

Title: Plasma cBIN1 Score (CS) Identifies HF_rEF and Can Predict Cardiac Hospitalization in Stable Ambulatory Patients

Short Title: Xie, CS prognosticates outcomes in stable HF_rEF

Authors: Yu Xie ^{1**}, Tara C. Hitzeman ^{1**}, Ronit H. Zadikany ¹, Andriana P. Nikolova ¹, Rachel Baum¹, Bing Xu ¹, Sosse Agvanyan ¹, Gil Y. Melmed ², Dermot T. McGovern ², Dael R. Geft ^{1,3}, David H. Chang^{1,3}, Jaime D. Moriguchi ^{1,3}, Antoine Hage ^{1,3}, Babak Azarbal ^{1,3}, Lawrence S. Czer ^{1,3}, Michelle M. Kittleson ^{1,3}, Jignesh K. Patel ^{1,3}, Alan H. B. Wu ⁴, Jon A. Kobashigawa ^{1,3}, Michele Hamilton ^{1,3}, TingTing Hong ^{1,3*}, Robin M. Shaw ^{1,3*}

** Co-first authors

¹ Division of Cardiology, Cedars-Sinai Heart Institute and Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

² Division of Gastroenterology, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

³ Division of Cardiology, Department of Medicine, University of California Los Angeles, Los Angeles, CA 90095, USA

⁴Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA 94110, USA

*Co-corresponding authors: TingTing Hong, MD, PhD and Robin M. Shaw, MD, PhD
8700 Beverly Blvd., Davis Bldg #1016
Los Angeles, CA 90048
Fax: 310-9673891
Tel: 310-9673842
Email: tingting.hong@cshs.org and robin.shaw@cshs.org

Number of figures and tables: 8 (6 figures, 2 tables)

Conflict of interest: None

ABBREVIATIONS:

AUC – area under the curve

BIN1 – bridging integrator 1

BMI – body mass index

BNP – brain natriuretic peptide

cBIN1 – cardiac bridging integrator 1

CS – cBIN1 score

HF – heart failure

HFrEF – heart failure with reduced ejection fraction

HR – hazard ratio

IQR – interquartile range

LVEF – left ventricular ejection fraction

NT-proBNP – N-terminal prohormone of brain natriuretic peptide

NYHA– New York Heart Association

ROC – receiver operating characteristic

SD – standard deviation

ABSTRACT

Objective: We determined, in stable ambulatory heart failure with reduced ejection fraction (HFrEF) subjects and matched controls, the capability of a novel blood based cardiac-specific cBIN1 Score (CS), which assesses the health of cardiac muscle, to identify patients with known heart failure (HF) and to prognosticate future hospitalization.

Background: Limited clinical tools are available in assessing cardiac muscle health in stable ambulatory patients. Cardiac bridging integrator 1 (cBIN1) is a cardiomyocyte t-tubule membrane scaffolding protein which regulates calcium signaling in cardiomyocytes, decreases in failing muscle, and is present in plasma in levels that correlate with cardiac content. We hypothesize that CS, a normalized index of plasma cBIN1 concentration, can function as a diagnostic and prognostic biomarker of HF.

Methods: Plasma cBIN1 concentration is measured by an ELISA test, and CS is calculated as the natural log of the ratio of a constant population mean cBIN1 to measured cBIN1 concentration. We determined CS among 125 clinically stable individuals with HFrEF (LVEF \leq 40%) (mean age 56 ± 10 years old, 79% men) and 125 age, sex matched volunteers with no known history of HF. We obtained plasma concentrations of NT-proBNP, a marker of volume status, as comparison. Baseline co-morbidities and 18-month longitudinal clinical information were obtained through electronic medical records.

Results: CS follows a normal distribution with a median of 0 in the control population and median is significantly increased among HFrEF patients to 1.8 (IQR 1.4 – 2.1, $p < 0.0001$). CS diagnosed HFrEF with a receiver operating characteristic (ROC) area under the curve (AUC) of 0.93 (AUC is 0.98 for NT-proBNP, and combined CS and NT-proBNP AUC is 0.99). Unlike NT-proBNP, CS does not correlate with body mass index (BMI) in either the control or HFrEF population (Pearson's $r = -0.15$, $p = 0.12$; Pearson's $r = 0.003$, $p = 0.97$, respectively). NT-proBNP significantly correlates

with renal function (Pearson's $r = -0.37$, $p = 0.001$), while CS also has no correlation (Pearson's $r = 0.03$, $p = 0.71$). During an 18-month follow-up, a high CS ≥ 1.8 at the initial visit predicted future cardiovascular hospitalizations (38% vs. 21%, $p = 0.04$, hazard ratio 2.0). NT-proBNP did not predict future cardiovascular hospitalizations.

Conclusions: Plasma cBIN1 based CS is insensitive to BMI and renal function and differentiates myocardial health between patients with HFrEF versus matched controls. An abnormally high CS reflected poor intrinsic myocardial health and can predict future 18-month cardiac hospitalization in stable ambulatory patients.

KEY WORDS: biomarker; heart failure with reduced ejection fraction (HFrEF); cBIN1; cBIN1 Score (CS)

INTRODUCTION

Heart failure (HF) is the most common clinical manifestation of abnormally functioning myocardium. There is a 20% lifetime risk of HF for Americans who are 40 years of age or older, half of whom develop HF with reduced ejection fraction (HFrEF) [1, 2]. Despite improvements in care, mortality remains at 50% within 5 years after diagnosis [3, 4]. There are an estimated 650,000 new HF cases diagnosed annually and 5.1 million persons in the US with clinically significant HF. Care is difficult for this population with multiple medical problems and a 1-month readmission rate of 25% and a total cost of care greater than \$30 billion per year (half of which is spent on hospitalizations). Given the current aging US population, this expense is expected to increase, and HF hospitalization is already the highest single cost to Medicare for Americans over the age of 65 [5, 6]. Furthermore, among patients with HFrEF, there is increased post-hospital discharge mortality [2, 7]. Being able to assess the health of cardiac muscle and use that information to accurately predict and limit future hospitalization are both health care and economic imperatives.

Current guidelines define HFrEF patients as having transthoracic echocardiogram obtained left ventricular ejection fraction (LVEF) less than 40% [8]. Traditional diagnostic tools for assessing the severity of HFrEF include transthoracic echocardiogram, New York Heart Association (NYHA) assessment, cardiopulmonary exercise testing, and cardiac index on right heart catheterization. These indices provide overall cardiac function and general health, but do not necessarily correlate to intrinsic muscle. Furthermore, traditional tools often require specialized equipment and medical staff which are not available in a general clinician's office, limiting accessibility. Clinicians could be well served with a quantitative blood test based tool that assesses the health and reserve of cardiac muscle.

The gold standard biomarkers to assess HF in patients with HFrEF are natriuretic peptides of the BNP family [9]. BNP and its more stable and non-active version N-terminal prohormone BNP

(NT-proBNP) are secreted by cardiomyocytes in response to pressure and stretch, which (with active BNP) results in a downstream effect of increased natriuresis, diuresis, and vasodilatation [10-13]. As stretch-response molecules, BNP biomarkers assess fluid status and perform extremely well in situations of diagnostic dilemmas regarding patients' symptoms of acute dyspnea [14, 15]. However, NT-proBNP assesses fluid status not muscle health and is less useful in the management of ambulatory patients with known HF [16]. Furthermore, renal dysfunction is associated with elevated concentrations of BNP markers [17] and BNP also has a negative correlation with body mass index (BMI) [18, 19]. Taken together, these data suggest that HF management could benefit from additional assessment of cardiac muscle health independent of volume status.

The cardiac isoform of bridging integrator 1 (cBIN1) is a t-tubule membrane sculpting protein, which organizes microdomains and proteins responsible for calcium release and excitation-contraction coupling [20, 21]. cBIN1 is transcriptionally decreased in failing hearts [22] and is also detectable in blood [23, 24]. In this study, we developed a cBIN1 score (CS) calculated as the natural log of the normalized reciprocal of plasma cBIN1 concentration, which provides a dimensionless index of muscle health and rises with worsening muscle failure. We explored, in a large ambulatory clinic population with diagnosed HFrEF and matched controls, the capability of CS in detecting failing heart muscle, its dependence of extracardiac parameters, and its ability to accurately predict future cardiac hospitalization.

METHODS

Study Design

All human studies were approved by the Institutional Review Board (IRB) at Cedars-Sinai Medical Center. Full informed consent was obtained from all subjects prior to participation in the study. The study involved two human populations including volunteers with no known history of HF and patients with known HFrEF.

The HFrEF cohort was followed longitudinally in the Advanced Heart Disease clinic at Cedars-Sinai Heart Institute, Los Angeles. Those in the clinic with a known diagnosis of HFrEF (LVEF \leq 40% and a history of HF), were eligible for inclusion in the Cedars-Sinai Medical Center Heart Institute Biobank and enrollment into this study. From July 2014 to November 2015, 125 patients were enrolled. A blood sample was obtained from patients at the time of scheduled clinic visits with planned phlebotomy. Patients with recovered HFrEF (LVEF $>$ 40%) at the time of the study were excluded. Patient demographics, clinical information (etiology of HF, NYHA, hospitalization, implantable cardioverter defibrillator firing), medications, laboratory results (BNP, creatinine, lipid profile), and diagnostic test results (echocardiogram, right heart catheterization, and cardiopulmonary exercise testing) were gathered from the hospital electronic medical records into the secure de-identified biobank database. Subsequent clinical information was updated from chart review occurring every three months.

