Plasma biomarkers to predict or rule out early post-discharge events in patients discharged after an acute heart failure hospital admission

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Abstract

Aim: Improved prediction of early post-discharge death or rehospitalization after admission for acute heart failure (AHF) is a major unmet need. We evaluated the value of biomarkers to predict either low or high risk for early post-discharge events.

Methods and results: A total of 1653 patients enrolled in the PROTECT trial who were discharged alive and with available blood samples were included. Forty-seven biomarkers were serially evaluated in these patients. Measurement closest to discharge was utilized to evaluate the predictive value of biomarkers for low and high post-discharge risk. Patients were classified as 'low risk' if post-discharge 30-day risk of death or heart failure rehospitalization was <5% while risk >20% was utilized to define 'high risk'. Cut-off values that yielded 95% NPV and 20% PPV were identified for each biomarker. Partial area under the ROC curve (pAUC) in the high sensitivity and high specificity regions was calculated to compare low and high risk predictive values. Of analyzed patients, 193 (11.7%) patients reached the 30-day death or heart failure rehospitalization outcome. We found marked differences between low and high risk predictors. cTnI was the strongest biomarker for low risk prediction (pAUC=0.552, 95% CI [0.52-0.58]) while ET-1 showed greater performance for high risk prediction (pAUC=0.560, 95% CI [0.53-0.59]). Several biomarkers (individually and in combination) provided added predictive value, on top of a clinical model, in both low and high risk regions.

Conclusion: Different biomarkers predicted low versus high risk of early post-discharge death or heart failure readmission in patients hospitalized for AHF.

Keywords: biomarker, acute heart failure, risk stratification, predictive value, low risk, high risk

Introduction

Nearly 20% of patients discharged after hospitalization for acute heart failure (AHF) need readmission within 30 days (1). Risk of short-term post-hospital discharge mortality is also at an unacceptably high level. In addition to significantly reducing survival and quality of life, the high rates of these post-discharge events (particularly readmissions) contribute substantially to the monetary cost of health care for heart failure patients. The total cost of heart failure care was estimated to be $31 billion in the US alone in 2012 and this is projected to increase to an unprecedented $70 billion in 2030 (2, 3). The majority of this cost is associated with rehospitalizations.

Several strategies have been proposed to reduce the enormous burden of early post-discharge events on patients and health care systems (4-9). Several observational data suggested that longer hospital stay and intensified post discharge care are amongst the strategies that can be potentially beneficial in terms of lowering 30-day readmission rates (10-12). Nevertheless, such strategies are very unlikely to benefit the whole spectrum of hospitalized AHF patients and do not appear to be feasible, particularly from a cost-effectiveness perspective. A targeted approach in which low risk patients are discharged early with less aggressive post-discharge monitoring while an extended, intensive inhospital and post-discharge care is implemented in high risk patients is a more feasible, and potentially cost-effective, strategy. Effective implementation of this strategy requires tools that can accurately identify subpopulations of patients at low or high risk for early post-discharge events. Nonetheless, prediction of these events, hospital readmissions in particular, remains a significant clinical challenge (1, 4, 13).

Biomarkers can play an essential role as objective tools for short-term post-discharge risk stratification in hospitalized AHF patients and, interestingly, several promising prognostic biomarkers are available. Traditionally, global measures of model performance like the C-statistics have been utilized to quantify prognostic performance of biomarkers in prognostic AHF research (14-18, 24). However, these parameters do not provide clear indication of the performance of biomarkers in

certain risk ranges deemed to be clinically relevant, like the low or high risk regions. There is evidence, particularly in diagnostic medicine, suggesting that the performance of biomarkers might significantly differ in specific clinically relevant regions of the receiver operating characteristic (ROC) curve, like the rule-out (high sensitivity) or rule-in (high specificity) regions (19). Subsequently, different sets of biomarkers might need to be utilized for more optimal identification of low versus high risk subpopulation of patients. However, there is a limited objective data on the value of biomarkers for the identification of hospitalized AHF patients who are at low or high risk for post-discharge events (20).

In this exploratory study, we aimed to assess the value of biomarkers measured close to discharge for the identification of AHF patients at low and high risk for short-term post-hospital discharge events.

Methods

Study population

Data from the 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 (PROTECT) trial was utilized in this study. The PROTECT trial enrolled 2033 hospitalized AHF patients with a history of heart failure. Patients with BNP≥500 pg/mL or NT-proBNP ≥2000 pg/mL and mild to moderate renal impairment were included in the trial. Details of the design and main results of the study have been published previously (21, 22). All patients provided written informed consent. The study was conducted in compliance with the Declaration of Helsinki and was approved by all relevant local ethics committees. Patients who were documented to have been discharged from the index hospitalization alive were considered for inclusion in the current study (N=1911).

