality or heart failure related hospitalization. Survival time was calculated from date of inclusion in BIOSTAT-CHF to date of death/heart failure hospitalization or date of censoring. Only patients who were at least followed for 3 months, were included in the present analysis.

Biomarkers

A total of 161 biomarkers were considered as treatment-selection markers. All markers were measured at inclusion of the patients. This included standard biochemical blood-parameters (hemoglobin, hematocrit, sodium, total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, serum creatinine, blood urea nitrogen (BUN), bilirubin, serum iron, potassium), heart failure markers (LVEF, NT-proBNP and BNP), 29 markers from the Luminex multiplexed bead-based immunoassay (Alere, San Diego, CA) heart failure panel (22, 23), and 92 peptide markers from a high-throughput technique using the Olink Proseek$^®$ Multiplex INF I^{96x96} kit, which measures 92 selected inflammation-related proteins simultaneously in 1μl plasma samples. The kit uses a proximity extension assay (PEA) technology, where 92 oligonucleotide-labeled antibody probe pairs are allowed to bind to their respective target present in the sample.

The 92 peptides measured by Olink$^{®}$ were normalized in arbitrary normalized protein expression units (NPX). Other biomarkers were normalized using Box-Cox transformations when deemed necessary. A complete list of all biomarkers and their summary statistics are shown in Online Table 1.

Statistical analysis

Imputation of missing data

Patients in whom >50% or more biomarker values were missing were not included in the analyses. Remaining missing values were imputed using random forests regression models implemented in the mice package (24) of the R statistical program (version 3.2.4) (25). Five completed data sets were created.

Indication bias

Since BIOSTAT-CHF is not a randomized study, we adjusted for treatment indication-bias. All analyses of the effect of successful up-titration treatment on mortality and/or hospitalization risk were inversely weighted with the probability of the given treatment. Given treatment is defined here as a successful up-titration to >50% of ESC recommended doses for ACE-inhibitor/ARB or beta-blocker or not or ≥50% ESC recommended MRA treatment dose. The probability of given treatment for a specific patient was modelled using a logistic regression model. All biomarkers were considered as predictor variables for successful up-titration. In addition, we considered 39 demographic and clinical predictor variables for prediction of the successful outcome of the up-titration (age, sex, race, BMI, blood pressure, heart rate, smoking, alcohol use, heart failure aetiology, heart failure duration, NYHA class, and several heart failure symptoms and comorbid conditions). We used lasso penalization to obtain sparse logistic models consisting of a limited number of predictor variables. Optimal penalty parameters were obtained

by 10-fold cross-validation. Analyses were performed for each imputed dataset and the calculated treatment probabilities were averaged per patient over the five imputed datasets. Performance of the logistic models was quantified using optimism-corrected c-statistics using 100 bootstrap samples, averaged over the imputed datasets.

Death and/or heart failure hospitalization and treatment-biomarker interaction

Mortality and/or heart failure hospitalization risk was modelled using the Cox regression model with given treatment as a stratum-variable. Therefore, we did not assume proportional hazards for the effect of treatment on mortality/hospitalization risk. The assumed proportional hazards assumption of the biomarkers was checked using Grambsch and Therneau's test implemented in the cox.zph function of the R statistical program (26).

We performed multivariable Cox regression with all 161 biomarkers. We used the split sample technique to obtain a *training sample* consisting of 80% of the patients in the original index cohort and the remaining 20% of the patients formed the *test sample*. The split-sample procedure was repeated 100 times. In all 100 training samples, we used lasso penalization to obtain sparse Cox regression models consisting of a limited number of the 161 biomarkers. Optimal penalty parameters were obtained by 10-fold cross-validation.

We performed separate analyses for patients who were successfully up-titrated to >50% of recommended treatment dose for either ACE-inhibitors/ARB's or beta-blockers and for patients who were non-responders as defined by lack of up-titration (≤50% of recommended treatment dose). This resulted in 6 different models predicting mortality and/or heart failure hospitalization; three models predicting mortality and/or heart failure hospitalization in successfully up-titrated patients for ACE-inhibitors/ARB's, beta-blockers and MRA's, and three for non-responding patients who were up-titrated to ≤50% of recommended ACE-inhibitor/ARB

and beta-blocker doses and <50% recommended MRA dose. We stratified on given treatment

and considered both the main effects of all biomarkers as well as all interactions of biomarkers

with treatment. In the 100 test samples, we subsequently evaluated the goodness of fit of the

selected sparse Cox regression models. We calculated both calibration and discrimination

statistics (c-statistic and shrinkage statistic). Moreover, the benefit of successful and not

successful up-titration was calculated for the patients in the test samples. All analyses were

inversely weighted with the probability of the given treatment to account for indication bias.

<u>Treatment benefit statistics</u>

We calculated the expected number of events at 24 months follow-up for three scenarios:

A) if all patients are successfully up-titrated to >50% of recommended doses according to the

ESC guidelines ($\geq$50% for MRA's); B) if all patients are up-titrated following a treatment-

strategy based on the biomarker values; C) if no patient is treated at >50% of recommended

doses according to the ESC heart failure guidelines ($\geq$50% for MRA's). We performed all

analyses for ACE-inhibitor/ARB and beta-blocker separately. For scenario B; we decided to up-

titrate when the probability of survival for mortality and/or hospitalization at 24 months for up-

titrating were higher than for not up-titrating, and vice versa.

