Multiple Biomarkers for Risk Prediction in Chronic Heart Failure

Ky et al: Multiple Biomarkers in Chronic Heart Failure

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Abstract

Background—Prior studies have suggested using a panel of biomarkers that measure diverse biological processes as a prognostic tool in chronic heart failure. Whether this approach improves risk prediction beyond clinical evaluation is unknown.

Methods and Results—In a multi-center cohort of 1513 chronic systolic heart failure patients, we measured a contemporary biomarker panel consisting of: high-sensitivity C-reactive protein (hsCRP), myeloperoxidase (MPO), B-type natriuretic peptide (BNP), soluble fms-like tyrosine kinase receptor-1 (sFlt-1), troponin I (TnI), soluble toll-like receptor-2 (ST2), creatinine, and uric acid. From this panel, we calculated a parsimonious multimarker score and assessed its performance in predicting risk of death, cardiac transplantation, or ventricular assist device (VAD) placement in comparison to an established clinical risk score, the Seattle Heart Failure Model (SHFM). During a median followup of 2.5 years, there were a total of 317 outcomes: 187 patients died; 99 were transplanted; and 31 had a VAD placed. In unadjusted Cox models, patients in the highest tertile of the multimarker score had a 13.7-fold increased risk of adverse outcomes compared to the lowest tertile (95%CI 8.75-21.5). These effects were independent of the SHFM (adjusted HR 6.80, 95%CI 4.18-11.1). Addition of the multimarker score to the SHFM led to a significantly improved AUC of 0.803 versus 0.756 (p=0.003) and appropriately reclassified a significant number of patients who experienced the outcome into a higher risk category (NRI 25.2%, 95%CI 14.2-36.2%, p<0.001).

Conclusions—In ambulatory chronic heart failure patients, a score derived from multiple biomarkers integrating diverse biologic pathways substantially improves prediction of adverse events beyond current metrics.

Key Words: biomarkers, chronic heart failure
Heart failure is a major public health burden that accounts for at least 290,000 deaths in the US alone each year.\(^1\) There is substantial variation in the severity and prognosis of heart failure, ranging from mild disease that is easily managed with neurohormonal blockade to advanced illness requiring therapy with mechanical support or heart transplantation.\(^2\) Accurate assessment of prognosis is critical to guide clinical management, and to identify high risk patients who should be considered for advanced therapy. Although established predictors such as the New York Heart Association (NYHA) Class, left ventricular ejection fraction (LVEF), natriuretic peptide levels, and risk scores such as the Seattle Heart Failure Model (SHFM) exist, they do not fully explain the risk of adverse outcomes in ambulatory chronic heart failure patients.\(^2\)

The progression of heart failure is complex and is driven by multiple biologic processes, including inflammation, oxidative stress, neurohormonal activation, vascular remodeling, myocyte injury, and renal impairment.\(^3\) As such, there has been growing interest in the measurement of a diverse biomarker profile, reflective of the underlying biology of heart failure, as a means to risk-stratify patients and improve our understanding of the underlying pathophysiology.\(^4\) We therefore evaluated the predictive utility of 8 biomarkers, reflective of diverse biologic pathways in heart failure: high-sensitivity C-reactive protein (hsCRP) (inflammation), uric acid and myeloperoxidase (MPO) (oxidative stress), B-type natriuretic peptide (BNP) (neurohormonal activation), soluble fms-like tyrosine kinase receptor-1 (sFlt-1) (vascular remodeling), troponinI (TnI) (myocyte injury), soluble toll-like receptor-2 (ST2) (myocyte stress), and creatinine (renal function) in a multi-center cohort of 1513 ambulatory chronic heart failure patients. We hypothesized that a biomarker score summarizing the activity
of multiple pathways implicated in heart failure would improve our ability to classify risk of adverse outcomes (cardiac transplantation, ventricular assist device [VAD] placement, or death) compared to a validated clinical risk prediction algorithm, the Seattle Heart Failure Model.

Methods

Study Population

The Penn Heart Failure Study (PHFS) is an NHLBI-sponsored multi-center cohort study of outpatients with chronic heart failure (HF) recruited from referral centers at the University of Pennsylvania (Philadelphia, PA), Case Western University (Cleveland, OH), and the University of Wisconsin (Madison, WI). The primary inclusion criterion is a clinical diagnosis of heart failure as determined by a heart failure specialist. The resultant cohort spans a full spectrum of heart failure severity ranging from mild disease to severe disease requiring advanced therapies. Participants are excluded if they have a non-cardiac condition resulting in an expected mortality of less than 6 months as judged by the treating physician, or if they were unable to provide consent.