Study procedures and measurements

Forty-three established and novel circulating biomarkers were evaluated at baseline and then daily until day 6 or discharge (whichever came first), days 7 and 14. Measurement closest to discharge was utilized to define predischarge biomarker value. Albumin, alanine transaminase, aspartate transaminase, 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 measured in a central laboratory (ICON Laboratories, Farmingdale, New York). A panel of 26 novel biomarkers was measured by Alere Inc., San Diego, CA, USA in available frozen serum samples. Galectin-3, myeloperoxidase (MPO) and neutrophil gelatinase-associated lipocalin (NGAL) were measured using sandwich enzyme-linked immonsorbent assays (ELISA) on a microtiter plate; angiogenin and C-reactive protein (CRP) were measured using competitive 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, pentraxin-3, periostin, polymeric immunoglobulin receptor (PIGR), pro-adrenomedullin (pro-ADM), prosaposin 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 measured using sandwich ELISAs on a Luminex® platform. 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. Four additional biomarkers – B-type natriuretic peptide (BNP), endothelin-1 (ET-1), Interleukin-6 (IL-6) and cardiac specific Troponin I (cTnI) – were measured in frozen plasma samples collected at baseline and on days 2, 7 and 14 using high sensitive single molecule counting (SMC™) technology (RUO, Erenna® Immunoassay System, Singulex Inc., Alameda, CA, USA). Details of assay information and the number of available measurements of each biomarker have been previously published (24). A maximum difference of ±3 days was allowed between time of measurement and time of hospital discharge. Subsequently, patients discharged from hospital after 17 days of baseline assessment (N=258) were excluded from the analysis by definition.

Study outcomes

Primary outcome evaluated in the current study was time-to-death or heart failure rehospitalization within 30 days of discharge. Time-to-all-cause mortality within 90 days of discharge was evaluated as a secondary outcome. Further sensitivity analysis was performed using time-to-death or rehospitalization for cardiovascular/renal causes by day 30 and time-to-all-cause mortality by 180 (from index hospital admission).

Definitions

A threshold of 5% was considered an acceptable level of risk to categorize patients as 'low risk' for the 30-day risk of death or heart failure rehospitalization outcome. Subsequently, a cut-off value of a biomarker under consideration yielding negative predictive value (NPV) of 95% (i.e. corresponding to 5% false omission rate) was used to define low risk status and, therefore, patients with biomarker values below this cut-off were classified as low risk. On the other hand, a risk level of 20% for 30-day death or heart failure rehospitalization was utilized as the threshold defining 'high risk' status. In this case, a cut-off value of a biomarker yielding 20% positive predictive value (PPV) was employed to categorize patients as high risk. Patients with biomarker values above this cut-off were classified as positive for high risk. For secondary analysis with the 90-day all-cause mortality, risk thresholds of 2.5% and 15% were utilized to define low and high risk status, respectively.

Statistical analysis

Biomarker levels were summarized by 30-day death or heart failure rehospitalization status. Mean (SD) or median (interquartile range) was presented for normally and non-normally distributed continuous variables, respectively. Groups were compared with the independent t-test or Wilcoxon rank-sum test based on normality of distribution.

To define optimal cut-off values of biomarkers for the identification of low and high risk patients, time-dependent sensitivity and specificity with corresponding negative and positive predictive values were estimated at all possible cut-off values for each biomarker using R package

survivalROC. A biomarker was considered to be predictive of low risk for 30-day death or heart failure rehospitalization if it had at least one cut-off value that reached a NPV of 95% or greater. Biomarkers fulfilling this criterion were then further evaluated in a comparative analysis. In this analysis, predictive performances were compared by estimating partial area under the receiver operating characteristic (ROC) curve (pAUC) for each of the biomarkers considered to be predictive of low risk. The pAUC summarizes the discriminatory performance of a marker in a portion of the ROC curve defined based on a pre-specified sensitivity/specificity range of interest (19). For the quantification of low-risk predictive value, the high sensitivity (i.e. rule-out) region of the ROC curve, defined as a sensitivity range from 0.9 to 1.0, was analyzed. Patients with available measurements of all biomarkers considered to be predictive of low risk were included in this analysis. Evaluation of high risk predictive value was performed by analyzing pAUC in the high specificity (i.e. rule-in) portion of the ROC curve, which was defined as a specifity range from 0.8 to 1.0. Patients with available measurements of all biomarkers considered to be predictive of high risk were included in this analysis. Here, a biomarker was considered predictive if it had at least one cut-off value reaching a PPV of 20% or greater. R package pROC was utilized for the calculation of pAUC.

Biomarkers that had cut-off values that classified at least 10% of patients to either low or high risk (for the 30-day death or heart failure rehospitalization outcome) were then further analyzed in multivariable analysis to quantify added predictive value in the rule-out or rule-in regions of the ROC curve on top of a clinical model. First, a clinical model encompassing baseline variables including systolic blood pressure, history of diabetes mellitus, atrial fibrillation, CABG, hyperlipidemia, past HF hospitalization and diuretic response (defined as weight loss in Kg per 40 mg of furosemide by day 4) was identified after implementation of a backward selection procedure on a logistic regression model that included candidate predictors associated with outcome at a significance level of 10%. A bootstrap analysis was incorporated in this procedure to assure stability of model selection. In a second step, the added value of low and high risk predictive biomarkers in the rule-out and rule-in regions of the ROC curve, respectively, was quantified with the percentage change in pAUC attained with the addition of the biomarker under consideration to the clinical model. In this analysis, linear

combination of biomarkers that maximizes the pAUC was identified based on the clinical model alone or clinical model plus biomarker and a linear predictor was calculated for each patient and this was entered as a marker for the calculation of pAUC. In a last step, the added value of a combination of biomarkers, on top of the clinical model, was evaluated by quantifying the gain the pAUC in the rule-out or rule-in regions. The combination of the smallest number of the top four best performing biomarkers that yielded the highest pAUC in the respective risk regions was considered optimal.