The comparison cohort consisted of 125 volunteers with no known history of HF, who were age and sex matched, were obtained with informed consent from the Cedars MIRIAD IBD Consortium and Innovative Research, under similar collection and plasma preparation protocol as the biobank. For confirmation of health status, a clinical history including height, weight, age, ethnicity, medical history, and current medications was obtained from each volunteer, and stored in same Heart Institute de-identified database.

Cardiac hospitalization defined as the primary outcome during follow-up

The cohort of 125 HFrEF patients underwent routine follow-up for the next 18 months at the Cedars-Sinai Heart Institute Advanced Heart Disease clinic. Cardiac hospitalization was predefined as any hospitalization after the time of cBIN1 blood draw, where the primary diagnosis was cardiac in origin. All chart review and adjudication were done by a two-physician panel who were not involved in the clinical care of the patients (YX and RZ). Patients who subsequently underwent heart transplantation or left ventricular assist device placement, for any reason, were excluded from our analysis.

Sample processing and CS Determination

Whole venous blood was obtained from consented patients and volunteers during standard clinic phlebotomy, drawn into EDTA lavender tubes, and stored immediately at 4°C for less than four hours. The specimen tubes were processed by centrifugation in a refrigerated centrifuge for 20 minutes at 2,250 g to separate plasma, then over ice plasma was aliquoted into one ml bar coded cryovials (0.5 ml per aliquot), flash frozen with dry ice and ethanol, and stored at -80°C freezer. All cryovials were de-identified and securely stored in the biobank until they were used for cBIN1 or NT-proBNP analysis.

The concentration of cBIN1 was determined using an assay provided by Sarcotein Diagnostics. Cryovials containing plasma were thawed on ice and then subjected to ELISA based analysis with the capture antibody, mouse monoclonal anti-BIN1 exon 17 (Sigma-Aldrich) and the detection antibody, HRP-conjugated recombinant anti-BIN1 exon 13 (Sarcotein Diagnostics). Concentration was obtained by standard curves generated from purified cBIN1 protein standards prepared from cells over-expressing cBIN1. To detect the full plasma cBIN1 content from plasma

microparticles, each plasma sample was subjected to osmotic shock to break up the microparticles by dilution with distilled water (3 volumes water, 1 volume plasma) before loaded to the ELISA plate [24]. The ELISA reagents were purchased from BD Biosciences (BD OptEIA reagents kit, catalog 550534). Using positive control plasma samples with known cBIN1 concentrations, this assay has an inter-plate variability of < 5%.

CS is the natural log of the ratio of median cBIN1 of the controls (10 ng/ml) to that of measured cBIN1:

$$CS = \ln\left(\frac{10}{cBIN1}\right)$$

We express our findings as CS, a normalized reciprocal, rather than an absolute cBIN1 value, to be consistent with clinical convention of an elevated biomarker of HF to correlate with worsening cardiac status. A natural log transformation is performed because cBIN1 levels and the normalized ratio have log normal distribution.

NT-proBNP assay

NT-proBNP values were obtained from the plasma of control and HFREF patients. The Cedars-Sinai Medical Center clinical laboratory sends out the plasma samples to Quest Diagnostics Laboratory to perform the NT-proBNP assay using electrochemiluminescence. NT-proBNP was chosen over BNP due to its superior stability and higher mean recovery (residual immunoreactivity) when obtained from stored frozen plasma [12, 13]. Similar to CS, a natural log transformation of NT-proBNP was also performed and used for analyses.

Statistical Analysis

Data distributions were assessed for normality based on the quantile-quantile plot and the Kolmogorov-Smirnov test. Continuous variables with a normal distribution were expressed and

analyzed as means and standard deviations and compared using a two-sided Student's t-test. Continuous variables with non-normal distributions were analyzed with medians and interquartile ranges, and compared using the Mann-Whitney U and Kruskal-Wallis tests. Categorical variables were compared using the chi-square test. Pearson's correlation analysis was performed to examine the relationship of CS or NT-proBNP with estimated glomerular filtration rate (eGFR) [25] and BMI. Receiver operating characteristic (ROC) analyses were performed to determine the sensitivity and specificity of CS to diagnose HF as well as for NT-proBNP. Kaplan-Meier analysis was used to compare the differences in hospital-free survival rates between patients with high and low values of CS using the log-rank test. Statistical analyses were conducted using SAS Version 9.3.1 software (SAS Institute Inc., Cary, NC) and RStudio Version 1.0.143 (RStudio, Inc., Boston, MA). Two-sided *p*-values were reported and a *p*-value <0.05 was considered statistically significant.

RESULTS

Study cohorts

Baseline characteristics of HFrEF patients and matched controls are compiled in **Table 1**. Between July 2014 and November 2015, a total of 125 HFrEF patients (mean age 56 ± 10 years old, 79% men) were enrolled in the study. HF etiology included 43 (34%) ischemic and 80 (64%) non-ischemic. Most of the non-ischemic patients were idiopathic (53 patients, 66%), with the remainder due to known valvular disease, toxin-mediated disease, or infiltrative disease. Most patients were classified as NYHA II or III (40% and 41%, respectively) per clinical notes. The prevalence of comorbidities was 45% hypertension, 38% diabetes, and 22% chronic kidney disease. The baseline median LVEF on transthoracic echocardiography was $25 \pm 8\%$. There is a high prevalence of background guideline directed medical therapy by the Cedars-Sinai Medical Center Advanced Heart Disease group, reflecting optimal medical management. Additionally, 125 age and sex matched volunteers were recruited for this study.

CS is elevated in patients with HFrEF

Violin plots of cBIN1 based CS and natural log NT-proBNP among HFrEF and controls are shown in **Figure 1**. The width of the violin plot is the density plot for the individuals in the cohorts for CS (**Panel 1A**) and ln NT-proBNP (**Panel 1B**). The HFrEF cohort median CS is elevated relative to the controls, 1.8 (IQR 1.4 – 2.1) compared to 0 (IQR -0.5 – 0.7), respectively ($p < 0.0001$). The HFrEF cohort median of ln NT-proBNP level, 7.1 (IQR 5.9 – 8.2) is also significantly elevated as compared to the median level of the control cohort, 3.3 (IQR 2.7 – 4.1), with $p < 0.0001$. In **Supplemental Figure 1**, the violin plot of NT-proBNP is shown for the matched controls and HFrEF groups (median 1153 pg/ml [IQR 380 – 3529 pg/ml] and median 28 pg/ml [IQR 15 – 58

pg/ml], respectively). The distribution of both CS and NT-proBNP are approximately normal with log-transformation (**Figure 2**).

Median CS and NT-proBNP values among different subgroups of race, age, sex, BMI and etiology were analyzed (**Table 2**). CS and NT-proBNP do not vary with sex or age. Similarly, CS does not differ among BMI categories (normal, overweight, obese and morbidly obese) in controls or HFrEF patients. Interestingly, there is a statistically significant difference in CS with respect to different racial and ethnic groups for the HFrEF patients, with White and Asian individuals having lower CS values than Hispanic and Black individuals ($p = 0.02$). We did not detect a statistically significant difference of CS among these same racial groups in the controls. CS value is not statistically different among HFrEF patients with normal versus decreased eGFR (1.8 and 1.9, respectively), while patients with worsening eGFR (< 60 mL/min) have higher NT-proBNP levels ($p = 0.02$).

Pearson's correlation analysis was performed to further examine the association of the biomarkers with obesity (**Figure 3**) and renal function (**Figure 4**). In both control and HFrEF groups, CS does not have a significant correlation with BMI (Pearson's $r = -0.15$, $p = 0.12$ and Pearson's $r = 0.003$, $p = 0.97$, respectively). NT-proBNP has an inverse correlation with BMI level in both the control and HFrEF cohorts (Pearson's $r = -0.34$, $p = 0.0002$ and Pearson's $r = -0.24$, $p = 0.04$, respectively). The association of renal function, as eGFR, was analyzed in the HFrEF patients. CS does not correlate with eGFR (Pearson's $r = 0.03$, $p = 0.71$), while NT-proBNP increases as eGFR decreases (Pearson's $r = -0.37$ $p = 0.001$).

CS Diagnoses HF

Since the CS level is higher in HFrEF patients than controls, we proceeded to assess the ROC performance of CS relative to NT-proBNP (**Figure 5**). Both CS and NT-proBNP distinguish

HF from control populations. Area under the curve (AUC) of ROC for CS (0.93, red curve) is close to that of NT-proBNP (AUC = 0.98, blue curve). Of note, combining CS with NT-proBNP provides even better performance (AUC = 0.99, green curve) indicating that CS (muscle health) and NT-proBNP (intracardiac volume) can be complementary and worth exploring in a more heterogeneous population.

Elevated CS prognosticates cardiovascular hospitalization in HFrEF patients

Next, we explored whether, in addition to its diagnostic value (**Figure 5**), CS can serve as a prognostic marker in predicting future clinical outcomes in patients with HFrEF. During the 18-months follow-up, we found 40 (32%) of the HFrEF patients had at least one HF-related hospitalization event. Other outcome events were: 4 (3%) patients had ICD firing, 2 (2%) patients went onto mechanical circulatory support (MCS), 5 (4%) patients died (3 of which were due to cardiac etiology and all had a cardiac admission prior to death). These additional outcome events occurred among patients with HF-related hospitalizations during the follow-up period.