At time of study entry, detailed clinical data were obtained using standardized questionnaires administered to the patient and physician, with verification via medical records. Blood samples were obtained at enrollment, processed, and stored at -80°C until time of assay. Follow-up events including all-cause mortality and cardiac transplantation were prospectively ascertained every 6 months via patient contact and verified through death certificates, medical records, or contact with patients’ families by research personnel.

All participants provided written, informed consent, and the PHFS protocol was approved by participating Institutional Review Boards.
Laboratory Analyses

All biomarkers were measured from banked plasma obtained at the time of study entry. sFlt-1 and MPO were measured using prototype ARCHITECT® chemiluminescent microparticle-based immunoassays (Abbott Laboratories, Abbott Park, IL). BNP, TnI, hsCRP, uric acid, and creatinine were measured using standard ARCHITECT immunoassays (Abbott Laboratories, Abbott Park, IL). ST2 was measured via a high sensitivity sandwich monoclonal immunoassay (Presage™ ST2 assay, Critical Diagnostics, New York, New York). Full details are provided in the Supplemental File.

Seattle Heart Failure Model

The Seattle Heart Failure Model (SHFM) is a multivariable risk prediction scoring system that has been validated in multiple heart failure populations as a predictor of mortality, urgent cardiac transplantation, or VAD placement. The version of the score used in this study was the SHFM-D, abbreviated as SHFM for simplicity, and is based upon clinically assessed variables including demographics, heart failure characteristics, laboratory values, and medications. The derivation and validation of the SHFM has been previously described.

Statistical Analysis

Baseline characteristics were summarized for the entire cohort using standard descriptive statistics. A log (base 2) transformation was applied to each biomarker to normalize its distribution. Spearman correlations were calculated between each biomarker pair. Cox regression models were used to determine the unadjusted association between each individual
biomarker and time to the combined outcome of all-cause death, cardiac transplantation, or VAD placement. For TnI, variables that indicated a detectable value and the log-transformed continuous value were included to estimate the risk associated with a detectable value and the risk associated with the absolute value if detectable. The proportional hazards assumption was evaluated using weighted residuals.

All biomarkers were included in a multivariable Cox model, and a parsimonious set of biomarkers was selected using a stepwise procedure based on the Akaike information criterion (AIC), in which a biomarker that reduced the AIC was retained. To estimate a multimarker score, a leave-one-out-jackknife approach was used such that the multimarker score for each individual was calculated as a weighted combination of his/her biomarker levels, with weights determined by regression coefficients, which were estimated from the parsimonious Cox model that fit the data for all other individuals. The jackknife approach ameliorates the potential for bias when applying a predictive score to the same dataset from which it was derived, and avoids arbitrarily splitting the data into derivation and validation cohorts.10

In primary analyses, we compared the multimarker score to the clinically used SHFM score. To ensure a fair comparison between the multimarker score and the SHFM, we used estimates of the baseline hazard function to recalibrate the SHFM within our cohort. Kaplan-Meier curves were used to contrast the survival distribution across tertiles of the multimarker and SHFM score. Hazard ratios were estimated from an unadjusted Cox regression model and a model that adjusted for the SHFM.

To evaluate the multimarker score as a discriminator of individual risk, time-dependent receiver operating characteristic (ROC) curves were used to compare the ability of the multimarker score and the SHFM to classify patients with regard to adverse event at 1 year.11
Differences in the area under the ROC curve (AUC) were used to quantify improvements in predictive accuracy. The incremental value of the multimarker score compared to the SHFM in predicting outcomes at 1 year was determined using net reclassification improvement (NRI), which represents the difference in the number of patients moving up or down clinical risk groups, stratified according to outcome. Here, clinically meaningful risk categories of 0%-%<10%, 10%-%<20%, 20%-%<50%, and ≥50% risk were defined a priori. Cox regression models were used to predict risk at 1 year. Because some subjects were censored before 1 year, the number of events and nonevents at 1 year was estimated from the Kaplan-Meier survival estimator.

Bootstrap resampling was used to compute standard error estimates upon which to base confidence intervals for the AUC and the NRI, as well as the Wald p values to test whether the difference between two AUCs and the NRI were equal to 0. Estimates of the AUC, the difference between two AUCs, and the NRI were obtained for each resampled dataset, and the standard deviation of the estimates across 1000 resampled datasets was used as the standard error.