A further analysis was performed at additional risk thresholds of 0%,1%, 2%,3% and 4% for low risk and at risk thresholds of 22.5%, 25%, 27.5% and 30% for high risk to determine cut-off values and associated proportion of patients that can be classified as low or high risk based on individual biomarkers, the clinical model and clinical model plus combination of biomarkers.

Secondary analysis with the 90-day all-cause mortality outcome was performed applying the same procedure described above. Here, further analysis was performed at risk thresholds of 0%, 0.5%, 1%, 1.5% and 2% for low risk and 17.5%, 20%, 22.5% and 25% for high risk. A similar analysis was also performed using 30-day death or rehospitalization for cardiovascular/renal causes and time-to-all-cause mortality by day 180 after index hospital admission outcomes as sensitivity. P-values <0.05 were considered statistically significant. Statistical analyses were performed with R: A Language and Environment for Statistical Computing, version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline clinical characteristics

Baseline clinical characteristics of patients included in the current analysis are presented in **supplementary table S1**. Analyzed patients were generally comparable to excluded patients (N=258) in terms of baseline characteristics with the exceptions of small differences on degree of edema, heart rate, BMI, histories of angina, hypercholesterolemia and beta-blocker use. Outcomes were also comparable between the two groups (**supplementary table S2**).

Discharge biomarker levels and outcomes

Summary of biomarker levels measured close to discharge by 30-day death or heart failure rehospitalization status is presented in **table 1**. A total of 193 (11.7%) and 129 (7.8%) patients reached the 30-day death or heart failure rehospitalization and 90-day all-cause mortality outcomes, respectively.

Low risk prediction

30-day death or heart failure rehospitalization

At a risk threshold of 5%, 13 biomarkers were found to be predictive of low risk. In a comparative analysis that included 1160 patients with available measurements of these 13 biomarkers, cTnI had the best predictive value with pAUC=0.552 and provided the greatest yield. At a cut-off value of 6.2 pg/mL, cTnI identified 251 (21.6%) patients with post-hospital discharge risk for 30-day death or heart failure rehospitalization below 5%. Syndecan-1, GDF-15, creatinine and BNP showed pAUC>0.53 in the rule-out region and had cut-off values that identified at least 10% of patients as low risk (**table 2**).

90-day all-cause mortality

Higher number of biomarkers (N=19) were predictive of low risk for 90-day all-cause mortality. Among these, cTnI and IL-6 showed greater predictive value with pAUC=0.564. IL-6, at a cut-off value of 6.0 pg/mL, identified 325 (28.0%) patients with post-discharge 90-day all-cause mortality risk below 2.5%, which was the greatest yield observed for this outcome. cTnI classified 251 (21.6%) patients to low risk for 90-day all-cause mortality at a cut-off value of 6.2 pg/mL. Eight additional biomarkers – syndecan-1, BNP, BUN, CRP, uric acid, GDF-15, creatinine and WAP-4c – identified at least 10% of patients as low risk for this outcome (**supplementary table S3**).

High risk prediction

30-day death or heart failure rehospitalization

Eighteen biomarkers were predictive of high risk for 30-day death or heart failure rehospitalization at a risk threshold of 20%. A total of 1129 patients had available measurements of these biomarkers. ET-1 and procalcitonin showed the best predictive value in the rule-in region with pAUC=0.560 At a cut-off value of 7.0 pg/mL, ET-1 identified 306 (27.1%) of these patients as high risk which was the highest yield observed among biomarkers predictive of high risk. On the other hand, procalcitonin, at a cut-off value of 0.04 ng/mL, categorized 265 (23.5%) patients as high risk for post-discharge 30-day death or heart failure rehospitalization. Six biomarkers – galectin-3, sST-2, RAGE, pro-ADM, BUN and VEGFR-1 – also had cut-off values that identified at least 10% of patients as high risk (**table 3**).

90-day all-cause mortality

Fifteen biomarkers were predictive of high risk for 90-day all-cause mortality at a threshold of 15%. Among these sST-2, ET-1 and pro-ADM had pAUC=0.60 in the rule-in region for 90-day all-cause mortality. The greatest yield was attained by sST-2 as it categorized 288 (25.5%) patients as high risk at a cut-off value of 5.0 pg/mL. ET-1, IL-6 and procalcitonin, at cut-off values of 7.5 pg/mL, 20 pg/mL and 0.05 ng/mL, respectively, identified ~20% of patients as high risk for 90-day all-cause mortality. Six additional biomarkers including pro-ADM, galectin-3, creatinine, VEGFR-1, BNP and BUN had cut-off values that categorized at least 10% of analyzed patients as high risk (**supplementary table S4**).