The survival probabilities were based on the difference of a patient's mean death and/or

heart failure hospitalization probability at 24 months follow-up ($S(t = 24|\dots)$) under both

treatments according to the sparse Cox regression models estimated for the associated training

sample:

$S(t = 24|successful\ uptitration, X = x) - S(t = 24|unsuccessful\ uptitration, X = x)$.

where 'X=x' represent specific levels of the biomarkers selected in the Cox models for predicting

mortality and/or heart failure hospitalization in the successfully and not-successfully up-titrated

patients, respectively. The difference was averaged over all test samples that included the specific patient, and was subsequently multiplied with total number of patients. This benefit-statistic can be interpreted as the number of deaths and/or heart failure hospitalizations that is prevented at 24 months by successful up-titrating to >50% of recommended doses according to the ESC guidelines.

Benefit-statistics were calculated for each test sample separately. The standard deviation of the benefit-statistics over the 100 test samples was then used as an estimate of the standard error of the mean benefit-statistic.

Results

Of the 2516 patients included in the index cohort, 151 patients died, 23 patients were censored before 3 months follow-up and 242 patients had a LVEF >40%; these patients were excluded from the current data-analysis. Of the remaining 2100 patients, there were 298 patients with missing values on more than 50% of the biomarkers. Subsequent analyses were done with data from the remaining 1802 patients. Because BIOSTAT-CHF is not a randomized trial, we corrected for the probability of being up-titrated to >50% of recommended treatment dose. Biomarkers predictive for up-titration and subsequent indication-bias correction is presented in supplementary data. Of the 1802 patients, 529 (29%) were up-titrated to >50% of recommended ACE-inhibitor/ARB dose and 318 (18%) to >50% of recommended beta-blocker dose. We have MRA treatment data for 1423 patients at 9 months after inclusion. Of these 1423 patients, 14% (195) patients were successfully up-titrated to ≥50% recommended treatment doses (2% (28) to >50% recommended doses). Patient characteristics of patients achieving >50 recommended ACE-inhibitor/ARB and beta-blocker dose and ≥50% recommended MRA dose and of those who did not respond to recommended treatment are presented in **Table 1**.

Multivariable treatment-selection markers

To distinguish patients who benefited from up-titration from those who did not, we created two models. From 161 biomarkers, we first identified the strongest biomarkers to predict clinical events (death of heart failure hospitalization) despite successful up-titration with either ACE-inhibitors/ARBs or beta-blockers. Most frequently selected biomarkers are reported in Online Table 2. BUN, FGF23 and pro-ENK were the strongest predictors of clinical events in patients that were successfully up-titrated with ACE-inhibitors/ARBs. Serum creatinine, galetin-3, ST2 and albumin were the strongest predictors of clinical events in patients that were successfully up-titrated with beta-blockers (Online Table 3). Predictive biomarkers for events in up-titrated patients with MRAs are presented in Online Table 4.

In the second model, we identified the strongest biomarkers to predict clinical events in patients who were NOT successfully up-titrated with either ACE-inhibitors/ARBs or beta-blockers. FGF23, BUN, cystatin C, ST2, WAP-4C and IGFBP-2 were the strongest predictors of clinical events in patients that were NOT successfully up-titrated with ACE-inhibitors/ARBs. FGF23, cystatin C, BUN, WAP4C and NT-proBNP were the strongest predictors of clinical events in patients that were NOT successfully up-titrated with beta-blockers.

The treatment-selection models had reasonable performances for the patients in the test sets. Averaged c-statistics for ACE-inhibitor/ARB models were 0.74 (0.68-0.80) in up-titrated patients, and 0.77 (95% CI 0.70-0.83) in not-up-titrated patients, respectively. Beta-blocker treatment-selection models averaged c-statistics were 0.75 (95% CI 0.70-0.82) in up-titrated patients, and 0.78 (95% CI 0.73-0.83) in not-up-titrated patients, respectively. C-statistic for MRA treatment-selection models were 0.65 (95% CI 0.56-0.74) and 0.77 (0.71-0.86) in up-titrated and NOT up-titrated patients.

Using both models, we were able to calculate survival probability at 24 months for both scenarios (successful or non-successful up-titration). The scenario with the highest probability was considered the most beneficial one for the individual patient. In 2% (n=42) of patients, the highest probability was found in patients who were not successfully up-titrated with ACE-inhibitors/ARBs. Patients characteristics of these patients are presented in Table 3. Patients not benefitting from ACE-inhibitor/ARB up-titration were younger, more frequent smokers, with less AF; higher haemoglobin and BUN, but lower heart rate and NT-proBNP levels. In 33% (n=592) of patients, the highest survival probability was found in patients who were not successfully up-titrated with beta-blockers. Patients characteristics of these patients are presented in Table 3. Patients not benefitting from beta-blocker up-titration were older, leaner, more frequently smoker or former smoker. The also had less ischemic HF, but more myocardial infarction, and other co-morbidities. They also had significantly higher LVEF, (NT-pro)BNP, BUN and creatinine, levels, and lower DBP, heart rate, haemoglobin and eGFR levels. Patients for whom up-titrating MRA treatment was not beneficial for 13% (n=184) of the patients.

Clinical events according to the three hypothetical scenarios

Kaplan-Meier curves for ACE-inhibitor/ARB scenarios are presented in **Figure 1**. Mortality and/or heart failure hospitalization was highest in the scenario where no patient was up-titrated to at least 50% of the recommended dose. Patients who were up-titrated based on their biomarker profile had the lowest risk of death and/or heart failure hospitalization.