Kaplan-Meier survival curves were generated. The red curve denotes patients with CS < 1.8 (median CS in the HFrEF population) and the blue curve denotes patients with CS \geq 1.8 at the initial visit (**Figure 6**). Of note, a high CS (\geq 1.8) predicted a higher hospitalization rate among HFrEF patients in the cohort during the 18-months of follow-up than those with a low CS (< 1.8), (38% vs. 21%, $p = 0.04$) (**Figure 6A**). This difference was even greater in the subgroup of patients with non-ischemic HFrEF (39% for CS \geq 1.8 versus 14% for CS < 1.8, $p = 0.01$) (**Figure 6B**). For NT-proBNP, the Kaplan-Meier survival curve using a NT-proBNP cutoff value of 300 pg/ml, which is the recommended cutoff value offering a 99% negative predictive value across all age groups at excluding acute chronic HF [26-28], did not significantly predict hospitalization. Additionally, we generated a NT-proBNP Kaplan-Meier survival curve using a NT-proBNP cutoff value of 100

pg/ml, and found the false positive rate goes from 2.4% to 12 %, and yet still NT-proBNP did not significantly predict hospitalization. Interestingly, among the ischemic HFrEF subgroup, hospitalization rates were equally frequent (37%), whether CS was low or high (**Figure 6C**), suggesting that hospitalization for patients with ischemic heart disease may occur for reasons other than primary failing heart muscle, such as ischemic myocardium. Hazard ratio for 18-month hospitalization for $CS \geq 1.8$ versus $CS < 1.8$ is 2.03 (CI 1.02 – 4.06, $p = 0.04$) for all HFrEF patients, 3.18 (CI 1.22 – 8.29, $p = 0.02$) for non-ischemic HFrEF patients, and 1.06 (CI 0.37 – 3.01, $p = 0.92$) for ischemic HFrEF patients.

DISCUSSION

While HF_{rEF} contributes to half of all HF in the United States, it remains a syndrome, and its diagnosis involves a combination of history taking, physical examination, labs, imaging and functional studies. There is currently no invasive or noninvasive tool that measures intrinsic cardiomyocyte health. cBIN1 is an integral protein in cardiomyocyte t-tubule biology [20, 21] and beat-to-beat calcium transients [29-31]. Previously, our group found that BIN1 is transcriptionally decreased in HF and is also detectable in blood [22, 23]. A generic BIN1 ELISA measured plasma BIN1 levels correlated with cardiac health in patients with arrhythmogenic right ventricular cardiomyopathy [23]. More recently, we cloned the cardiac specific t-tubule isoform cBIN1 [20], which is enclosed in microparticles and released into blood [23, 24, 31]. In the present study, we introduce CS, which is derived from the inverse of plasma cBIN1 concentration measured by a specific ELISA. In an optimally managed heterogeneous HF_{rEF} patient cohort, we found that a positive CS not only diagnoses HF but also prognosticates future HF-related hospitalization events.

CS as a new diagnostic and prognostic marker of HF_{rEF}

At present, we are not aware of another assay that measures the biochemical health of individual cardiomyocytes. In this current study, CS accurately diagnosed HF (AUC = 0.93) and had additive accuracy when combined with NT-proBNP (AUC = 0.99) (**Figure 5**), indicating that CS is a test complimentary to natriuretic peptides. Inspection of the distribution of CS compared to NT-proBNP reveals both markers separate the control from the HF_{rEF} populations. Furthermore, whereas the large dynamic range for NT-proBNP is in the HF_{rEF} population, the large dynamic range for CS is in the control population (**Figure 1**). It is well understood that pre-clinical asymptomatic patients with structural heart disease are at risk for HF (such as ACC/AHA Class B patients). Such patients are difficult to diagnose but would benefit the most from early medical

therapy [32-34]. The data from **Figure 1** indicate that CS may be able to detect negative cardiomyocyte remodeling prior to the onset of clinical symptoms. CS could be a powerful screening tool for diagnosing early stages of HF or those with pre-clinical disease.

A high CS cutoff value of greater than 1.8 accurately predicted lower cardiac event-free survival during an 18-month follow-up period (**Figure 6**). Large cohort studies of Medicare patients have demonstrated increased post-hospital discharge 30-day mortality among patients with HFrEF [2, 7], suggesting there is still a gap in identifying the patients who are most at risk and not optimized, despite costly hospital care. In our study, we found that screening CS among stable HFrEF clinical patients offers future prognostic power in determining which patients are at highest risk of cardiac events. For those clinical patients, a high CS may be the added prognostic information needed to help push clinical decisions towards more aggressive surveillance or the next step in advanced therapies. Conversely, an advanced HFrEF patient with a low (normal) CS may indicate the ability to postpone advanced therapies and continue monitoring with periodic clinic visits.

Note in our subgroup analysis, CS is consistently higher than the controls in either ischemic or non-ischemic HFrEF patients (median 1.9 and 1.7, respectively). In both subgroups, a higher CS (≥ 1.8) predicts increased rate of future cardiac hospitalization. However, unlike non-ischemic HFrEF patients, low CS (< 1.8) fails to predict a low rate of future cardiac hospitalization in ischemic HFrEF patients. The ischemic HFrEF patients at the Cedars-Sinai Advanced Heart Disease clinic often have multiple revascularization needs and still have acute or chronic ischemic events. Hospitalization for revascularization can explain why such patients require frequent hospitalization independent of cardiac muscle health status evaluated by CS. We are encouraged that CS is detecting general myocardial health rather than regional ischemia. In ischemic patients, evaluating a

patient with both CS and a perfusion assay should help distinguish patient illness due to overall failing muscle function versus that requiring focal revascularization.

CS distinguishes failing cardiac muscle from HF comorbidities

HF is the result of multiple complex pathways, which can be reflected by different biomarkers. Natriuretic peptides (BNP and NT-proBNP) have the most robust data and reflect myocardial wall stress [35]. The BNP peptides are effective at diagnosing HF among patients with dyspnea [14], ruling out acute decompensated HF [15], and future adverse cardiovascular events and mortality among patients [36, 37]. They are also used to guide HF therapy [8, 38]. High sensitivity troponin release in the absence of coronary hypoperfusion is also a marker of ongoing myocyte injury and necrosis [39-43]. A scientific consensus is that natriuretic peptide and high sensitivity troponins add prognostic information for predicting new-onset HF [44]. Additional promising biomarkers include a family of inflammatory mediators and oxidative stress markers, which participate in the inflammation cascade that leads to tissue injury, remodeling and fibroblast proliferation. Soluble ST2 (sST2) [45, 46], galectin-3 (Gal-3) [47, 48] and growth differentiating factor 15 (GDF-15) [49] have been described as biomarkers to assess HF. The 2017 ACC/AHA Guideline states a IIB level of evidence for use of sST2 and Gal-3 as additive risk stratification in chronic as well as acute HF [50]. Current AHA guidelines suggest the use of these newer biomarkers may be useful for risk stratification [44].

However, none of these HF biomarkers have the capability in identifying the intrinsic health and recovery potential of cardiac muscle cells. Markers of inflammatory pathways are not HF specific and often associated with diseases with systemic inflammation. Natriuretic peptides reflect volume status and require adjustment based on age and sex [51], as well as obesity and renal dysfunction. For instance, it can be difficult to use natriuretic peptides to distinguish between those

with severe HFrEF versus other volume overload states such as in chronic kidney disease. In a recent trial, use of NT-proBNP failed as a guide to medical management of HFrEF patients [16]. From the patient's perspective, an inability to assess changes in muscle health may require additional cardiac testing and delays in appropriate treatment. In contrast, cBIN1 derived CS is cardiomyocyte specific and does not detect BIN1 from other organs such as skeletal muscle [20]. Because CS measures cardiac muscle cell health, CS is stable and independent of fluctuations induced by intracardiac volume, inflammatory state, or body habitus. Of note, cBIN1 is enclosed inside microparticles formed by t-tubule origin lipid vesicles [24] which are more likely to be cleared by the liver than kidneys and can explain in our cohort why CS is not affected by renal function (**Figure 4**).

Taken together, the newly developed CS is a promising HF biomarker with the capability to distinguish cardiac muscle health from systemic symptoms contributed by both failing heart muscle and other HF associated comorbidities. In the era of staggering health care costs, CS is a parameter, present in blood, that can help triage ambulatory with primary failing hearts from those with extracardiac disease. As seen in **Figure 5**, when CS was combined to NT-proBNP, the AUC of ROC improved, suggesting these tests evaluate different pathophysiological features (muscle health for cBIN1-based CS and intracardiac volume for NT-proBNP) and are complimentary. Furthermore, the wide range of CS values among controls speaks to the future usefulness of this test in diagnosing cardiac muscle disease prior to the onset of volume overload and symptoms.