Added predictive value of individual and combination of biomarkers for low risk prediction

The clinical model encompassing baseline systolic blood pressure, history of diabetes mellitus, atrial fibrillation, CABG, hyperlipidemia, past HF hospitalization and diuretic response had a pAUC of 0.628 and 0.625 for 30-day death/HF rehospitalization and 90-day all-cause mortality, respectively, in the low risk region. Individual biomarkers provided modest incremental predictive value to the clinical model for the 30-day death/HF rehospitalization as evidenced by the ~1.0% gain in the pAUC attained with the additions of cTnI, creatinine and BNP. Similarly, the gain attained with a combination of biomarkers was also limited for this endpoint. A combination of cTnI, creatinine and BNP to the clinical model increased the pAUC to 0.652, only a 2.4% increment. The added value of

biomarkers was more pronounced for the 90-day all-cause mortality. cTnI and IL-6 provided a 4.0% increment to the pAUC in the rule-out region. Addition of a combination of cTnI, IL-6 and BNP to the clinical model increased the pAUC to 0.718; a marked 9.3% increment (**table 4**).

Added predictive value of individual and combination of biomarkers for high risk prediction

The clinical model had pAUCs of 0.618 and 0.570 for 30-day death/HF rehospitalization and 90-day all-cause mortality, respectively, in the high risk region. Similar to low risk prediction, the added value of biomarkers was very limited for the 30-day death/HF rehospitalization outcome in the high risk region. A combination of ET-1, sST-2, galectin-3 and BUN increased the pAUC to 0.634, which is only 1.6% increment in the pAUC. On the other hand, several biomarkers provided significant incremental predictive value for 90-day all-cause mortality. Individual additions of IL-6, proADM, BNP, BUN and VEGFR-1 to the clinical model yielded >6.0% increment in the pAUC. Addition of a combination of ET-1, galectin-3, sST-2 and proADM to the clinical model increased the pAUC to 0.662, a 9.2% gain (**table 5**).

Cut-off values and yields at other risk thresholds

Figure 1 shows proportion of patients classified as low or high risk for the two outcomes at the different analyzed risk thresholds for the three top performing biomarkers (identified from the primary analysis), the clinical model and clinical model plus combination of biomarkers. For the 30-day death/HF rehospitalization outcome, cTnI remained the biomarker with the highest yield for low risk prediction at all risk thresholds evaluated. However, the proportion of patients categorized as low risk decreased significantly with lower risk thresholds. The proportion of patients identified as low risk was <5% for risk thresholds below 3%. The yield was very low (<1.0%) for BNP and creatinine at all risk thresholds below 5%. For high risk prediction, on the other hand, ET-1 provided the highest yield at risk thresholds of 22.5% and 25% as well. It classified ~10%-15% of patients as high risk at these thresholds. Nevertheless, all the three top performing biomarkers for high risk prediction provided very low yield at risk thresholds of 27.5% and 30%. However, the clinical model alone classified ~15-20% of patients as either low risk or high risk at thresholds as low as 1.% for low risk

prediction and as high as 30.0% for high risk prediction. It must be noted that addition of combination of biomarkers to the clinical model did not add much compared to the yield of the latter (**figure 1**).

For the 90-day all-cause mortality outcome, the yield attained with individual biomarkers was also significantly reduced at lower or higher risk thresholds for low and high risk prediction, respectively. Nonetheless, the model encompassing clinical variables and combination of biomarkers markedly increased the proportion of patients classified as either low or high risk at all risk thresholds. Even at a risk threshold of 0%, this model classified >20% of patients as low risk for 90-day all-cause mortality, while at a threshold of 25%, it classified >10% of patients as high risk for this outcome (**figure 1**).

Sensitivity analysis

As presented in **supplementary tables S5 and S6**, the best performing low and high risk predictive biomarkers for 30-day death or rehospitalization for cardiovascular/renal causes were generally comparable to that of the 30-day death or heart failure rehospitalization outcome. Similarly, there were no significant differences in the set and ranking of low versus high risk predictive biomarkers for the 180-day all-cause mortality outcome compared to the findings for 90-day all-cause mortality (**supplementary tables S7 and S8**).

Discussion

In this comparative study we evaluated the value of 47 established and novel biomarkers measured close to discharge for low and high post-discharge risk prediction in hospitalized AHF patients. We found a remarkable difference between those markers that showed good performance for the prediction of low risk and those that predicted high risk for two early post-discharge outcomes. For the 30-day death or heart failure rehospitalization outcome, cTnI provided the greatest performance for low risk prediction while ET-1 and procalcitonin performed best for high risk prediction. On the other hand, cTnI and IL-6 were the best performing biomarkers for low risk prediction using the 90-day all-cause mortality outcome while several biomarkers including ET-1, sST-2 and pro-ADM showed good performance for high risk. Several biomarkers (both individually and in combination)

provided added predictive value, on top of a clinical model, in both the low and high risk regions, which was more pronounced for the mortality outcome.