Estimated event rate and averaged expected events at 24 months for each of the three hypothetical scenarios are presented in **Table 2.** If all patients were successfully up-titrated to >50% of recommended doses ACE-inhibitor/ARBs (Scenario A), estimated death and/or hospital admission occurred in 297 (260-335) patients. If patients were up-titrated with ACE-

inhibitor/ARBs following a treatment-strategy based on the biomarker values (Scenario B), estimated death and/or hospital admission occurred in 296 (260-333) patients. If no patient was treated with >50% of recommended doses ACE-inhibitor/ARBs (Scenario C), estimated death and/or hospital admission occurred in 474 (438-511) patients. Up-titrating ACE-inhibitor/ARBs to >50% of recommended dose compared to ≤50% less than recommended dose resulted in 174 fewer events (95% CI 128-227) ; p-value= 0.0003). Per 100 treated patients, this means that 9.8 (95% CI 7.1-12.6) fewer events were seen in this scenario. The biomarkers-based approach led to 178 fewer events (95% CI 130-226 ; p-value=0.0003) compared to the ≤50% recommended dose group. Per 100 treated patients this resulted in 9.9 (95% CI 7.2-12.6) fewer events.

Kaplan-Meier curves for beta-blocker scenarios are presented in **Figure 2**. Mortality and/or heart failure hospitalization was highest in the scenario where no patient was up-titrated to at least 50% of the recommended dose. Patients who were up-titrated based on their biomarker profile had the lowest risk of death and/or heart failure hospitalization, which was slightly lower compared to a scenario in which all patients were up-titrated to >50% of the recommended dose of ACE-inhibitor/ARBs.

Estimated event rate and averaged expected events at 24 months for each of the three hypothetical scenarios are presented in Table 2. If all patients were successfully up-titrated to recommended beta-blocker doses (Scenario A), estimated death and/or hospital admission occurred in 404 (95% CI 332-477) patients. If patients were up-titrated with beta-blockers following a treatment-strategy based on the biomarker values (Scenario B), estimated death and/or hospital admission occurred in 345 (95% CI 300-389) patients. If no patient was treated with recommended doses beta-blockers (Scenario C), estimated death and/or hospital admission occurred in 428 (95% CI 391-466) patients. Up-titrating beta-blockers to >50% of recommended

dose compared to ≤50% resulted in 24 less events (95% CI -54-103); p-value=0.50). The biomarkers-based approach led to 84 fewer events (95% CI 40-128; p-value=0.01) compared to the ≤50% recommended dose group. This means that 1.3 (95% CI -3-5.7) and 4.7 (95% CI 2.2-7.1) events could be prevented per 100 treated patients in both scenarios.

When considering up-titrating to both 50% of recommended ACE-inhibitor/ARB and beta-blocker dose we estimated that 222 (95% CI 147-298) event could be prevented when all patients would be up-titrated to at least 50% recommended treatment dose for both ACE-inhibitors/ARBs and beta-blockers. Another 14 events (95% CI -52-80) could be prevented when the decision to up-titrated was based on a biomarker-based model (Online Appendix).

For MRA treatment we estimated that not up-titrating patients to ≥50% of recommended MRA dose would result in 437 (95% CI 405-469) events. When we would up-titrate all patients this would be reduced with 222 (95% CI 147-298; p=0.0001) events to 215 (95% CI 150-280). Our biomarker-based model resulted in 236 (95% CI 170-303; p=0.0004) less events than when no patient would be up-titrated to ≥50% of recommended MRA dose.

Discussion

We hypothesized that not every patient with HFrEF will benefit from maximal up-titration with either ACE-inhibitors/ARBs or beta-blockers. We therefore tested 3 hypothetical scenarios: A) all patients were up-titrated to >50% of recommended ACE-inhibitor/ARB or beta-blocker dose, B) all patients were up-titrated or not based on a biomarker model, and C) no patient was up-titrated to >50% of recommended ACE-inhibitor/ARB or beta-blocker dose. Our models estimated that the highest number of events would have occurred in Scenario C) and the lowest number of events in Scenario B). The present results from this novel approach suggest that some patients do not benefit from maximally recommended doses.

There are many biomarkers known to influence therapeutic response and survival (27,28), and there have been many attempts to use biomarker levels for evaluating treatment response and outcome (29). However, no models were developed using a multitude of biomarkers to estimate and compare the risk of mortality and/or heart failure hospitalization in up-titrated and not up-titrated patients.

We recently published a meta-analysis on all prognostic heart failure models and an average c-statistics for predicting mortality and/or heart failure related hospitalization of 0.68 (30). Thus, the biomarker-based treatment-selection models in the present paper have similar predictive performance compared to existing models. Most of these prognostic models were based on clinical and biographical patient-characteristics with few biomarkers. The association of some biomarkers that we identified (e.g. NT-proBNP, BUN, ST2, and hemoglobin) with mortality or heart failure hospitalization-risk in heart failure patients is well known (9, 10, 31-38), but a differential predictive value in patients who were successfully up-titrated versus those who were not, was as yet unknown. This observation may be useful to identify residual heart failure disease and additional treatment targets in heart failure patients. Although our biomarker-based treatment-selection models have comparable performance to other prediction models, the performance of these models is still modest and they have large confidence bounds. In this study, we only looked at benefit, and did not take harm into account. Not up-titrating might be more beneficial for a patient, however up-titrating might not do harm.

We decided to dichotomize up-titration into successful or not. In clinical practice, the actual doses of ACE inhibitors/ARB's and beta-blockers vary substantially. Since we recently published data from BIOSTAT-CHF showing that up-titrating patients to 50-99% of recommended ACE-inhibitor/ARB and beta-blocker doses results in comparable survival and/or

heart failure related hospitalization reduction (21), we considered patients successfully up-titrated when >50% of recommended dose was achieved after 3 months of up-titration.