Implication of CS for Population Health

Most often, clinical recognition of HF occurs at the time of advanced and symptomatic disease. The most effective treatment window is earlier in disease progression [52]. A muscle health

biomarker such as CS provides the opportunity for earlier primary care-oriented diagnosis of failing muscle. A muscle specific biomarker also informs basic clinical decision making. For instance, patients with diabetes and renal insufficiency may develop hypervolemia, detectable on examination and with a biomarker such as BNP [53, 54]. A low CS in this patient population would indicate commitment of scarce resources to treating the renal failure. However, a high CS in this cohort could suggest that the myocytes are pathologically remodeling and will require a cardiac-oriented regimen or a referral to a cardiac specialist. In this manner, CS could assist with management of complex, multi-organ disease syndromes. Conversely, patients with obesity may have false negative BNP, yet with failing heart muscle [19]. CS in this instance would help identify the failing heart muscle.

This report is the first introducing CS as a marker of myocardial health. While statistically compelling, the next step is to generalize our study to multi-center national cohorts. At the same time, we will explore the use of CS as a biomarker for management of early disease. Not unlike the use of liver function tests, thyroid function tests, eGFR for renal function, and hemoglobin A1C for glucose regulation, we expect CS to provide organ specific detail on the health of the heart.

ACKNOWLEDGMENTS

We thank Dr. Eduardo Marbán and Dr. Prediman Krishan Shah for review of this manuscript and helpful suggestions, and Sarcotein Diagnostics for providing the recombinant anti-BIN1 exon 13 antibody required for the cBIN1-ELISA assay. Funding for this research including NIH/NHLBI HL133286, HL094414, HL138577, and American Heart Association grants 16BGIA27770151, 16IRG27780031, and 13EIA4480016. The MIRIAD IBD Biobank is supported by the Widjaja Foundation Inflammatory Bowel and Immunobiology Research Institute, NIH/NIDDK grant P01DK046763, The European Union Grant 305479, NIH/NIDDK grant DK062413, and The Leona M. and Harry B. Helmsley Charitable Trust.

FIGURE LEGENDS

Figure 1. Distribution of CS and NT-proBNP among HFrEF and controls. The vertical axis is the cBIN1 based CS and NT-proBNP value, and the width of each violin graph depicts the density plot at each measured value.

Figure 2. Overlap between HFrEF and control populations. (A) is the histogram distribution of CS and (B) is the histogram distribution of Ln NT-proBNP among HFrEF (red) and matched controls (green), both of which have a normal distribution.

Figure 3. Relationship between BMI and biomarkers. (A) scatter plot of CS against BMI (Pearson's $r = -0.15$, $p = 0.12$) and scatter plot of Ln NT-proBNP against BMI (Pearson's $r = -0.34$, $p = 0.0002$) among controls. (B) Scatter plot of CS against BMI (Pearson's $r = 0.003$, $p = 0.97$) and scatter plot of Ln NT-proBNP against BMI (Pearson's $r = -0.24$, $p = 0.04$) among HFrEF.

Figure 4. Relationship between renal function and biomarkers. (A) scatter plot against CS and eGFR (Pearson's $r = 0.03$, $p = 0.71$) and (B) scatter plot of Ln NT-proBNP against eGFR (Pearson's $r = -0.37$, $p = 0.001$).

Figure 5. Receiver operating characteristic (ROC) curves. ROC curve containing the various sensitivities and specificities of the NT-proBNP (blue, AUC = 0.98), CS (red, AUC = 0.93), and combined NT-proBNP and CS (green, AUC = 0.99) tests to diagnose disease in our control and HFrEF cohorts. The line of identity, where $x = y$ for every point on the curve, is shown as a dotted solid line.

Figure 6. Kaplan-Meier of HFrEF patients who are free of cardiac hospitalization during 18-months follow-up. Kaplan-Meier survival curve is shown here for (A) all HFrEF, (B) Ischemic HFrEF, and (C) Non-ischemic HFrEF patient cohort. The red line demonstrates patients with $CS < 1.8$ and the blue line demonstrate patients with $CS \geq 1.8$. Event free survival is defined as patients who did not have a HF-related hospitalization event during 18-months follow-up. A low CS (< 1.8)

predicted a higher event-free survival among all HFrEF patients ($p = 0.04$) as well as non-ischemic HFrEF patients ($p = 0.01$). Among ischemic HFrEF patients, a low CS (< 1.8) still portends a lower event-free survival than the entire HFrEF cohort, however a high CS (≥ 1.8) did as well.

Supplemental Figure 1. Violin plot of NT-proBNP among HFrEF and controls. The vertical axis is the NT-proBNP value, and the width of each violin graph depicts the density plot at each measured value.

TABLES

Table 1. Baseline characteristics of HFrEF and matched controls

Table 2. CS distribution among subgroups

REFERENCES

1. Djousse, L., J.A. Driver, and J.M. Gaziano, *Relation between modifiable lifestyle factors and lifetime risk of heart failure*. JAMA, 2009. **302**(4): p. 394-400.
2. Owan, T.E. and M.M. Redfield, *Epidemiology of diastolic heart failure*. Prog Cardiovasc Dis, 2005. **47**(5): p. 320-32.
3. Roger, V.L., et al., *Trends in heart failure incidence and survival in a community-based population*. JAMA, 2004. **292**(3): p. 344-50.
4. Levy, D., et al., *Long-term trends in the incidence of and survival with heart failure*. N Engl J Med, 2002. **347**(18): p. 1397-402.
5. Krumholz, H.M., et al., *Patterns of hospital performance in acute myocardial infarction and heart failure 30-day mortality and readmission*. Circ Cardiovasc Qual Outcomes, 2009. **2**(5): p. 407-13.
6. Go, A.S., et al., *Heart disease and stroke statistics--2014 update: a report from the American Heart Association*. Circulation, 2014. **129**(3): p. e28-e292.
7. Bueno, H., et al., *Trends in length of stay and short-term outcomes among Medicare patients hospitalized for heart failure, 1993-2006*. JAMA, 2010. **303**(21): p. 2141-7.
8. Yancy, C.W., et al., *2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines*. J Am Coll Cardiol, 2013. **62**(16): p. e147-239.
9. Sun, R.R., et al., *Biomarkers and heart disease*. Eur Rev Med Pharmacol Sci, 2014. **18**(19): p. 2927-35.
10. Cowie, M.R. and G.F. Mendez, *BNP and congestive heart failure*. Prog Cardiovasc Dis, 2002. **44**(4): p. 293-321.
11. Calzetta, L., et al., *Brain natriuretic peptide: Much more than a biomarker*. Int J Cardiol, 2016. **221**: p. 1031-8.
12. Mueller, T., et al., *Long-term stability of endogenous B-type natriuretic peptide (BNP) and amino terminal proBNP (NT-proBNP) in frozen plasma samples*. Clin Chem Lab Med, 2004. **42**(8): p. 942-4.
13. Nowatzke, W.L. and T.G. Cole, *Stability of N-terminal pro-brain natriuretic peptide after storage frozen for one year and after multiple freeze-thaw cycles*. Clin Chem, 2003. **49**(9): p. 1560-2.
14. Burke, M.A. and W.G. Cotts, *Interpretation of B-type natriuretic peptide in cardiac disease and other comorbid conditions*. Heart Fail Rev, 2007. **12**(1): p. 23-36.
15. Januzzi, J.L., Jr., et al., *The N-terminal Pro-BNP investigation of dyspnea in the emergency department (PRIDE) study*. Am J Cardiol, 2005. **95**(8): p. 948-54.
16. Felker, G.M., et al., *Effect of Natriuretic Peptide-Guided Therapy on Hospitalization or Cardiovascular Mortality in High-Risk Patients With Heart Failure and Reduced Ejection Fraction: A Randomized Clinical Trial*. JAMA, 2017. **318**(8): p. 713-720.
17. Dhar, S., et al., *Natriuretic peptides and heart failure in the patient with chronic kidney disease: a review of current evidence*. Postgrad Med J, 2009. **85**(1004): p. 299-302.
18. Tang, W.H., et al., *National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: clinical utilization of cardiac biomarker testing in heart failure*. Clin Biochem, 2008. **41**(4-5): p. 210-21.
19. van Kimmenade, R., et al., *Is brain natriuretic peptide production decreased in obese subjects?* J Am Coll Cardiol, 2006. **47**(4): p. 886-7.
20. Hong, T., et al., *Cardiac BIN1 folds T-tubule membrane, controlling ion flux and limiting arrhythmia*. Nat Med, 2014. **20**(6): p. 624-32.
21. Hong, T.T., et al., *BIN1 localizes the L-type calcium channel to cardiac T-tubules*. PLoS Biol, 2010. **8**(2): p. e1000312.
22. Hong, T.T., et al., *BIN1 is reduced and Cav1.2 trafficking is impaired in human failing cardiomyocytes*. Heart Rhythm, 2012. **9**(5): p. 812-20.