In a secondary analysis, we re-derived the SHFM in our cohort. We entered the component variables into a Cox regression model and used a leave-one-out jackknife approach to re-derive the SHFM. We then compared the multi-marker score to the re-derived SHFM. In addition, because the SHFM-D score has been validated only for mortality, we performed a sensitivity analysis in which we used all-cause mortality alone as the outcome, and compared the multimarker score to both the SHFM and its component variables. Calibration for the SHFM was assessed using the Grønnesby and Borgan statistic. All statistical analyses were completed using R 2.12.0, including the survival, survivalROC, and pec extension packages.
Results

Baseline characteristics of the 1513 patients in the study cohort are shown in Table 1. The mean age of patients at time of enrollment was 56±15 years. Sixty-six percent of the cohort were male, 74% were Caucasian, and 22% were African American. Over a maximum follow-up period of 5 years (median 2.5 years, interquartile range 1.4 to 4.0 years), there were a total of 317 outcomes: 187 deaths, 99 cardiac transplantations, and 31 VAD implantations.

Median biomarker levels are provided in Table 1. Among the 8 biomarkers, there were moderate correlations between BNP and sFlt-1 (R=0.54), BNP and ST2 (R=0.40), and BNP and TnI (R=0.55) (Supplementary Figure 1). There were also moderate correlations between sFlt-1 and TnI (R=0.43) and between creatinine and uric acid (R=0.51) (p<0.001 for all comparisons).

Consistent with previously published studies, each of the 8 biomarkers was individually associated with the combined risk of all-cause death, cardiac transplantation, or VAD implantation. Creatinine and uric acid showed nonlinear associations with outcome (Supplementary Figure 2) and were modeled using quadratic terms. A multivariable Cox model including all biomarkers together was fit, and a parsimonious set was selected based on a stepwise model selection procedure (Supplementary Table 1). The following 7 markers remained in the multimarker score: BNP, sFlt-1, hsCRP, ST2, TnI (detectable versus not), uric acid, and creatinine (Table 2).

Figure 1 displays the risk of transplant or VAD-free survival according to tertiles of the multimarker or SHFM score. In unadjusted models, patients in the middle tertile of multimarker score had a 4.69-fold increased risk of adverse outcomes, and those in the highest tertile had a 13.7-fold elevated risk. These findings remained robust after adjustment for the SHFM (Table
2). Hazard ratios were similar in subgroups defined by systolic (n=1305) or diastolic (n=198) heart failure, but our power to detect subtle differences between these subgroups was limited.

The AUC for the multimarker score was 0.798 at 1 year (95% CI 0.763-0.833), which indicated a strong ability to discriminate individual risk, and which was superior to that of the SHFM (AUC 0.756, 95% CI 0.717-0.795). As shown in Figure 2, addition of the multimarker score to the SHFM significantly improved predictive accuracy compared to the SHFM (AUC 0.803, 95% CI 0.769-0.837, p=0.003). Risk reclassification analyses revealed that use of the multimarker score in combination with the SHFM significantly improved classification of 1-year risk compared to the SHFM (NRI 24.1%, 95% CI 11.6%-36.7%, p<0.001) (Table 3). The improvement was limited to patients who experienced an adverse event (NRI 25.2%, 95% CI 14.2%-36.2%, p<0.001), signifying a better ability to classify patients at higher risk.

We obtained similar results in secondary analyses comparing the multimarker score to the SHFM components. The AUC for the SHFM components was 0.776 (95% CI 0.735-0.818) at 1 year (Supplementary Figure 3). Addition of the multimarker score to the SHFM components significantly improved predictive accuracy compared to the SHFM components (AUC 0.811, 95% CI 0.771-0.850, p=0.019). In addition, the multimarker score and SHFM components in combination significantly improved classification of 1-year risk compared to the SHFM components (NRI 12.2%, 95% CI 0.6%-23.8%, p=0.039) (Supplementary Table 2).

We also obtained similar results with all-cause mortality as the outcome. The SHFM was well-calibrated in this cohort for the outcome of all-cause mortality with a predicted versus observed 1-year survival of 93.7% versus 94.0% (p=0.21). The AUC for the multimarker score was 0.808 (95% CI 0.762-0.854); for the SHFM, 0.761 (95% CI 0.708-0.813); and for SHFM components, 0.749 (95% CI 0.690-0.808). Addition of the multimarker score improved
predictive accuracy compared to the SHFM (AUC 0.809, 95% CI 0.763-0.854, p=0.038) and compared to the SHFM components (AUC 0.790, 95% CI 0.735-0.846, p=0.065). The multimarker score also improved classification of 1-year risk of all-cause mortality in combination with the SHFM (NRI 18.0, 95% CI 2.4%-33.6%, p=0.023) and in combination with the SHFM components (NRI 13.7%, 95% CI -3.7%-31.1%, p=0.12). Although point estimates were similar, power was reduced in our sensitivity analysis due to the decreased event rate for all-cause mortality compared to the combined outcome of mortality, transplantation, and VAD implantation.