The BIOSTAT-CHF population mainly consists of patients with advanced heart failure who may be more likely to have limited benefit from up-titration of ACE-inhibitor/ARB and beta-blocker therapy. These patients may be worsened by even small doses of beta-blockers, or they may experience excessive hypotension and worsening renal function from ACE-inhibitor/ARBs. BIOSTAT-CHF was specifically designed to record reasons for not up-titrating to recommended treatment doses. Only in 26 and 22% of the patients for ACE-inhibitors/ARBs and beta-blockers, this was caused by intolerance to the drug, either because of organ dysfunction. In the majority of patients, no specific reason was provided (21). This analysis supports the concept that even less clinical ill patients may not be helped by ACE-inhibitor/ARB and beta-blocker up-titration.

There were significant hemodynamic differences (heart rate and blood pressure) between patients who were up-titrated >50% of recommended treatment dose and those who were not. This might suggest that these and other variables were at least partly responsible for the different achieved up-titration doses. We corrected for these difference by propensity score matching and inverse probability of treatment weighing.

Limitations

One major important limitation of the present study is that heart failure treatment was not randomly assigned in our study. Up-titration of ACE inhibitor/ARB's and beta-blockers has been shown to be beneficial on average in many randomized clinical trials and has been adopted into the ESC heart failure guidelines. It is striking, however, that in clinical practice so many patients are not up-titrated to >50% of recommended dose. We tried to adjust for this treatment-

indication bias, introduced in this cohort type BIOSTAT-CHF study, by two generally accepted

advanced statistical methods: propensity scoring and inverse probability of treatment weighing.

Whether this corrected the treatment-indication bias sufficiently is unfortunately not testable.

A second limitation is the large number of biomarkers that we analysed which increased

the chance of false positive findings. We used Bonferroni correction of p-values and we used

sparse regression models to minimize the risk of overfitting. Lasso penalization is known to yield

too large regression models (with too many predictor variables) (39), so our models might still be

somewhat larger than necessary (on average > 23 biomarkers). We used a repeated split-sample

technique to cross-validate benefit- and fit-statistics to reduce the effect of overfitting.

A third limitation of our analyses is that we ignored patients who died in the first three

months of up-titration period. We excluded 151 deaths and the survival at three months was only

93%. We made a prediction model for the risk of death within 3 months and found that FGF23,

NT-proBNP, BNP, low haemoglobin, TNI, ET1, ST2, WAP4C and CRP were the most

important predictors of death within 3 months. This selection of biomarkers coincided largely

with the set of biomarkers that we identified as prognostic in the patients who were not

successfully up-titrated for both ACE-inhibitors/ARBs and beta-blockers. Therefore, we assume

that the presented results were not largely biased by the removal of the 151 deaths. We only had

MRA dose data available after 9 months follow-up. This introduces additional bias because

excluded even more patients than for the ACE-inhibitor/ARB and beta-blockers analyses. We

tried to correct for this by inverse probability weighting. Although, we cannot test if this was

sufficient, we think the MRA data adds important information to our models.

Because not all biomarkers used in our treatment-selection models were measured in the

validation cohort of BIOSTAT-CHF existing of 1728 patients, we unfortunately could not

validate our results in this cohort. In the future, and when funding is available we aim to measure the missing biomarkers and validate our treatment-selection models in this cohort as well.

We found substantial differences between patients of which the model assumed not to benefit from ACE-inhibitor/ARB up-titration and patients of which the model assumed not to benefit from beta-blocker up-titration. Patients not benefitting from ACE-inhibitor/ARB up-titration were younger, with lower BNP and NT-proBNP, higher haemoglobin levels. Patients not benefitting from beta-blocker up-titration, conversely, were more often older, had higher BNP and NT-proBNP, lower haemoglobin compared to patients benefitting from beta-blocker up-titration. Blood urea nitrogen was elevated and heart rate was lower in both patients not benefitting from ACE-inhibitor/ARB up-titration and patients not benefitting from beta-blocker up-titration.

Other possible limitations that could not be addressed in our cohort are the fact that our data is unfortunately limited to Caucasian patients only, and that there was a very low use of device therapy. This would possibly limit the use of our biomarker-selection model in a more heterogeneous population. The percentage of device therapy is nevertheless comparable to EMPHASIS-HF which recruited patients at the same time as BIOSTAT-CHF (40).

Biomarkers predictive for mortality and/or hospitalization, were also markedly different between patients who were successfully up-titrated or not. This might have been expected because biomarkers related to ACE inhibition/ARB and beta-blocking pathways are likely to change substantially as a result of up-titration (41).

Conclusion

A biomarker-based treatment up-titration choice in patients with heart failure was favourable over both a hypothetical scenario in which all patients would have been successfully

up-titrated to >50% of recommended of ACE-inhibitor/ARB and beta-blocker dose and ≥50% MRA dose. We estimated that 1 in 50, 1/3 and 1/8 patients will not benefit from ACE-inhibitor/ARB, beta-blocker or MRA up-titration, but their mortality/hospitalization hazards do not increase much by up-titration. Because of the nature of this study, and the small differences between biomarker-based treatment choice and the scenario in which all patients would have been successfully up-titrated, we suggest that up-titration should always be attempted in heart failure patients, which should lead to improved treatment of life-saving therapies across Europe.