23. Hong, T.T., et al., *Plasma BIN1 correlates with heart failure and predicts arrhythmia in patients with arrhythmogenic right ventricular cardiomyopathy*. Heart Rhythm, 2012. **9**(6): p. 961-7.
24. Xu, B., et al., *The ESCRT-III pathway facilitates cardiomyocyte release of cBIN1-containing microparticles*. PLoS Biol, 2017. **15**(8): p. e2002354.
25. Levey, A.S., et al., *A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group*. Ann Intern Med, 1999. **130**(6): p. 461-70.
26. DeFilippi, C., R.R. van Kimmenade, and Y.M. Pinto, *Amino-terminal pro-B-type natriuretic peptide testing in renal disease*. Am J Cardiol, 2008. **101**(3A): p. 82-8.
27. Januzzi, J.L., et al., *NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the International Collaborative of NT-proBNP Study*. Eur Heart J, 2006. **27**(3): p. 330-7.
28. van Kimmenade, R.R., et al., *The value of (NT-pro) BNP in the diagnosis, prognosis and treatment of congestive heart failure*. Neth Heart J, 2004. **12**(2): p. 61-63.
29. Fu, Y., et al., *Isoproterenol Promotes Rapid Ryanodine Receptor Movement to Bridging Integrator 1 (BIN1)-Organized Dyads*. Circulation, 2016. **133**(4): p. 388-97.
30. Fu, Y. and T. Hong, *BIN1 regulates dynamic t-tubule membrane*. Biochim Biophys Acta, 2016. **1863**(7 Pt B): p. 1839-47.
31. Hong, T. and R.M. Shaw, *Cardiac T-Tubule Microanatomy and Function*. Physiol Rev, 2017. **97**(1): p. 227-252.
32. Kostis, J.B., et al., *Prevention of heart failure by antihypertensive drug treatment in older persons with isolated systolic hypertension. SHEP Cooperative Research Group*. JAMA, 1997. **278**(3): p. 212-6.
33. Levy, D., et al., *The progression from hypertension to congestive heart failure*. JAMA, 1996. **275**(20): p. 1557-62.
34. Sciarretta, S., et al., *Antihypertensive treatment and development of heart failure in hypertension: a Bayesian network meta-analysis of studies in patients with hypertension and high cardiovascular risk*. Arch Intern Med, 2011. **171**(5): p. 384-94.
35. Iwanaga, Y., et al., *B-type natriuretic peptide strongly reflects diastolic wall stress in patients with chronic heart failure: comparison between systolic and diastolic heart failure*. J Am Coll Cardiol, 2006. **47**(4): p. 742-8.
36. McKelvie, R.S., et al., *Baseline plasma NT-proBNP and clinical characteristics: results from the irbesartan in heart failure with preserved ejection fraction trial*. J Card Fail, 2010. **16**(2): p. 128-34.
37. Jhund, P.S., et al., *Changes in N-terminal pro-B-type natriuretic peptide levels and outcomes in heart failure with preserved ejection fraction: an analysis of the I-Preserve study*. Eur J Heart Fail, 2015. **17**(8): p. 809-17.
38. Maisel, A., et al., *Primary results of the HABIT Trial (heart failure assessment with BNP in the home)*. J Am Coll Cardiol, 2013. **61**(16): p. 1726-35.
39. Hudson, M.P., et al., *Implications of elevated cardiac troponin T in ambulatory patients with heart failure: a prospective analysis*. Am Heart J, 2004. **147**(3): p. 546-52.
40. Horwich, T.B., et al., *Cardiac troponin I is associated with impaired hemodynamics, progressive left ventricular dysfunction, and increased mortality rates in advanced heart failure*. Circulation, 2003. **108**(7): p. 833-8.
41. Sato, Y., et al., *Persistently increased serum concentrations of cardiac troponin t in patients with idiopathic dilated cardiomyopathy are predictive of adverse outcomes*. Circulation, 2001. **103**(3): p. 369-74.
42. Missov, E., C. Calzolari, and B. Pau, *Circulating cardiac troponin I in severe congestive heart failure*. Circulation, 1997. **96**(9): p. 2953-8.

43. Setsuta, K., et al., *Clinical significance of elevated levels of cardiac troponin T in patients with chronic heart failure*. Am J Cardiol, 1999. **84**(5): p. 608-11, A9.
44. Chow, S.L., et al., *Role of Biomarkers for the Prevention, Assessment, and Management of Heart Failure: A Scientific Statement From the American Heart Association*. Circulation, 2017. **135**(22): p. e1054-e1091.
45. Ky, B., et al., *High-sensitivity ST2 for prediction of adverse outcomes in chronic heart failure*. Circ Heart Fail, 2011. **4**(2): p. 180-7.
46. Anand, I.S., et al., *Changes in brain natriuretic peptide and norepinephrine over time and mortality and morbidity in the Valsartan Heart Failure Trial (Val-HeFT)*. Circulation, 2003. **107**(9): p. 1278-83.
47. Lok, D.J., et al., *Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: data from the DEAL-HF study*. Clin Res Cardiol, 2010. **99**(5): p. 323-8.
48. Anand, I.S., et al., *Baseline and serial measurements of galectin-3 in patients with heart failure: relationship to prognosis and effect of treatment with valsartan in the Val-HeFT*. Eur J Heart Fail, 2013. **15**(5): p. 511-8.
49. Kalogeropoulos, A., et al., *Inflammatory markers and incident heart failure risk in older adults: the Health ABC (Health, Aging, and Body Composition) study*. J Am Coll Cardiol, 2010. **55**(19): p. 2129-37.
50. Yancy, C.W., et al., *2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America*. J Card Fail, 2017.
51. Galasko, G.I., et al., *What is the normal range for N-terminal pro-brain natriuretic peptide? How well does this normal range screen for cardiovascular disease?* Eur Heart J, 2005. **26**(21): p. 2269-76.
52. Udelson, J.E. and L.W. Stevenson, *The Future of Heart Failure Diagnosis, Therapy, and Management*. Circulation, 2016. **133**(25): p. 2671-86.
53. Spanaus, K.S., et al., *B-type natriuretic peptide concentrations predict the progression of nondiabetic chronic kidney disease: the Mild-to-Moderate Kidney Disease Study*. Clin Chem, 2007. **53**(7): p. 1264-72.
54. Seki, S., et al., *Fetal and postnatal development of Ca²⁺ transients and Ca²⁺ sparks in rat cardiomyocytes*. Cardiovasc Res, 2003. **58**(3): p. 535-48.

Table 1. Baseline Characteristics of HFrEF and Matched Controls

Characterisitcs	HFrEF (n=125)	Controls (n=125)	p-value
Age	56 ± 10.3	54 ± 6.0	NS
Male (%)	99 (79)	95 (76)	NS
White (%)	71 (57)	74 (59)	NS
BMI (kg/m ²)	30.4 ± 5.9	29.0 ± 5.7	NS
Hypertension (%)	56 (45)	13 (10)	<0.0001
Diabetes (%)	47 (38)	9 (7)	<0.0001
CKD (%)	28 (22)		
IBD (%)	3 (2)		
LVEF (%)	25 ± 8.4		
Medications			
Beta-Blockers (%)	115 (92)		
ACE-I / ARB (%)	97 (78)		
Diuretics (%)	105 (84)		
NYHA			
I (%)	21 (17)		
II (%)	50 (40)		
III (%)	51 (41)		
IV (%)	3 (2)		
Subtype of Heart Failure			
Ischemic (%)	43 (34)		
Non-Ischemic (%)	80 (64)		
Valvular (%)	9 (11)		
Dilated (%)	8 (10)		
Toxin (%)	8 (10)		
Infiltrative (%)	2 (3)		
Other (%)	53 (66)		

HFrEF = heart failure with reduced ejection fraction; BMI = body mass index; CKD = chronic kidney disease; IBD = inflammatory bowel disease; LVEF = left ventricular ejection fraction; ACE-I = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; NYHA FC = New York Heart Association Functional Class.

Table 2. CS and NT-proBNP among subgroups

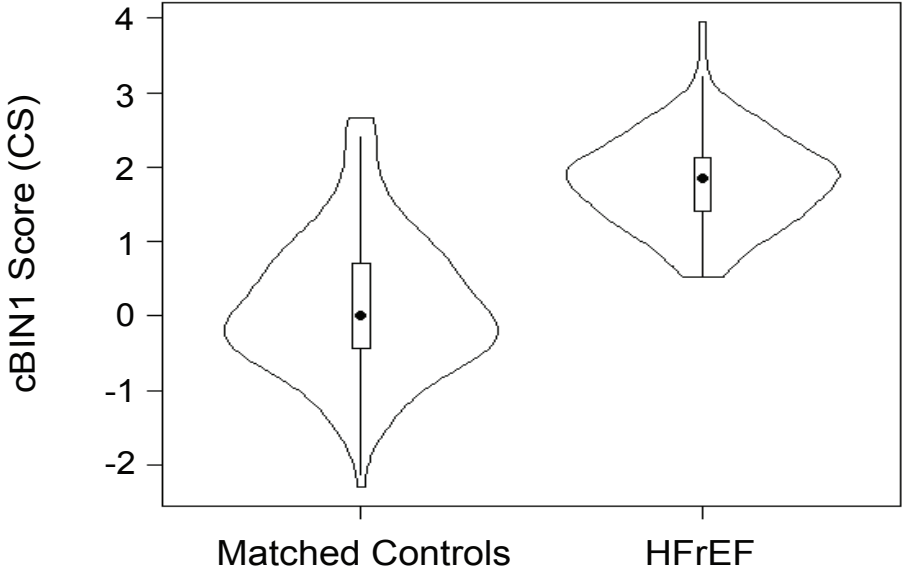
HFrEF Patients						
Characteristics	CS median n = 125	IQR (25% - 75%)	p-value	NT-proBNP median (pg/ml) n = 77*	IQR (25% - 75%)	p-value
All HFrEF patients	1.8	0.5 - 2.1		1153	380 - 3529	
Race/Ethnicity						
White	1.7	1.3 - 2.0	0.02	924	322 - 2770	NS
Black	2	1.7 - 2.4		2559	187 - 5763	
Hispanic	2	1.4 - 2.4		1913	503 - 6001	
Asian	1.5	1.3 - 1.9		1633	1081 - 7059	
Age (years)			NS			NS
< 55	1.7	1.4 - 2.0		1114	271 - 3323	
≥ 55	1.9	1.3 - 2.3		1375	409 - 4321	
Sex			NS			NS
Male	1.8	1.4 - 2.1		1531	409 - 3907	
Female	1.7	1.2 - 2.4		591	218 - 2055	
BMI (kg/m²)			NS			NS
Normal (< 25)	1.8	1.4 - 2.3		1794	1146 - 3907	
Overweight (25 - 29.9)	1.7	1.4 - 2.0		1796	430 - 5196	
Obese (30 - 34.9)	2	1.7 - 2.4		1531	525 - 3529	
Morbidly Obese (≥ 35)	1.7	1.1 - 2.1		381	257 - 1153	
Etiology			NS			NS
Ischemic HFrEF	1.9	1.4 - 2.3		1045	322 - 3424	
Non-ischemic HFrEF	1.7	1.3 - 2.1		1276	426 - 3925	
eGFR (ml/min/m²)			NS			0.01
< 60	1.8	1.3 - 2.4		815	349 - 2055	
> 60	1.9	1.4 - 2.1		3371	1002 - 6353	