A demand-based approach that combines earlier discharge with less intensive post-discharge care in low risk AHF patients and more extended and intensive inhospital and post-discharge care in those with high risk is appealing as it might lead to improved outcomes. This strategy might also promote efficient distribution of scarce health care resources potentially reducing the high monetary cost associated with the care of heart failure patients. Development and effective implementation of such strategies requires tools that can accurately identify low risk patients that can be safely discharged and treated less aggressively post-discharge and high risk patients for whom more intensive inhospital and post-discharge management is justified. Nevertheless, prediction of post-discharge events, particularly readmissions, with readily available clinical variables and standard laboratory parameters remains challenging (1, 4). Biomarkers can play an essential role towards improving objective predischarge risk stratification and several promising prognostic biomarkers are available in AHF currently thanks to significant advances in proteomic research over the past years (23-27). However, there is a very limited data on the potential clinical utility of biomarkers for the identification of patients at low and high risk for short-term post-discharge complications.

Our study suggests that different sets of biomarkers need to be utilized for optimal low and high risk prediction. Interestingly, cTnI was the most promising biomarker for the identification of patients who are at low risk for post-discharge complications. This is consistent with a recently reported study by Pang et al, which indicated that low levels of hs-TnT evaluated within 16 hours of presentation could identify AHF patients at very low risk for cardiovascular mortality within 180 days of hospital admission (28). Although further investigation is required, the pivotal role of troponins in the management of acute coronary syndrome appears to have the potential to extend to the management of AHF patients. Regarding high risk prediction, ET-1 is, possibly, the most optimal individual biomarker as it showed consistently good performance for both outcomes evaluated. ET-1 is a potent endogenous vasoconstrictor that had been indicated to have increased activity in heart failure through the upregulation of ET receptor A (29). Perez et al evaluated the prognostic value of

elevated ET-1 in hospitalized AHF patients included in the ASCEND trial. They showed that patients with baseline ET-1 levels in the highest tertile had the highest rate of 180-day all-cause mortality and, interestingly, risk persisted if ET-1 levels were maintained at the highest tertile after 48-72 hours and 30 days of the baseline assessment (30). Our findings further support the notion that persistence of endothelin-mediated vasoconstriction after inhospital treatment could be an important marker of susceptibility for post-hospital discharge adverse outcomes. It must be emphasized that several other biomarkers reflecting diverse pathophysiologic pathways also showed promising low risk (e.g. IL-6, syndecan-1, GDF-15, BNP, BUN and creatinine) and high risk (e.g. procalcitonin, sST-2, galectin-3, IL-6 and pro-ADM) prediction values. Plenty of biomarkers also provided added predictive value, on top of a clinical model, in both the low and high risk regions. Interestingly, both low and high risk prediction were further enhanced with combinations of biomarkers that encompassed markers reflecting the diverse pathophysiologic pathways involved in heart failure.

The choice of outcome utilized to define risk did not show significant effect on the selection of best performing biomarkers for both low and high risk prediction. Aside from small changes in ranking, biomarkers that showed good performance for the 30-day death or heart failure rehospitalization outcome also performed well for the 90-day all-cause mortality. However, the inflammatory biomarkers (IL-6 in particular) are notable exceptions here. IL-6 was among the best predictors of both low and high risk for 90-day all-cause mortality yet it had poor performance for the 30-day composite outcome. This is not unexpected as we, in a post-hoc analysis of biomarker data from the PROTECT trial, showed that IL-6 showed poor overall predictive value for 30-day death or rehospitalization for cardiovascular or renal causes despite having the highest C-index for both short and intermediate term all-cause mortality among 48 biomarkers evaluated (24). Moreover, the added value of both individual and combinations of biomarkers, on top of the clinical model, was more pronounced for the 90-day all-cause mortality outcome. This, again, is consistent with our previous analysis which showed that biomarkers are generally better at predicting mortality compared to rehospitalization outcomes as quantified by overall predictive value.

Evaluation of performance of biomarkers in clinically relevant risk regions, as we did in this study, is (probably) more important than the mere assessment of overall discriminatory accuracy for facilitating the clinical utility of the plenty of available prognostic biomarkers in AHF. However, defining these clinically relevant risk regions is a challenging task as the clinical and health economic implications of stratification into different risk categories remains unclear at this stage. Ideally, event rate should be close to zero in low risk subpopulation of patients and significantly high enough to warrant more intensive treatment in high risk subpopulation of patients. This must, however, be offset against the proportion of patients categorized as low or high risk for this risk stratification to have meaningful clinical impact. For the primary analysis of this study, we utilized risk thresholds of 5% and 20% for 30-day death or heart failure rehospitalization to define low and high risk, respectively. At this thresholds ~20%-25% of patients were categorized as low or high risk with the best performing biomarkers. In a sensitivity analysis we evaluated several risk thresholds <5% for low risk and >20% for high risk. The proportion of patients categorized as low risk significantly declined at lower risk thresholds; even with cTnI, <5% of patients were identified as low risk at risk thresholds below 3%. Similarly the number of patients identified as high risk was lower with the higher risk thresholds, particularly for thresholds above 25%. Subsequently, the risk thresholds utilized for the primary analysis appear to provide a reasonable balance between level of risk and proportion of patients categorized into low or high risk. Interestingly these parameters were significantly improved with models that utilized clinical prognosticators together with a combination of biomarkers.