**Discussion**

In 2008, Braunwald classified circulating biomarkers into categories based upon their pathophysiologic effects in heart failure, and hypothesized that multiple biomarkers in combination would provide a valuable means for risk stratification. Our findings strongly support this hypothesis. In a multi-center cohort of 1513 ambulatory heart failure patients, we found that a multimarker score comprised of 7 biomarkers, reflective of diverse biologic axes, was a strong predictor of risk and significantly improved the prediction of outcomes compared to the most commonly used clinical risk score in heart failure, the SHFM. Patients in the highest multimarker tertile had a nearly 14-fold unadjusted risk of death, transplant, or VAD placement compared to the lowest tertile, and this risk remained nearly 7-fold after adjustment for the SHFM. The multimarker score showed a substantial ability to discriminate individual patient risk at 1 year (AUC 0.798) that was again superior to the SHFM. Addition of the multimarker score to the SHFM appropriately reclassified a large proportion (24.1%) of patients as higher
risk. These findings support the usefulness of multiple biomarkers as part of an algorithm for assessing prognosis in heart failure.

Assessing risk of future adverse events is a fundamental task in clinical medicine. Research in community-based cohorts has led to the development of clinical scores that estimate the risk of new onset cardiovascular disease, the most widely used being the Framingham risk score. These scores are simple, easily calculated, and have been a major advance for preventive medicine. Attempts to improve risk prediction by adding a diverse panel of biomarkers and genetic variants to clinical risk scores is an active area of research, with mixed results depending upon the study population.

Once cardiovascular disease is established, however, approaches to assessing risk of adverse events (i.e. prognosis) are less well defined, although still critically important as an objective means to guide treatment strategy. The SHFM is the most widely validated clinical risk score to predict prognosis in chronic heart failure, and is easily calculated from readily available clinical data and well-calibrated in our cohort, but has been shown to underestimate risk and overestimate survival. Biomarkers can improve upon the use of primarily clinical data (i.e. SHFM) and offer advantages of rapid availability (within minutes to hours), reproducibility, and quantitative insight into the underlying disease mechanisms. A number of previous studies have investigated the prognostic utility of one or two biomarkers in chronic heart failure. Several of these have demonstrated associations with outcome, but their clinical value remains a matter of debate. As recently reviewed, one explanation for the slow translation of biomarkers to clinical use relates to overly optimistic risk estimates in initial publications. An alternative explanation is that individual biomarkers may be insufficient to assess multi-system disorders. By contrast, our findings demonstrate that a diverse multimarker panel, which
provides a more comprehensive signature of the underlying pathophysiology, offers substantial ability to determine individual patient prognosis.

These findings support the concept of a multimarker tool for prognosis, but identifying an ‘optimal’ panel of biomarkers for assessing heart failure, or any other disease, is a formidable task. We chose biomarkers that quantify known biological abnormalities in heart failure that affect outcome, including myocyte injury (TnI\textsuperscript{29-31}), neurohormonal activation (BNP\textsuperscript{32}), myocyte stress (ST2\textsuperscript{33}), vascular growth and remodeling (sFlt-1\textsuperscript{34}), inflammation (CRP\textsuperscript{32}), oxidative stress (uric acid\textsuperscript{35}, MPO\textsuperscript{36, 37}), and renal dysfunction (creatinine). There are certainly other biomarkers that were not included, such as galectin-3, adrenomedullin, adiponectin, GDF-15, or high sensitivity TnI\textsuperscript{24,38,39} and inclusion of these could improve our score. We also found substantial correlations among many of the biomarkers measured, strongly suggesting that there is no single ‘optimal panel.’ Rather, there are probably a number of biomarker panels that may perform equivalently, and choosing which to deploy in clinical practice will depend on factors such as cost, ease of assay, and potential therapeutic implications. As technology for proteomic and metabolomic profiling continues to advance, unbiased biomarker screens will undoubtedly reveal a large number of candidates for risk assessment in heart failure, and this challenge will be magnified.

We acknowledge the strengths and limitations of our study. First, our population was recruited from heart failure referral centers with broad inclusion criteria. As a result, the study cohort has a high prevalence of comorbid conditions and a broad spectrum of heart failure. The high burden of comorbidity parallels the complexity of heart failure patients in clinical practice, and stands in contrast to biomarker studies in clinical trial populations, which typically have fewer comorbid conditions. Furthermore, the broad spectrum of heart failure enabled biomarker
assessment over the full range of disease, from mild to severe. Second, the multimarker score was derived within our dataset, and as such, there is a potential to have results appear more optimistic for this score. To address this concern, we performed rigorous analyses that included the implementation of a leave-one-out jackknife estimation approach, which avoids self-influence, arbitrary splitting of the data, and provides a highly robust measure to assess the validity of our findings. Our supplemental analyses using the SFHM and its components and using death as the primary outcome yielded qualitatively similar findings. Although we recalibrated the SHFM for our composite endpoint for risk reclassification analyses, recalibration did not affect estimation of the AUC (discrimination). Finally, our NRI estimates may be sensitive to the number and selection of cutpoints.