Matched Controls						
Characteristics	CS median n = 125	IQR (25% - 75%)	p-value	NT-proBNP median (pg/ml) n = 122*	IQR (25% - 75%)	p-value
All Controls	0	-0.5 - 0.7	----	28	15 - 58	----
Race/Ethnicity			NS			0.02
White	0.1	-0.6 - 0.7		31	19 - 67	
Black	-0.1	-0.4 - 0.7		27	13 - 58	
Hispanic	-0.1	-0.4 - 0.3		17	12 - 41	
Asian	----	----		----	----	
Age (years)			NS			NS
< 55	-0.1	-0.5 - 0.7		29	15 - 60	
≥ 55	0	-0.5 - 0.7		27	16 - 58	
Sex			NS			NS
Male	0	-0.5 - 0.8		27	14 - 58	
Female	0	-0.4 - 0.4		29	19 - 68	
BMI (kg/m²)			NS			0.001
Normal (< 25)	0	-0.5 - 0.7		58	22 - 91	
Overweight (25 - 29.9)	0	-0.4 - 0.7		28	15 - 47	
Obese (30-34.9)	-0.2	-0.8 - 0.4		26	12 - 58	
Morbid Obesity (≥ 35)	-0.2	-0.5 - 0.0		15	13 - 25	

* We tested NT-proBNP on 77 out of the 125 HFrEF patients, and on 122 out of the 125 healthy volunteers. CS = cBIN1 score HFrEF = heart failure with reduced ejection fraction; HFI = Heart Failure Index; NT-proBNP = N-terminal pro-B-type natriuretic peptide; BMI = body mass index.

Figure 1

A



B

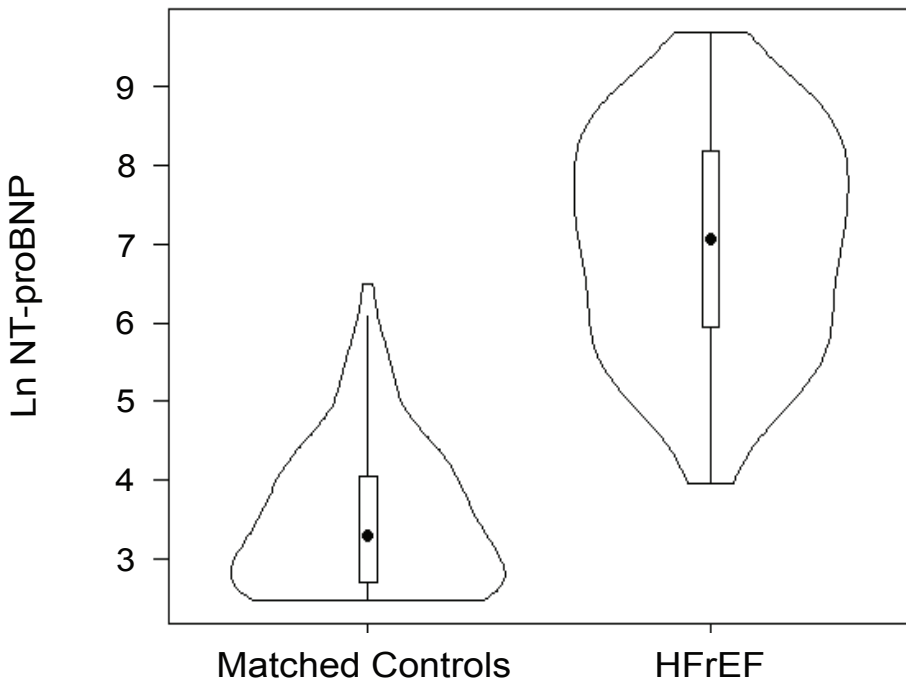
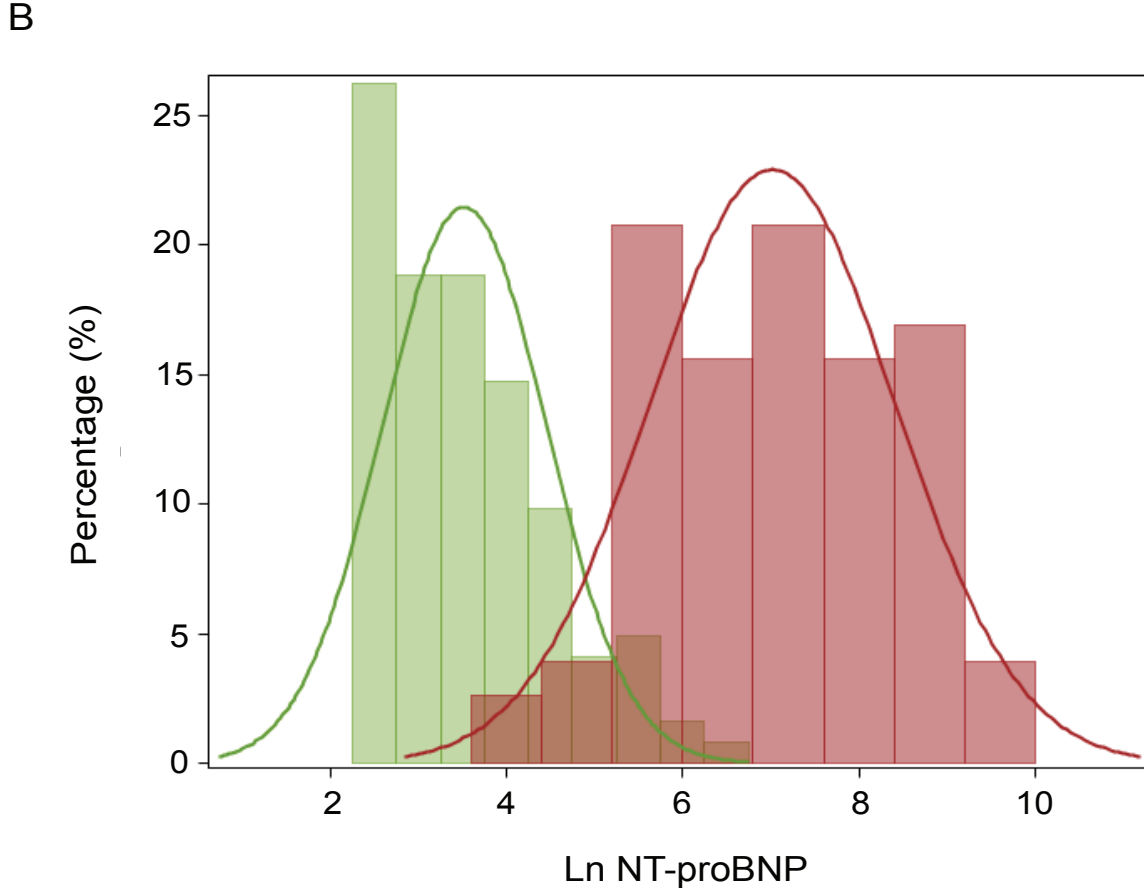
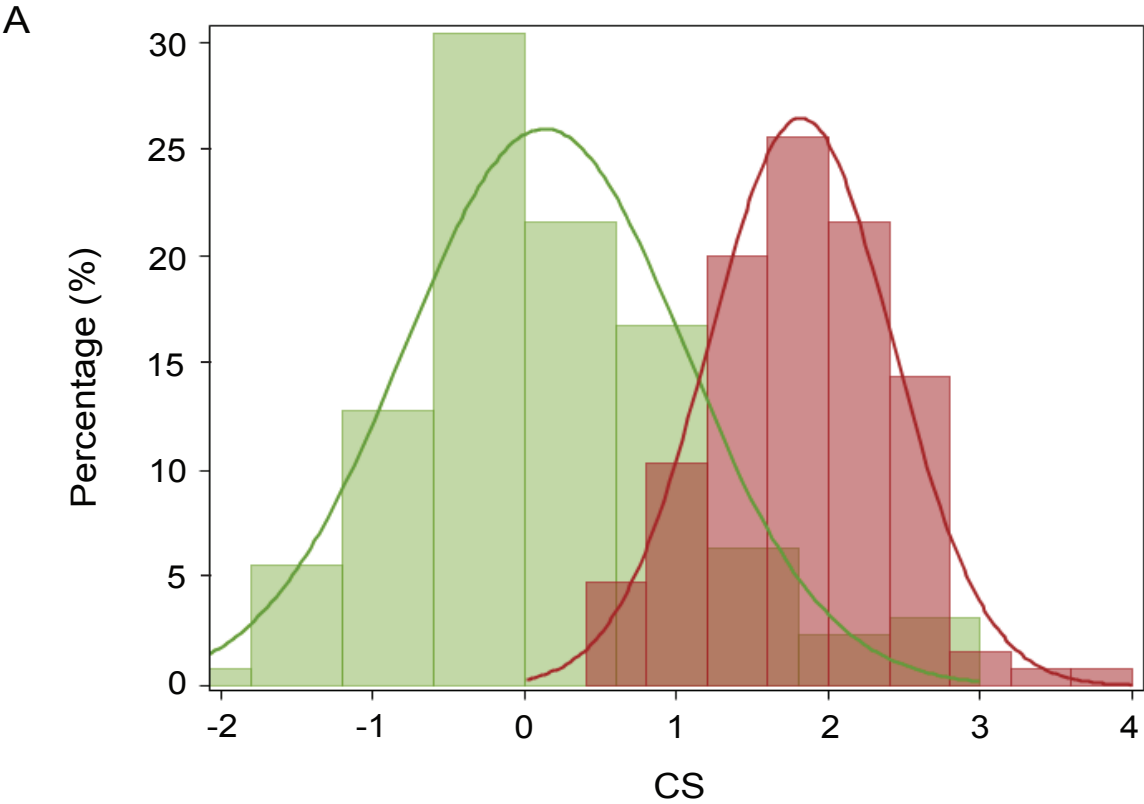