Strengths and limitations

We evaluated 47 established and novel biomarkers measured close to hospital discharge in in a fairly large, well-characterized cohort of hospitalized AHF patients. Our study has several limitations. This is a post-hoc analysis of data from a randomized controlled trial which was primarily designed to evaluate efficacy of rolofylline. Therefore, our analysis was exploratory and findings should only be considered as hypothesis generating. The PROTECT trial included sicker subpopulation of AHF patients with significantly elevated natriuretic peptide levels, mild to moderate renal dysfunction and previous history of heart failure hospitalization during admission. Subsequently, extrapolation of our

findings to the whole AHF patient population needs further evaluation. The importance of prospective validation of our findings could not be overstated at this point. Moreover, several of the biomarkers evaluated, in particular cTnI and BNP, were measured using assays that were not standardized to available commercial assays. Subsequently, cut-off values identified in the current analysis might not be generalized and need to be interpreted cautiously. Significant proportions of sST-2 and GDF-15 measurements available were at the lower and upper assay detection limits, respectively. This could potentially underestimate the performance of the two biomarkers for low (sST-2) and high (GDF-15) risk prediction.

Future steps

Currently, there appears to a disconnect between disease severity and intensity of treatment in hospitalized AHF patients. An interesting analysis by Cotter et al that utilized data from the VERITAS trial showed that length of hospital stay was only partially explained by baseline disease severity (10). Another study by Davison et al, utilizing data from the PROTECT trial also showed a similar finding (31). We further explored whether several potential surrogates for physicians' judgment of disease severity (including total IV diuretic dose, length of hospital stay and IV inotrope/vasopressor administration) differed in groups of patients classified as low versus high risk for post-discharge outcomes based on the most optimal discharge models we identified. As presented in the supplementary table S9, aside from a significant difference on total IV diuretic dose received through day 7 or discharge (if earlier), length of hospital stay and inotrope/vasopressor administration did not differ based on post-discharge risk of complications. These further support the assertion that objective risk stratification tools like biomarkers are highly needed in AHF patients.

Although biomarkers could effectively identify low and high risk patients, there is a lack of guideline recommended, risk-based interventions that could be implemented to reduce both the high rates of adverse outcomes and cost associated with hospitalization for AHF. This significantly limits the clinical application of risk stratification tools in general and biomarkers in particular. Subsequently, randomized controlled trials evaluating the effectiveness of targeted, risk-based

interventions are needed in the future. Length of hospital stay is one area that might be of interest. There is some observational data suggesting that longer hospital stay might be beneficial, particularly in terms of reducing rates of readmissions (10-12). It will be highly interesting to evaluate the effectiveness and cost-effectiveness of an intervention that combines early discharge in low risk patients and more extended inhospital treatment in high risk patients in a randomized controlled trial. Another potential target for intervention, particularly in high risk patients, involves intensity of post-discharge care. Although available evidence is inconclusive, intensive monitoring strategies like telemonitoring and natriuretic peptide guided monitoring have been indicated to be beneficial in some studies (5-8). It might be of particular interest to evaluate the effectiveness of such intensive monitoring strategies in selected high risk group of patients in a randomized controlled trial.

External validation of findings reported in this study in an independent cohort of AHF patients is another essential step that is needed to facilitate the clinical applicability of the current results. Last but not least, cost and availability of biomarker measurements is another important area that needs to be addressed in the future to facilitate the clinical utility of prognostic biomarkers. Quantification of cost-effectiveness of biomarker analysis, both individually and in tandem, is challenging currently as the clinical implications of information attained with prognostic biomarkers is less clear so far. It could, however, be speculated that the advent of new technology that could enhance the speed and availability of biomarker measurements and reduce costs (e.g. point-of-care devices) will significantly facilitate the clinical utility of biomarkers.

Conclusion

Different sets of biomarkers predict low versus high risk of early post-discharge events in patients hospitalized for AHF. Therefore, this needs to be taken into consideration for optimizing biomarker-guided predischarge risk stratification strategies in hospitalized AHF patients. Future studies are needed to prospectively validate our observations in the general AHF patient population.

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Figure legends

Figure 1: Size of low risk (left) and high risk (right) subpopulation of patients as a function of risk threshold for 30-day death or heart failure rehospitalization (top) and 90-day all-cause mortality (bottom); results for top three biomarkers, clinical model and clinical model + combination of biomarkers are presented

Tables

Table 1: Discharge biomarker levels by 30-day death or heart failure rehospitalization status