PERSPECTIVES

Competency in Medical Knowledge: Not all patients benefit from up-titrating ACE-inhibitor/ARB, beta-blocker and MRA to high evidence based recommended treatment dose. Predicting benefit based on treatment-selection models using individual biomarker profiles results in a higher reduction of mortality and/or heart failure related hospitalization than when all patients are up-titrated, although the difference is small.

Competency in Patient Care: A patient should be up-titrated to evidence based recommended treatment dose or not based on a biomarker profile using our treatment-selection models. This reduces the change of death and/or heart failure related hospitalization.

Translational Outlook: We have developed treatment-selection models in which patients should be up-titrated or not based on biomarker profiles. These biomarker treatment-selection models should be tested in a randomized fashion. The biomarkers selected in our treatment-selection models might lead to more insight into heart failure pathogenesis, and may lead to new treatment options.

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Figure Legends

Central Illustration: Biomarker Guided treatment in heart failure. A multitude of biomarker values determine if a patient should be treated to evidence based recommended treatment or not.

Figure 1. Estimated Kaplan-Meier survival curves based on three scenarios for up-titrating ACE-inhibitors/ARBs. Estimated Kaplan-Meier survival curves with the expected event-free survival rate and time in months based on three scenarios (green, blue and red lines): A) if all patients were up-titrated to >50% of recommended ACE-inhibitor/ARB dose (green); B) if all patients were up-titrated according to biomarker-selection model (blue); C) if no patient was up-titrated to >50% of recommended ACE-inhibitor/ARB dose (red), with 95% confidence interval.

Figure 2. Estimated Kaplan-Meier survival curves based three scenarios for up-titrating beta-blockers. Estimated Kaplan-Meier survival curves with the expected event-free survival rate and time in months based on three scenarios (green, blue and red lines): A) if all patients were up-titrated to >50% of recommended beta-blocker dose (green); B) if all patients were up-titrated according to biomarker-selection model (blue); C) if no patient was up-titrated to >50% of recommended of beta-blocker dose (red), with 95% confidence interval.

Figure 3. Estimated Kaplan-Meier survival curves based three scenarios for up-titrating MRA's. Estimated Kaplan-Meier survival curves with the expected event-free survival rate and time in months based on three scenarios (green, blue and red lines): A) if all patients were up-titrated to ≥50% of recommended MRA dose (green); B) if all patients were up-titrated according to biomarker-selection model (blue); C) if no patient was up-titrated to ≥50% of recommended of MRA dose (red), with 95% confidence interval.

Table 1: Baseline characteristics of the patients who were up-titrated to >50% of recommended ACE-inhibitor/ARB and beta-blocker and ≥50% MRA dose and those who were not.

	ACE-inhibitor/ARB			beta-blocker			MRA		
	successful up-titration	no successful up-titration	P - value	successful up-titration	no successful up-titration	P - value	successful up-titration	no successful up-titration	P - value
Number of patients: n (%)	529	1273		318	1484		195	1228	
% of recommended ACE-inhibotor/ARB dose: mean (SD)	100 (28)	29 (18)		61 (39)	48 (38)		54 (38)	52 (39)	
% of recommended beta-blocker dose: mean (SD)	45 (32)	34 (30)		93 (18)	25 (17)		38 (30)	37 (31)	
Age (years): mean (SD)	66.36 (11.85)	68.15 (12.12)	0.004	66.14 (12.63)	67.94 (11.92)	0.02	63.21 (12.35)	67.71 (11.89)	<0.00001
Male gender: n (%)	395 (75%)	967 (76%)	0.56	235 (74%)	1127 (76%)	0.44	161 (83%)	914 (74%)	0.01
Caucasian ethnicity: n (%)	523 (99%)	1259 (99%)	0.29	314 (99%)	1468 (99%)	0.04	187 (96%)	1219 (99%)	0.0006
BMI (kg/m2): mean (SD)	28.93 (6.02)	27.49 (5.26)	<0.00001	28.41 (5.57)	27.81 (5.51)	0.09	28.84 (5.55)	27.87 (5.51)	0.02
Systolic blood pressure: mean (SD)	130.04 (22.37)	121.24 (20.5)	<0.00001	125.47 (21.7)	123.46 (21.37)	0.13	121.62 (18.39)	125.68 (21.24)	0.006
Diastolic blood pressure (mmHg): mean (SD)	79.03 (13.71)	73.68 (12.6)	<0.00001	78.35 (14.45)	74.58 (12.77)	0.00002	75.03 (11.24)	76.36 (13.35)	0.14
Heart rate (bpm): mean (SD)	79.88 (20.33)	79.97 (19.19)	0.93	85.3 (22.25)	78.8 (18.7)	<0.00001	80.66 (18.97)	79.87 (20.29)	0.59
Smoking (current/ever/never): n	197/256/76	450/630/193	0.73	101/177/40	546/709/229	0.04	63/94/38	450/602/176	0.14
Alcohol use: n (%)	368 (70%)	909 (71%)	0.45	203 (64%)	1074 (72%)	0.003	132 (68%)	872 (71%)	0.33
Ischemic HF etiology: n (%)	261 (49%)	563 (44%)	0.05	163 (51%)	661 (45%)	0.03	100 (51%)	584 (48%)	0.33
HF duration (years): median (IQR)	8.81 (4.43-14.09)	7.59 (3.34-13.2)	0.5	8.54 (3.77-17.02)	7.64 (3.49-12.72)	0.39	10.52 (5.86-15.42)	6.76 (2.89-12.93)	0.27
NYHA class III/IV: n (%)	244 (46%)	509 (40%)	0.02	134 (42%)	619 (42%)	0.89	83 (43%)	566 (46%)	0.36
LVEF: median (IQR)	29 (24-34)	28 (22-34)	0.0005	29 (24-34)	29 (24-34)	0.3	24 (19-29)	29 (24-34)	0.00001
NT-proBNP, ng/L: median (IQR)	32109 (29824-34465)	33454 (30868-35940)	0.00001	32593 (30378-35101)	32919 (30630-35676)	0.19	32008 (29504-34411)	32704 (30398-35513)	0.03
Oedema, % (n)	228 (43%)	603 (47%)	0.1	156 (49%)	675 (45%)	0.25	86 (44%)	526 (43%)	0.74
Orthopnoea, % (n)	150 (28%)	431 (34%)	0.02	82 (26%)	499 (34%)	0.006	62 (32%)	366 (30%)	0.58
Rales >1/3 up lung fields, % (n)	44 (19%)	125 (19%)	0.98	17 (12%)	152 (20%)	0.03	12 (13%)	104 (18%)	0.21
Jugular venous pressure, % (n)	111 (29%)	281 (31%)	0.45	63 (28%)	329 (31%)	0.37	43 (30%)	240 (27%)	0.43
Hepatomegaly, % (n)	60 (11%)	184 (14%)	0.07	39 (12%)	205 (14%)	0.45	39 (20%)	125 (10%)	0.00007
Hypertension, % (n)	349 (66%)	731 (57%)	0.0007	195 (61%)	885 (60%)	0.58	107 (55%)	750 (61%)	0.1
Atrial fibrillation, % (n)	209 (40%)	564 (44%)	0.06	163 (51%)	610 (41%)	0.0009	80 (41%)	518 (42%)	0.76
Myocardial infarction, % (n)	188 (36%)	491 (39%)	0.23	113 (36%)	566 (38%)	0.38	61 (31%)	441 (36%)	0.21
PCI, % (n)	106 (20%)	285 (22%)	0.27	72 (23%)	319 (21%)	0.65	39 (20%)	260 (21%)	0.71