We caution that the most rigorous comparison is to assess both the biomarker score and the SHFM in completely independent cohorts, emphasizing the need for future research. We focused on a select panel of markers, but it is certainly plausible that there remain additional markers that could have further improved prognosis. The optimal panel of markers, the change in these markers over time, and how these changes might help guide therapeutic interventions remain to be defined, as does the cost-effectiveness of such an approach are necessary studies before its clinical application.

In summary, we demonstrate that a multimarker score comprised of seven circulating biomarkers showed a substantial ability to predict risk in chronic heart failure, and out-performed the most widely validated clinical risk score in heart failure, the SHFM. These findings strongly support ongoing efforts to use unbiased proteomic and metabolomic technologies to develop signatures of heart failure progression.
Sources of Funding

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Disclosures

Dr. Cappola reports receiving research support from Abbott Diagnostics. Drs. Ky and Cappola are co-inventors on a pending patent application for the use of sFlt-1 as a biomarker in heart failure. Dr. Levy received research support for the SHFM from NIH, General Electric, Epocrates, and HeartWare and honoraria from Glaxo SmithKline.

References

American Heart Association Statistics Committee and Stroke Statistics Subcommittee.


Table 1. Baseline characteristics of study participants*

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>All participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 1513</td>
<td></td>
</tr>
</tbody>
</table>

| Medical History and Risk Factors             |                  |
| History of hypertension                     | 877 (58)         |
| History of diabetes                         | 426 (28)         |
| Tobacco use                                  |                  |
| Current                                      | 138 (9)          |
| Former                                       | 806 (54)         |
| Hypercholesterolemia                         | 752 (50)         |

| Heart Failure Characteristics                |                  |
| NYHA classification                          |                  |
| I                                            | 258 (17)         |
| II                                           | 690 (46)         |
| III                                          | 444 (29)         |

Demographic Characteristics

- Age, mean (SD), yr: 56 (15)
- Male: 1000 (66)
- Race:
  - Caucasian: 1114 (74)
  - African American: 330 (22)
  - Other: 69 (4)

Medical History and Risk Factors

- History of hypertension: 877 (58)
- History of diabetes: 426 (28)

Tobacco use

- Current: 138 (9)
- Former: 806 (54)

Heart Failure Characteristics

- NYHA classification:
  - I: 258 (17)
  - II: 690 (46)
  - III: 444 (29)
IV 114 (8)
Ischemic heart failure 455 (30)
Systolic heart failure 1305 (86)
Cardiac resynchronization therapy 381 (25)
Defibrillator 636 (42)

**Medication Use**

ACE inhibitors or ARBs 1314 (87)
Aldosterone antagonists 511 (34)
Aspirin 825 (55)
Beta-blockers 1328 (88)
Digoxin 586 (39)
Diuretics 1185 (78)
HMG CoA reductase inhibitors 756 (50)
Inotrope 50 (3)

**Clinical Measures**

Mean (standard deviation)

Body mass index, kg/m² 30 (7.3)
Systolic blood pressure, mmHg 114 (20)
Diastolic blood pressure, mmHg 70 (12)
Estimated Glomerular Filtration Rate, ml/min/1.73 m² 84 (32)
Sodium – meq/L 139 (3.4)
Ejection fraction – % 34 (17)
Seattle Heart Failure Model score -0.07 (1.0)
<table>
<thead>
<tr>
<th>Biomarkers or Clinical Risk Scores</th>
<th>Median (interquartile range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP – pg/ml</td>
<td>171 (47, 576)</td>
</tr>
<tr>
<td>sFlt-1 – pg/ml</td>
<td>308 (260, 381)</td>
</tr>
<tr>
<td>hsCRP – mg/l</td>
<td>0.35 (0.14, 0.89)</td>
</tr>
<tr>
<td>MPO – pmol/l</td>
<td>138 (95, 227)</td>
</tr>
<tr>
<td>ST2 – ng/ml</td>
<td>27.5 (19.9, 40.8)</td>
</tr>
<tr>
<td>TnI</td>
<td></td>
</tr>
<tr>
<td>Detectable, no. (%)</td>
<td>973 (64)</td>
</tr>
<tr>
<td>TnI – ng/ml</td>
<td>0.02 (0.01, 0.04)</td>
</tr>
<tr>
<td>Uric acid – mg/dl</td>
<td>7.0 (5.7, 8.8)</td>
</tr>
<tr>
<td>Creatinine – mg/dl</td>
<td>0.92 (0.76, 1.25)</td>
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</tbody>
</table>