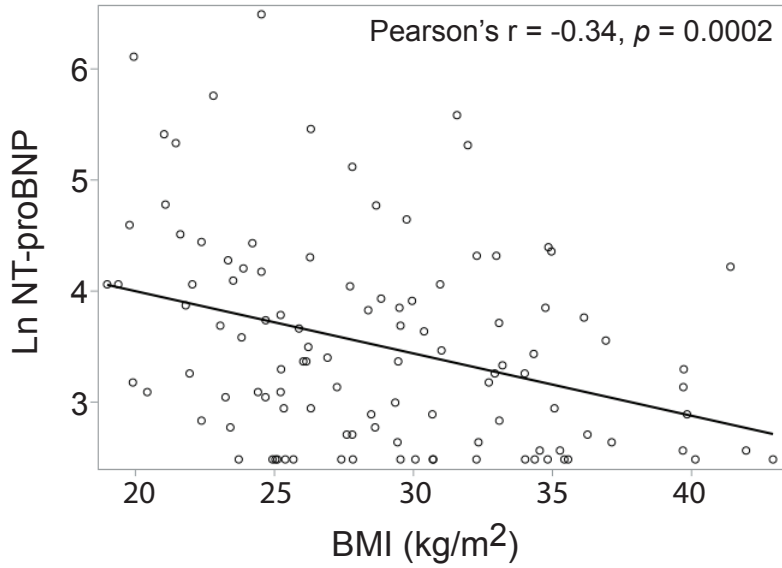
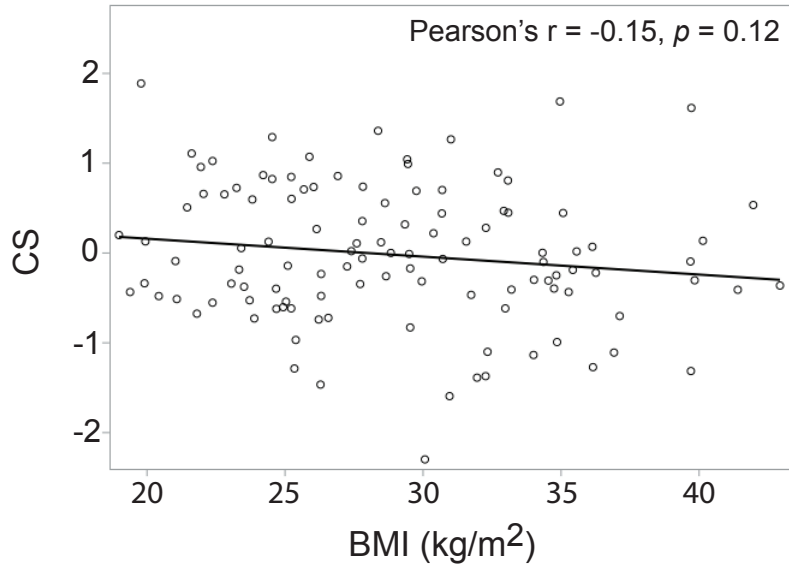
Figure 2



- HF rEF
- Matched Controls

Figure 3

A. Matched Controls



B. HFrEF

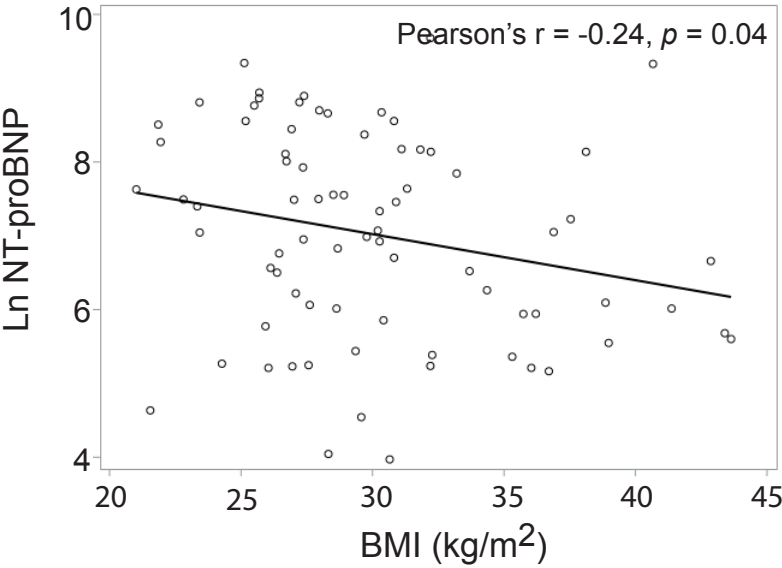
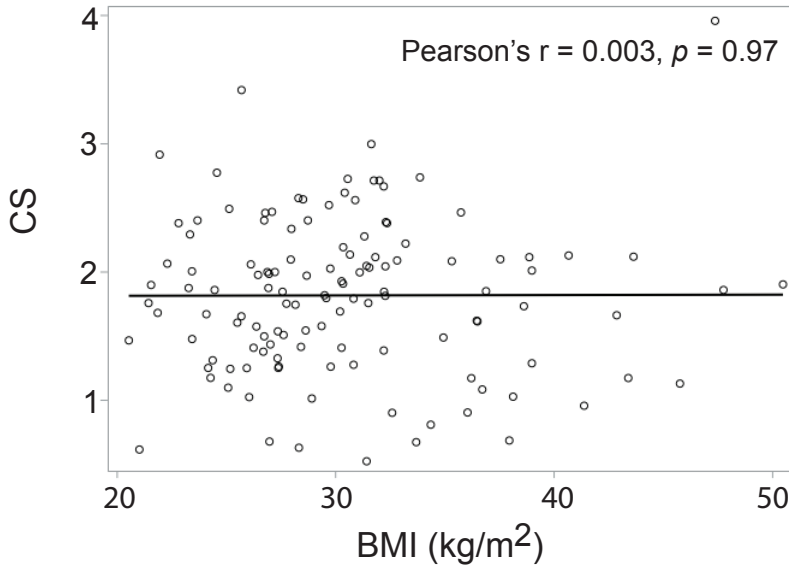
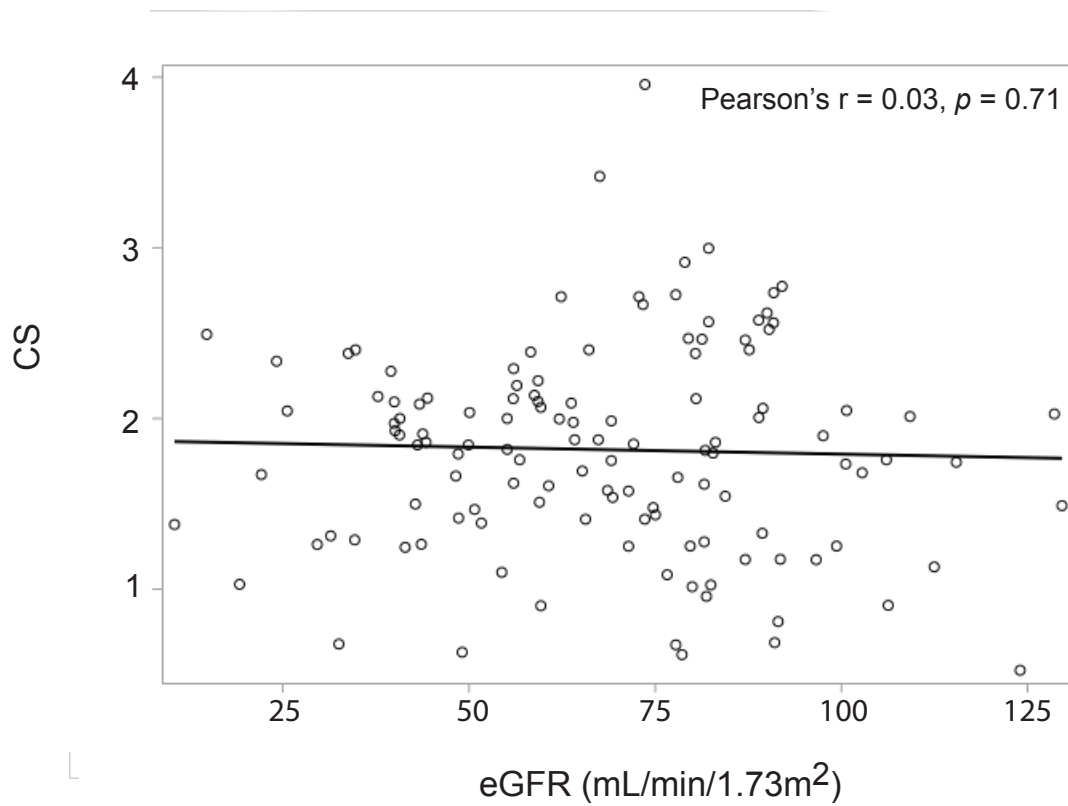