CABG, % (n)	70 (13%)	220 (17%)	0.03	47 (15%)	243 (16%)	0.48	23 (12%)	183 (15%)	0.25
None	427 (24%)	932 (52%)	0.02	234 (13%)	1125 (62%)	0.52	136 (10%)	969 (68%)	0.004
Pacemaker only	28 (2%)	89 (5%)		16 (1%)	101 (6%)		8 (1%)	80 (6%)	
ICD only	31 (2%)	121 (7%)		30 (2%)	122 (7%)		25 (2%)	84 (6%)	
CRT only	11 (1%)	24 (1%)		7 (0%)	28 (2%)		5 (0%)	19 (1%)	
ICD and CRT	31 (2%)	102 (6%)		30 (2%)	103 (6%)		20 (1%)	72 (5%)	
Other	1 (0%)	5 (0%)		1 (0%)	5 (0%)		1 (0%)	4 (0%)	
Diabetes mellitus, % (n)	182 (34%)	389 (31%)	0.11	97 (31%)	474 (32%)	0.62	63 (32%)	351 (29%)	0.29
COPD, % (n)	70 (13%)	220 (17%)	0.03	42 (13%)	248 (17%)	0.12	28 (14%)	185 (15%)	0.8
Stroke, % (n)	40 (8%)	122 (10%)	0.17	20 (6%)	142 (10%)	0.06	12 (6%)	112 (9%)	0.17
Peripheral artery disease, % (n)	46 (9%)	142 (11%)	0.12	27 (8%)	161 (11%)	0.21	16 (8%)	121 (10%)	0.47
Aldosterone antagonists, % (n)	267 (50%)	719 (56%)	0.02	150 (47%)	836 (56%)	0.003	156 (80%)	621 (51%)	<0.00001
Loop diuretics, % (n)	526 (99%)	1268 (100%)	0.61	317 (100%)	1477 (100%)	0.7	194 (99%)	1221 (99%)	0.92
Digoxin, % (n)	82 (16%)	242 (19%)	0.08	54 (17%)	270 (18%)	0.61	50 (26%)	206 (17%)	0.003
Haemoglobin, g/dL: mean (SD)	12.69 (1.73)	12 (2)	<0.00001	12.52 (1.81)	12 (2)	0.13	12.71 (1.79)	13 (2)	0.14
Creatinine, Œºmol/L: median (IQR)	481 (470-500)	491 (470-515)	<0.00001	484 (467-506)	487 (467-510)	0.19	482 (463-497)	484 (463-508)	0.09
BUN, mmol/L: median (IQR)	25.5 (24.2-31.6)	29 (24-35)	<0.00001	26.7 (23.5-32.4)	28 (23-34)	0.005	28 (22.7-32.5)	27 (23-33)	0.69
GFR MDRD formula, mL/min.1.73m2: mean (SD)	71 (22)	64 (24)	<0.00001	68 (24)	65 (23)	0.09	73 (20)	67 (23)	0.001
Sodium, mmol/L: mean (SD)	138.85 (3.55)	138.06 (3.81)	0.00004	138.62 (3.46)	138.22 (3.81)	0.07	138.56 (3.84)	138.53 (3.6)	0.91
Potassium, mmol/L: mean (SD)	3.24 (0.53)	3.29 (0.56)	0.07	3.24 (0.51)	3.28 (0.56)	0.2	3.19 (0.5)	3.28 (0.56)	0.01
BNP, pg/mL: median (IQR)	3931 (3624-4227)	4010 (3624-4438)	0.04	3966 (3496-4482)	3984 (3496-4343)	0.92	3991 (3418-4172)	3937 (3418-4319)	0.84

BNP: brain natriuretic peptide; BUN: blood urea nitrogen; CRT: cardiac resynchronization therapy ; GFR: glomerular filtration rate; ICD: implantable cardioverter-defibrillator IQR: interquartile range; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association; N-terminal prohormone of BNP; SD: standard deviation

Table 2: Estimation of mortality and/or heart failure hospitalizations at 24 months three scenarios: Scenario A) if all patients are successfully up-titrated to more than 50% of recommended dose; scenario B) if up-titration was based on the biomarker treatment-selection model; scenario C) if no patient was successfully up-titrated for ACE-inhibitors/ARB's.