Biomarker	30-day death or heart failure rehospitalization		
	No (N=1460)	Yes (N=193)	P-value
Albumin (g/dL)	3.9 [3.6-4.2]	3.8 [3.6-4.2]	0.065
ALT(U/L)	21 [15-30]	21 [14-30]	0.542
Angiogenin (ng/ml)	2275.8 [1557.9-3483]	2089.1 [1494.2-3070.7]	0.063
AST (U/L)	24 [18-32]	24 [19-31.5]	0.683
Bicarbonate (mEq/L)	24.6±3.8	24.4±4.3	0.55
BNP (pg/ml)	228 [126-419]	299 [178-543]	<0.001
BUN (mg/dL)	32 [23-43]	39 [27-55]	<0.001
Chloride (mEq/L)	100 [97-103]	98 [95-102]	<0.001
Creatinine (mg/dL)	1.4 [1.1-1.8]	1.6 [1.3-2.1]	<0.001
CRP (ng/ml)	11582.8 [5513.2-23608.7]	13537.2 [7527.2-25424.5]	0.062
cTnI(pg/ml)	12.3 [6.6-24.1]	15.7 [8.4-31.3]	0.007
D-Dimer (ng/ml)	179.5 [90.6-366.2]	196.3 [90.6-438.8]	0.189
ESAM (ng/ml)	62 [55.8-69.3]	62.2 [57.1-70]	0.563
ET1 (pg/ml)	5.2 [3.9-7.0]	6.3 [4.8-9.0]	<0.001
Galectin-3 (ng/ml)	35.7 [27.2-46.9]	38.2 [28.4-56.9]	0.01
GDF-15 (ng/ml)	3.8 [2.7-5.8]	4.6 [2.9-6.3]	<0.001
Glucose (mg/dL)	115 [95-151]	126 [96.5-169]	0.047
Hemoglobin (g/dL)	12.7±2.0	12.3±2.0	0.005
IL-6 (pg/ml)	9.2 [5.4-16.3]	11.1 [7.2-17.2]	0.031
LTBR (ng/ml)	0.4 [0.3-0.6]	0.4 [0.3-0.6]	0.028
Mesothelin (ng/ml)	86.4 [73.2-99.5]	87 [75.7-101.6]	0.254
Myeloperoxidase (ng/ml)	32.1 [16.8-62.6]	24.8 [15.2-53.1]	0.015
Neuropilin (ng/ml)	10.6 [6.8-15]	12.2 [8.1-16]	0.022
NGAL(ng/ml)	91.2 [58.6-155.8]	98.6 [59.1-171.9]	0.297
NT-proCNP (pg/ml)	0 [0-0.1]	0 [0-0.1]	0.009
Osteopontin (ng/ml)	113.8 [80.7-167]	115.3 [84.4-173]	0.354
PCT (pg/ml)	0 [0-0]	0 [0-0.1]	<0.001
Pentraxin-3 (ng/ml)	3.3 [2.2-5]	3.4 [2.5-5.3]	0.18
Periostin (ng/ml)	4.2 [2.4-6.8]	5.3 [3-8.4]	0.001
PIGR (ng/ml)	369.5 [243.4-640.8]	419.7 [303-757.4]	0.002

Platelet count (*10^9/l)	231 [184.8-287]	215 [169.8-266]	0.044
Potassium (mmol/L)	4.5±0.6	4.4±0.6	0.072
proADM (ng/ml)	2.0 [1.1-3.3]	2.4 [1.3-4.3]	0.003
PSAP-B(ng/ml)	33.3 [24.1-47.3]	38.4 [28.4-51.8]	0.003
RAGE (ng/ml)	4 [2.7-5.5]	4.5 [3.2-6.3]	<0.001
RBC count (*10^9/L)	4.4±0.7	4.2±0.7	0.006
Sodium (mmol/L)	139 [136-141]	137 [134-140]	<0.001
sST-2 (ng/ml)	0.9 [0.9-2.7]	1.4 [0.9-4.9]	<0.001
Syndecan-1 (ng/ml)	8.1 [6.8-9.8]	8.7 [7.3-10.5]	0.004
TNF-R1a (ng/ml)	3.1 [2.1-4.5]	3.6 [2.3-5.1]	0.008
Total cholesterol (mg/dL)	156 [129-189]	137 [110-165]	<0.001
Troy (pg/ml)	0.1 [0.1-0.1]	0.1 [0.1-0.2]	0.01
Uric acid (mg/dL)	8.7 [7-10.4]	9.2 [7.6-11]	0.004
VEGFR (ng/ml)	0.3 [0.2-0.4]	0.3 [0.2-0.5]	0.003
WAP4C(ng/ml)	23.6 [11.7-44.5]	31.3 [17.3-58.4]	<0.001
WBC (*10^9/l	7.1 [5.9-8.6]	7.1 [5.7-8.9]	0.941

Table 2: Performance of biomarkers in the rule-out region of the ROC curve, cut-off values at a NPV of 95.0% and size of patients classified as 'low risk' for 30-day death/heart failure rehospitalization

Biomarker	Partial AUC [95% CI]	Cut-off value	Number of 'low risk' patients (%)
cTnI, pg/mL	0.552 [0.52-0.58]	6.20	251 (21.6)
Syndecan-1, ng/mL	0.540 [0.51-0.57]	6.40	224 (19.3)
Creatinine, mg/dL	0.536 [0.50-0.57]	0.90	147 (12.7)
BNP, pg/mL	0.532 [0.51-0.56]	76	129 (11.1)
GDF-15, ng/mL	0.527 [0.50-0.56]	2.24	151 (13.0)
Pentraxin-3, ng/mL	0.526 [0.51-0.56]	1.32	95 (8.2)
IL-6, pg/mL	0.526 [0.50-0.56]	3.3	104 (9.0)
Neuropilin, ng/mL	0.522 [0.50-0.55]	4.20	110 (9.5)
WAP-4c, ng/mL	0.521 [0.50-0.55]	4.95	87 (7.5)
pro-ADM, ng/mL	0.516 [0.50-0.54]	0.16	46 (4.0)
CRP, ng/mL	0.514 [0.50-0.55]	1676.0	44 (3.8)
BUN, mg/dL	0.515 [0.50-0.54]	14.0	32 (2.8)
Galectin-3, ng/mL	0.502 [0.49-0.53]	11.7	4 (0.03)