Abbreviations: ACE, Angiotensin-converting enzyme; ARBs, Angiotensin receptor blocker; BNP, B-type natriuretic peptide; sFlt-1, soluble fms-like tyrosine kinase receptor-1; hsCRP, high-sensitivity C-reactive protein; MPO, myeloperoxidase; ST2, soluble toll-like receptor-2; TnI, troponin I.

*Values are expressed as number (percentage) unless otherwise noted.
Table 2. Association of multimarker score with risk of all-cause death, cardiac transplantation, or VAD placement

<table>
<thead>
<tr>
<th>Risk category†</th>
<th>Kaplan-Meier 1-year risk</th>
<th>Unadjusted Hazard Ratio (95% CI); P value</th>
<th>Adjusted* Hazard Ratio (95% CI); P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>1.6% (0.5%, 2.7%)</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>&lt;−0.51‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>6.4% (4.3%, 8.6%)</td>
<td>4.69 (2.92, 7.54); &lt; 0.001</td>
<td>3.50 (2.17, 5.67); &lt; 0.001</td>
</tr>
<tr>
<td>−0.51 to &lt;0.52‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>26% (22%, 30%)</td>
<td>13.7 (8.75, 21.5); &lt; 0.001</td>
<td>6.80 (4.18, 11.1); &lt; 0.001</td>
</tr>
<tr>
<td>≥0.52‡</td>
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</table>

Abbreviation: CI, confidence interval
*Adjusted for SHFM score
†Bottom tertile of multimarker score defined as low risk, middle tertile defined as moderate risk, and top tertile defined as high risk
‡Multimarker score calculated as 0.210 × log₂ BNP (pg/ml) + 0.176 × log₂ sFlt-1 (pg/ml) + 0.067 × log₂ hsCRP (mg/l) + 0.274 × log₂ ST2 (ng/ml) + 0.772 × detectable TnI (vs. not) − 1.718 × log₂ uric acid (mg/dl) + 0.298 × (log₂ uric acid)² + 0.267 × log₂ creatinine (mg/dl) − 0.138 (log₂ creatinine)², where log₂ denotes log (base 2) transformation
Table 3. Reclassification of 1-year risk of all-cause death, cardiac transplantation, or VAD placement with the addition of multimarker score to SHFM score*

<table>
<thead>
<tr>
<th>Reclassification</th>
<th>SHFM score plus multimarker score</th>
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<tbody>
<tr>
<td>SHFM score</td>
<td>0%-&lt;10%</td>
</tr>
<tr>
<td>Events, no. (row %)†</td>
<td></td>
</tr>
<tr>
<td>0%-&lt;10%</td>
<td>22 (50%)</td>
</tr>
<tr>
<td>≥10%-&lt;20%</td>
<td>4 (7%)</td>
</tr>
<tr>
<td>≥20%-&lt;50%</td>
<td>0</td>
</tr>
<tr>
<td>≥50%-100%</td>
<td>0</td>
</tr>
<tr>
<td>Non-events, no. (row %)†</td>
<td></td>
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<tr>
<td>0%-&lt;10%</td>
<td>764 (88%)</td>
</tr>
<tr>
<td>≥10%-&lt;20%</td>
<td>121 (36%)</td>
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<td>≥20%-&lt;50%</td>
<td>11 (9%)</td>
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<td>≥50%-100%</td>
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<table>
<thead>
<tr>
<th>Net reclassification</th>
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<tbody>
<tr>
<td>Up</td>
</tr>
<tr>
<td>Events†</td>
</tr>
<tr>
<td>Non-events†</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Abbreviations: SHFM, Seattle Heart Failure Model score; CI, confidence interval; NRI, net reclassification index
*Multimarker score calculated as described in Table 2
†Number of events and non-events estimated from 1-year Kaplan-Meier risk estimates; row percentages may not sum to 100% due to estimation
Figure Legends

Figure 1. Event-free survival according to multimarker score category and tertiles of SHFM*

Kaplan-Meier curves illustrating the incidence of all-cause death, cardiac transplantation, or VAD placement among Penn Heart Failure Study participants according to (A) multimarker score category tertiles (P < 0.001 by log rank test) and (B) Seattle Heart Failure Model score tertiles (P < 0.001 by log rank test)