ACE-inhibitor/ARB	Scenario A	Scenario B	Scenario C
Estimated event rate at 24 months	16%	16%	26%
Estimated number of events (95% CI)	297 (260-335)	296 (260-333)	474 (438-511)
Estimated event reduction compared to scenario C (95% CI)	177 (128-227)	178 (130-226)	-
Estimated event reduction compared to scenario C (95% CI) per 100 treated patients	9.8 (7.1-12.6)	9.9 (7.2-12.6)	

Beta-blocker	Scenario A	Scenario B	Scenario C
Estimated event rate at 24 months	23%	19%	24%
Estimated number of events (95% CI)	404 (332-477)	345 (300-389)	428 (391-466)
Estimated event reduction compared to scenario C (95% CI)	24 (-54-102.55)	84 (40-128)	-
Estimated event reduction compared to scenario C (95% CI) per 100 treated patients	1.3 (-3-5.7)	4.7 (2.2-7.1)	

MRA	Scenario A	Scenario B	Scenario C
Estimated event rate at 24 months	12%	11%	24%
Estimated number of events (95% CI)	215 (150-280)	201 (147-255)	437 (405-469)
Estimated event reduction compared to scenario C (95% CI)	222 (147-298)	236 (170-303)	-
Estimated event reduction compared to scenario C (95% CI) per 100 treated patients	12.3 (8.1-16.5)	13.1 (9.4-16.8)	

Scenario A) if all patients are successfully up-titrated; scenario B) if up-titration was based on the biomarker treatment-selection model; scenario C) if no patient was successfully up-titrated; CI: confidence interval

Table 3: Characteristics of patients who did benefit from ACE-inhibitor/ARB, beta-blocker or MRA up-titration and those who did not.

	ACE-inhibitor/ARB			beta-blocker			MRA		
	benefit up-titration	NOT benefit up-titration	P-value	benefit up-titration	NOT benefit up-titration	P-value	benefit up-titration	NOT benefit up-titration	P-value
Number of patients: n (%)	1760	42		1210	592		1573	229	
% of recommended ACE-inhibotor/ARB dose: mean (SD)	50 (39)	57 (41)		51 (38)	47 (40)		49 (39)	59 (39)	
% of recommended beta-blocker dose: mean (SD)	37 (31)	41 (32)		37 (31)	36 (32)		37 (32)	37 (28)	
Age (years): mean (SD)	67.72 (12)	63.37 (14)	0.05	65.93 (12.13)	71.08 (11.18)	<0.00001	68.04 (12.04)	64.77 (11.88)	0.0001
Male gender: n (%)	1331 (76%)	31 (74%)	0.79	922 (76%)	440 (74%)	0.38	1219 (77%)	143 (62%)	<0.00001
Caucasian ethnicity: n (%)	1742 (99%)	40 (95%)	0.08	1194 (99%)	588 (99%)	0.54	1555 (99%)	227 (99%)	0.42
BMI (kg/m2): mean (SD)	27.89 (5.55)	28.82 (4.22)	0.18	28.37 (5.72)	26.99 (4.98)	<0.00001	27.86 (5.55)	28.27 (5.39)	0.3
Systolic blood pressure: mean (SD)	123.67 (21.46)	129.81 (19.94)	0.06	124.26 (21.77)	122.9 (20.73)	0.2	123.26 (21.44)	127.61 (21.09)	0.004
Diastolic blood pressure (mmHg): mean (SD)	75.18 (13.15)	78.07 (13.62)	0.18	76.23 (13.53)	73.23 (12.13)	<0.00001	74.87 (13.11)	77.8 (13.26)	0.002
Heart rate (bpm): mean (SD)	80.05 (19.63)	75.24 (13.49)	0.03	80.91 (19.36)	77.97 (19.72)	0.003	80.62 (19.68)	75.29 (17.72)	0.00004
Smoking (current/ever/never): n	626/866/268	21/20/1	0.03	417/584/209	230/302/60	0.0003	560/779/234	87/107/35	0.72
Alcohol use: n (%)	1246 (71%)	31 (74%)	0.68	857 (71%)	420 (71%)	0.94	1094 (70%)	183 (80%)	0.0009
Ischemic HF etiology: n (%)	801 (46%)	23 (55%)	0.23	582 (48%)	242 (41%)	0.004	715 (45%)	109 (48%)	0.54
HF duration (years): median (IQR)	8.02 (3.55-13.4)	3.54 (1.6-6.59)	0.2	8.34 (3.78-13.54)	6.38 (2.61-12.46)	0.31	8.3 (3.27-13.74)	5.8 (5.03-8.84)	0.28
NYHA class III/IV: n (%)	731 (42%)	22 (52%)	0.16	498 (41%)	255 (43%)	0.44	618 (39%)	135 (59%)	<0.00001
LVEF: median (IQR)	29 (24-34)	29 (24-34)	0.19	27 (23-34)	29 (24-34)	0.00001	28 (23-34)	29 (24-36)	<0.00001
NT-proBNP, ng/L: median (IQR)	32900 (30630-35620)	27928 (26980-32965)	0.01	32635 (30247-35086)	33593 (31140-36655)	0.00003	33143 (30708-35788)	31303 (29506-33500)	0.00001
Oedema, % (n)	818 (46%)	13 (31%)	0.05	558 (46%)	273 (46%)	1	753 (48%)	78 (34%)	0.00009
Orthopnoea, % (n)	567 (32%)	14 (33%)	0.88	404 (33%)	177 (30%)	0.14	538 (34%)	43 (19%)	<0.00001
Rales >1/3 up lung fields, % (n)	166 (19%)	3 (14%)	0.58	108 (18%)	61 (20%)	0.43	159 (20%)	10 (12%)	0.1
Jugular venous pressure, % (n)	387 (31%)	5 (16%)	0.07	256 (30%)	136 (31%)	0.8	365 (32%)	27 (16%)	0.00001
Hepatomegaly, % (n)	240 (14%)	4 (10%)	0.44	166 (14%)	78 (13%)	0.75	224 (14%)	20 (9%)	0.02
Hypertension, % (n)	1052 (60%)	28 (67%)	0.37	713 (59%)	367 (62%)	0.21	940 (60%)	140 (61%)	0.69
Atrial fibrillation, % (n)	763 (43%)	10 (24%)	0.01	517 (43%)	256 (43%)	0.84	716 (46%)	57 (25%)	<0.00001
Myocardial infarction, % (n)	668 (38%)	11 (26%)	0.12	415 (34%)	264 (45%)	0.00002	595 (38%)	84 (37%)	0.74
PCI, % (n)	385 (22%)	6 (14%)	0.24	248 (20%)	143 (24%)	0.08	345 (22%)	46 (20%)	0.53
CABG, % (n)	282 (16%)	8 (19%)	0.6	180 (15%)	110 (19%)	0.04	254 (16%)	36 (16%)	0.87
None	1326 (74%)	33 (2%)	0.31	927 (51%)	432 (24%)	0.39	1177 (65%)	182 (10%)	0.37
Pacemaker only	116 (6%)	1 (0%)		70 (4%)	47 (3%)		105 (6%)	12 (1%)	