Table 3: Performance of biomarkers in the rule-in region of the ROC curve, cut-off values at a PPV of 20.0% and size of patients classified as 'high risk' for 30-day death/heart failure rehospitalization

Biomarker	Partial AUC [95% CI]	Cut-off value	Number of 'high risk' patients (%)
Procalcitonin, ng/mL	0.565 [0.53-0.60]	0.04	265 (23.5)
ET-1, pg/mL	0.560 [0.53-0.59]	7.0	306 (27.1)
sST-2, ng/mL	0.546 [0.52-0.58]	5.0	165 (14.6)
Galectin-3,ng/mL	0.544 [0.51-0.58]	54.0	193 (17.1)
BUN, mg/dL	0.544 [0.51-0.58]	60.0	133 (11.8)
pro-ADM, ng/mL	0.542 [0.51-0.57]	5.4	138 (12.2)
VEGFR-1, ng/mL	0.540 [0.51-0.57]	0.58	121 (10.7)
RAGE, ng/mL	0.536 [0.51-0.57]	6.8	143 (12.7)
Uric acid, mg/dL	0.535 [0.51-0.57]	12.5	96 (8.5)
BNP, pg/mL	0.534 [0.51-0.57]	720	118 (10.4)
Periostin, ng/mL	0.531 [0.50-0.56]	12.0	76 (6.7)
Neuropilin, ng/mL	0.520 [0.49-0.55]	23.5	70 (6.2)
ALT, u/L	0.519 [0.49-0.55]	68	40 (3.5)
NGAL, ng/mL	0.515 [0.49-0.54]	335.0	56 (5.0)
Pentraxin-3, ng/mL	0.513 [0.49-0.54]	9.7	47 (4.2)
Glucose, mg/dL	0.510 [0.49-0.54]	258	43 (3.8)
Troy, ng/mL	0.510 [0.49-0.54]	0.32	19 (1.7)

Table 4: Added predictive value of biomarkers in the rule-out region of the ROC curve on top of a clinical model*

Biomarker	30-day death/HF rehospitalization		90-day all-cause mortality	
	Corrected pAUC	Absolute change in pAUC (%)	Corrected pAUC	Absolute change in pAUC (%)
BNP	0.641	1.30	0.653	2.80
BUN	-	-	0.647	2.20
Creatinine	0.642	1.40	0.622	-0.30
CRP	-	-	0.645	2.00
cTnI	0.640	1.20	0.664	3.90
GDF-15	0.624	-0.04	0.631	0.60
IL-6	-	-	0.665	4.00
Syndecan-1	0.635	0.70	-	-
Uric acid	-	-	0.653	2.80
WAP-4C	-	-	0.641	1.60
Combination of biomarkers**	0.652	2.40	0.718	9.30

* clinical model included SBP, history of DM, AFIB, CABG, hyperlipidemia, past HF hospitalization and diuretic response; partial AUC of the clinical model in the rule-out region is 0.628 for 30-day death/HF rehospitalization and 0.625 for 90-day all-cause mortality; **combination of biomarkers include cTnI, creatinine and BNP for 30-day death/HF rehospitalization and cTnI, BNP and IL-6 for 90-day all-cause mortality

Table 5: Added predictive value of biomarkers in the rule-in region of the ROC curve on top of a clinical model*

Biomarker	**30-day death/HF rehospitalization**		**90-day all-cause mortality**	
	Corrected pAUC [95% CI]	Absolute change in pAUC (%)	Corrected pAUC [95% CI]	Absolute change in pAUC (%)
BNP	0.621	0.30	0.632	6.20
BUN	0.627	0.90	0.631	6.10
Creatinine	-	-	0.611	4.10
ET-1	0.623	0.50	0.626	5.60
Galectin-3	0.619	0.10	0.624	5.40
IL-6	-	-	0.634	6.40
pro-ADM	0.623	0.50	0.641	7.10
Procalcitonin	0.613	-0.50	0.585	1.50
RAGE	0.615	-0.30	-	-
sST-2	0.612	-0.60	0.624	5.40
VEGFR-1	0.626	0.80	0.633	6.30
Combination of biomarkers**	0.634	1.60	0.662	9.20

* Clinical model included SBP, history of DM, AFIB, CABG, hyperlipidemia, past HF hospitalization and diuretic response; partial AUC of the clinical model in the rule-in region is 0.618 for 30-day death/HF rehospitalization and 0.570 for 90-day all-cause mortality; **combination of biomarkers include ET-1, galectin-3, sST-2 and BUN for 30-day death/HF rehospitalization and ET-1, galectin-3, sST-2 and proADM for 90-day all-cause mortality