*Multimarker score calculated as described in Table 2

Figure 2. ROC curves for events at 1 year

ROC curves comparing the ability of the SHFM and the multimarker score to correctly classify patients who died, required cardiac transplantation, or VAD placement by 1 year of followup*

*Multimarker score calculated as described in Table 2
Multiple Biomarkers for Risk Prediction in Chronic Heart Failure
Bonnie Ky, Benjamin French, Wayne C. Levy, Nancy K. Sweitzer, James C. Fang, Alan H.B. Wu,
Lee R. Goldberg, Mariell Jessup and Thomas P. Cappola

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Supplemental Material

Biomarker Assays

ST2 Assay

ST2 was measured from banked plasma via a highly sensitive sandwich monoclonal immunoassay (Presage™ ST2 assay, Critical Diagnostics, New York, New York). This platform offers improved accuracy in quantifying ST2 levels, particularly at lower concentrations. The antibodies used in the Presage™ assay were generated from a recombinant protein based upon the human cDNA clone for the complete soluble ST2 sequence. The intra- and inter-assay coefficients of variation (CV) were less than 4.0% and 2.5%, respectively. The lower limit of detection of ST2 was 2 ng/ml and the upper limit was 200 ng/ml.

sFlt-1

sFlt-1 was measured using prototype ARCHITECT immunoassays (Abbott Laboratories, Abbott Park, IL). The sFlt-1 immunoassay measures both free and bound sFlt-1, with an assay range of 15 to 50,000pg/ml. The intra- and interassay coefficients of variation (CV) ranged from 1.3% to 5.2% and 1.9% to 5.9%, respectively.

BNP

BNP was measured using the ARCHITECT® BNP chemiluminescent microparticle immunoassay (Abbott Laboratories, Abbott Park, IL) as previously described. The assay range was from 10 to 5,000pg/ml. The intra- and interassay CV ranged from 0.9% to 5.6% and 1.7% to 6.7%, respectively.
MS ID#: CIRCHF/2011/965020

TnI

TnI was measuring using a chemiluminescent microparticle immunoassay on the ARCHITECT® system (Abbott Laboratories, Abbott Park, IL). The limits of detection were <0.01 ng/ml. The total CV was ≤ 10%.

hsCRP

hsCRP was measured using the MULTIGENT Vario assay on the ARCHITECT® system (Abbott Laboratories, Abbott Park, IL), which is a latex immunoassay. The lower limit of detection if 0.01mg/dL. The total CV was ≤ 6%.

MPO

MPO was measured using a chemiluminescent microparticle immunoassay on the ARCHITECT® system (Abbott Laboratories, Abbott Park, IL) using a 2-step immunoassay. The total CV was ≤ 10%.

Uric Acid

The uric acid assay was based on the methods of Trivedi and Kabasakalian and measured on the ARCHITECT® platform (Abbott Laboratories, Abbott Park, IL). The intra- and interassay CV were <0.5% and <1.6%, respectively.

Creatinine

Creatinine was measured on the ARCHITECT® MULTIGENT platform (Abbott Laboratories, Abbott Park, IL). The intra- and interassay CV were ≤ 4%.
**Supplementary Table 1: Association of biomarkers with risk of all-cause death, cardiac transplantation, or VAD placement**

<table>
<thead>
<tr>
<th></th>
<th>Univariable</th>
<th>Multivariable</th>
<th>Parsimonious</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>(\log_2) BNP – pg/ml</td>
<td>1.46 (1.39, 1.54)</td>
<td>1.23 (1.15, 1.32)</td>
<td>1.23 (1.15, 1.32)</td>
</tr>
<tr>
<td>(\log_2) sFlt-1 – pg/ml</td>
<td>1.68 (1.54, 1.84)</td>
<td>1.17 (0.99, 1.38)</td>
<td>1.19 (1.02, 1.40)</td>
</tr>
<tr>
<td>(\log_2) hsCRP – mg/l</td>
<td>1.26 (1.19, 1.34)</td>
<td>1.06 (0.99, 1.14)</td>
<td>1.07 (0.99, 1.14)</td>
</tr>
<tr>
<td>(\log_2) MPO – pmol/l</td>
<td>1.20 (1.09, 1.32)</td>
<td>1.04 (0.93, 1.15)</td>
<td>—</td>
</tr>
<tr>
<td>(\log_2) ST2 – ng/ml</td>
<td>2.05 (1.84, 2.30)</td>
<td>1.32 (1.14, 1.52)</td>
<td>1.31 (1.14, 1.52)</td>
</tr>
<tr>
<td>TnI – ng/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detectable (vs. not)</td>
<td>3.79 (2.58, 5.70)</td>
<td>2.00 (1.32, 3.04)</td>
<td>2.16 (1.48, 3.18)</td>
</tr>
<tr>
<td>(\log_2) TnI</td>
<td>1.14 (1.07, 1.22)</td>
<td>1.04 (0.96, 1.12)</td>
<td>—</td>
</tr>
<tr>
<td>(\log_2) Uric Acid – mg/dl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.12 (0.02, 0.99)</td>
<td>0.17 (0.02, 1.36)</td>
<td>0.18 (0.02, 1.42)</td>
</tr>
<tr>
<td>Quadratic</td>
<td>1.65 (1.15, 2.36)</td>
<td>1.36 (0.95, 1.95)</td>
<td>1.35 (0.94, 1.93)</td>
</tr>
<tr>
<td>(\log_2) Creatinine – mg/dl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>2.69 (2.08, 3.47)</td>
<td>1.30 (0.97, 1.76)</td>
<td>1.31 (0.97, 1.76)</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.78 (0.67, 0.90)</td>
<td>0.87 (0.73, 1.03)</td>
<td>0.87 (0.74, 1.03)</td>
</tr>
</tbody>
</table>