ICD only	151 (8%)	1 (0%)		101 (6%)	51 (3%)		133 (7%)	19 (1%)	
CRT only	34 (2%)	1 (0%)		25 (1%)	10 (1%)		34 (2%)	1 (0%)	
ICD and CRT	127 (7%)	6 (0%)		83 (5%)	50 (3%)		118 (7%)	15 (1%)	
Other	6 (0%)	0 (0%)		4 (0%)	2 (0%)		6 (0%)	0 (0%)	
Diabetes mellitus, % (n)	560 (32%)	11 (26%)	0.44	367 (30%)	204 (34%)	0.08	506 (32%)	65 (28%)	0.25
COPD, % (n)	287 (16%)	3 (7%)	0.11	194 (16%)	96 (16%)	0.92	259 (16%)	31 (14%)	0.26
Stroke, % (n)	162 (9%)	0 (0%)	0.04	101 (8%)	61 (10%)	0.17	148 (9%)	14 (6%)	0.1
Peripheral artery disease, % (n)	185 (11%)	3 (7%)	0.48	120 (10%)	68 (11%)	0.31	173 (11%)	15 (7%)	0.04
Aldosterone antagonists, % (n)	966 (55%)	20 (48%)	0.35	692 (57%)	294 (50%)	0.003	854 (54%)	132 (58%)	0.34
Loop diuretics, % (n)	1752 (100%)	42 (100%)	0.66	1203 (99%)	591 (100%)	0.22	1567 (100%)	227 (99%)	0.3
Digoxin, % (n)	321 (18%)	3 (7%)	0.06	231 (19%)	93 (16%)	0.08	296 (19%)	28 (12%)	0.02
Haemoglobin, g/dL: mean (SD)	12.36 (1.85)	13 (1)	0.004	12.79 (1.73)	12 (2)	<0.00001	12.4 (1.87)	12.23 (1.68)	0.17
Creatinine, mmol/L: median (IQR)	486 (461-510)	488 (461-508)	0.55	482 (476-502)	500 (476-527)	<0.00001	489 (451-514)	472 (451-491)	<0.00001
BUN, mmol/L: median (IQR)	28 (26.9-33.6)	33 (27-36)	0.002	27.4 (24.4-32.8)	29 (24-35)	0.0001	28.4 (21.5-34)	25.2 (21.5-30.5)	<0.00001
GFR MDRD formula, mL/min.1.73m2: mean (SD)	66 (23)	70 (25)	0.29	70 (22)	56 (23)	<0.00001	64 (23)	78.16 (21.68)	<0.00001
Sodium, mmol/L: mean (SD)	138.28 (3.75)	138.95 (3.88)	0.27	138.42 (3.64)	138.03 (3.97)	0.04	138.11 (3.84)	139.58 (2.79)	<0.00001
Potassium, mmol/L: mean (SD)	3.27 (0.55)	3.29 (0.53)	0.86	3.26 (0.53)	3.31 (0.58)	0.08	3.27 (0.55)	3.33 (0.52)	0.1
BNP, pg/mL: median (IQR)	3985 (2090-4357)	3124 (2090-3823)	0.04	3914 (3744-4282)	4182 (3744-4457)	0.008	3999 (2631-4394)	3403 (2631-3877)	0.004