**Abbreviations:** HR, Hazard ratio; CI, Confidence interval

\(\log_2\) denotes \(\log\) (base 2) transformation
Supplementary Table 2: Reclassification of 1-year risk of all-cause death, cardiac transplantation, or VAD placement with the addition of multimarker score to the individual SHFM components

**Reclassification**

<table>
<thead>
<tr>
<th>SHFM score plus multimarker score</th>
<th>SHFM score</th>
<th>0%--&lt;10%</th>
<th>≥10%--&lt;20%</th>
<th>≥20%--&lt;50%</th>
<th>≥50%--100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events, no. (row %)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%--&lt;10%</td>
<td>26 (63%)</td>
<td>15 (37%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>≥10%--&lt;20%</td>
<td>6 (14%)</td>
<td>20 (49%)</td>
<td>16 (37%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>≥20%--&lt;50%</td>
<td>0</td>
<td>12 (17%)</td>
<td>45 (64%)</td>
<td>13 (18%)</td>
<td></td>
</tr>
<tr>
<td>≥50%--100%</td>
<td>0</td>
<td>3 (16%)</td>
<td>16 (84%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-events, no. (row %)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%--&lt;10%</td>
<td>856 (91%)</td>
<td>82 (9%)</td>
<td>2 (&lt;1%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>≥10%--&lt;20%</td>
<td>87 (35%)</td>
<td>114 (46%)</td>
<td>44 (18%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>≥20%--&lt;50%</td>
<td>2 (1%)</td>
<td>31 (22%)</td>
<td>94 (68%)</td>
<td>12 (9%)</td>
<td></td>
</tr>
<tr>
<td>≥50%--100%</td>
<td>0</td>
<td>0</td>
<td>7 (41%)</td>
<td>10 (59%)</td>
<td></td>
</tr>
</tbody>
</table>

**Net reclassification**

<table>
<thead>
<tr>
<th>Up</th>
<th>Down</th>
<th>NRI</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events†</td>
<td>43</td>
<td>21</td>
<td>13.2</td>
<td>(2.1%, 24.3%)</td>
</tr>
<tr>
<td>Non-events†</td>
<td>140</td>
<td>127</td>
<td>-1.0</td>
<td>(-4.3%, 2.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>127</td>
<td></td>
<td>12.2</td>
<td>(0.6%, 23.8%)</td>
</tr>
</tbody>
</table>

Abbreviations: SHFM, Seattle Heart Failure Model score; CI, confidence interval; NRI, net reclassification index

†Number of events and non-events estimated from 1-year Kaplan-Meier risk estimates; row percentages may not sum to 100% due to estimation
Supplementary Figure 1: Pairwise Spearman rank correlation between biomarkers

The Spearman rank correlation coefficients are represented in each ellipsoid. The shape of the ellipsoid and intensity of the color reflects the degree of correlation. P < 0.001 for all.
Supplementary Figures 2A and 2B: Fitted values for uric acid and creatinine

Nonlinearity of relationship between uric acid and creatinine and risk of all-cause death, cardiac transplantation, or VAD implantation. Solid lines show estimated association. Shaded regions demonstrate pointwise confidence regions.
Supplementary Figure 3: ROC curves for events at 1 year

ROC curves comparing the ability of the individual components of the SHFM score and the multimarker score to correctly classify patients who died, required cardiac transplantation, or VAD placement by 1 year of followup.
REFERENCES