Figure 4

A



B

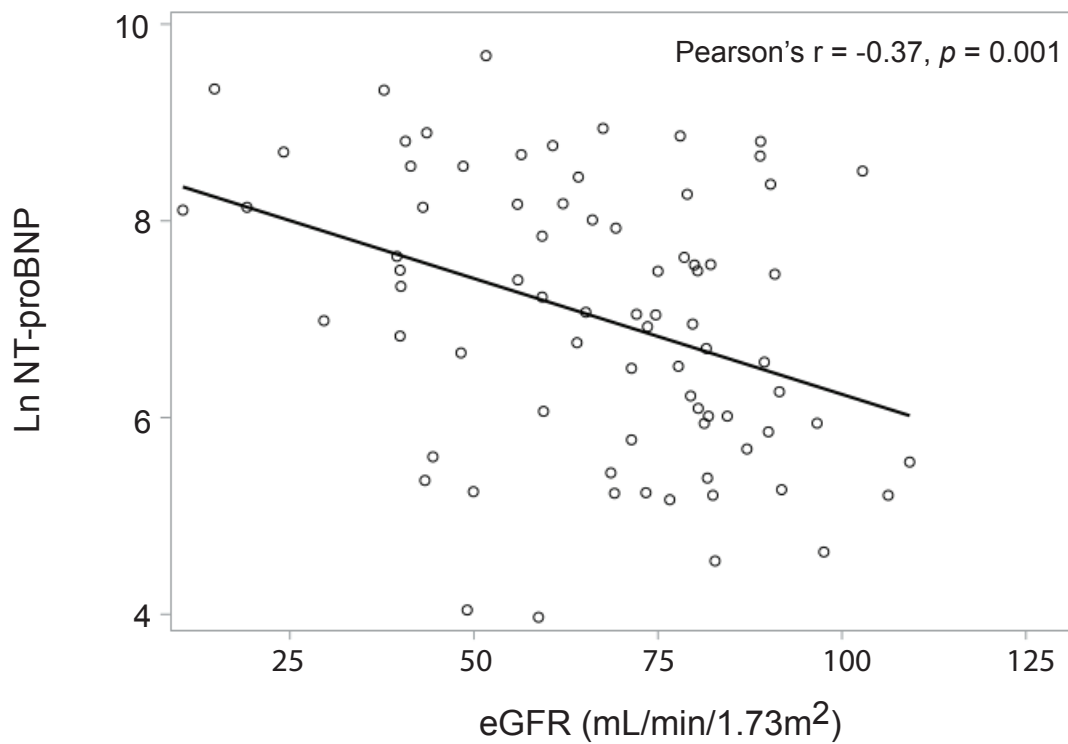


Figure 5

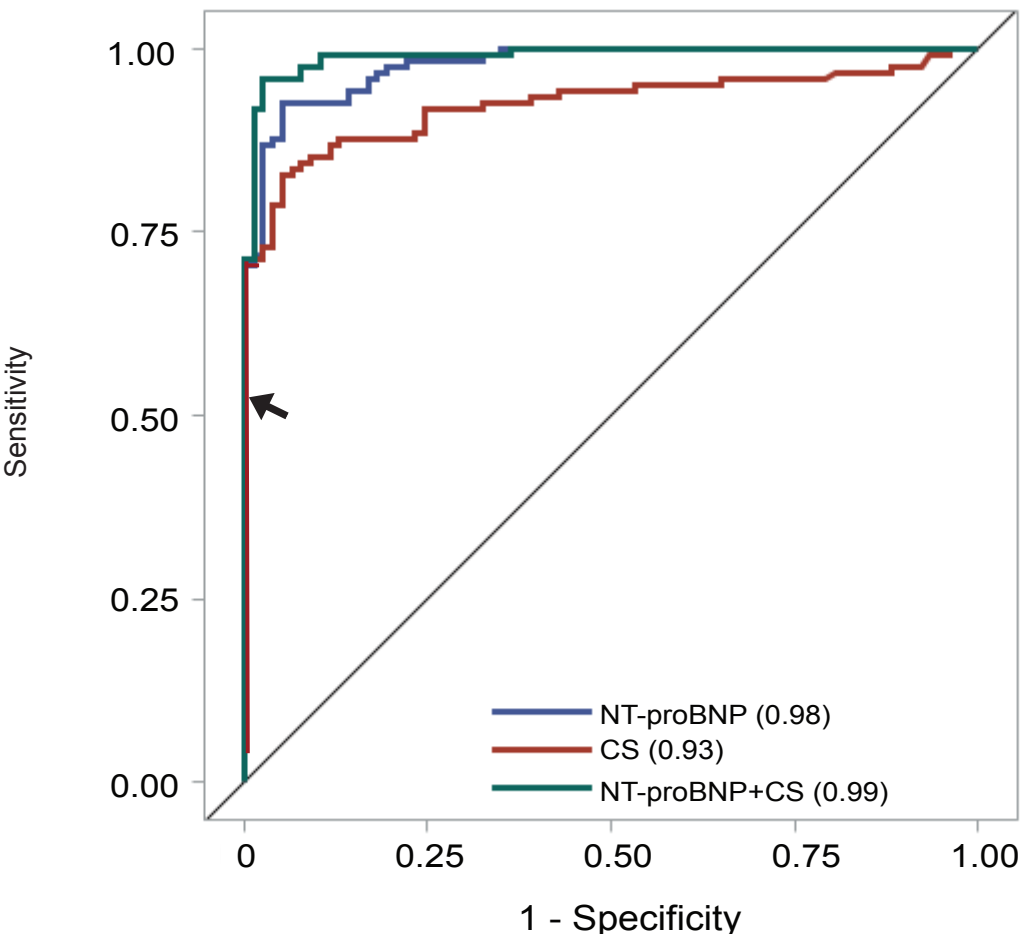
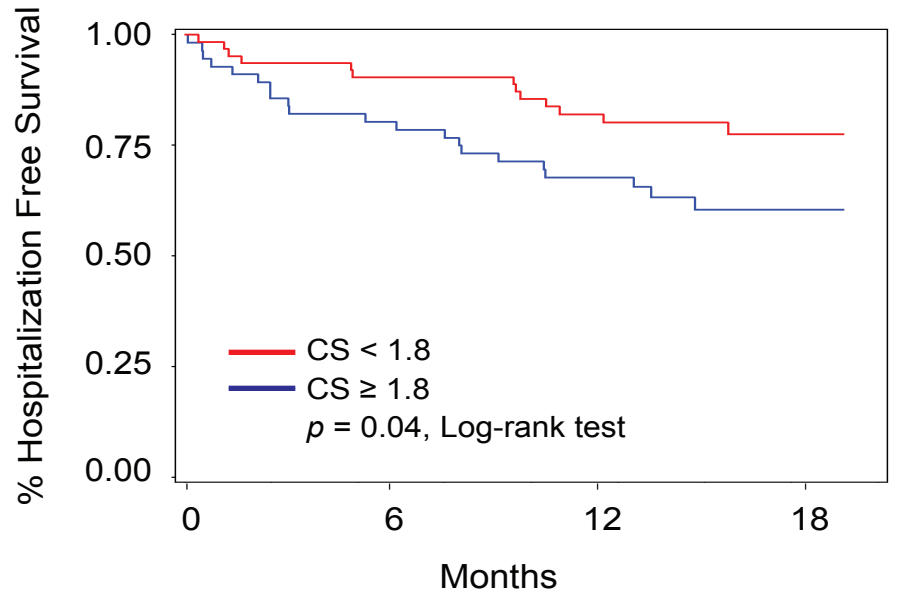
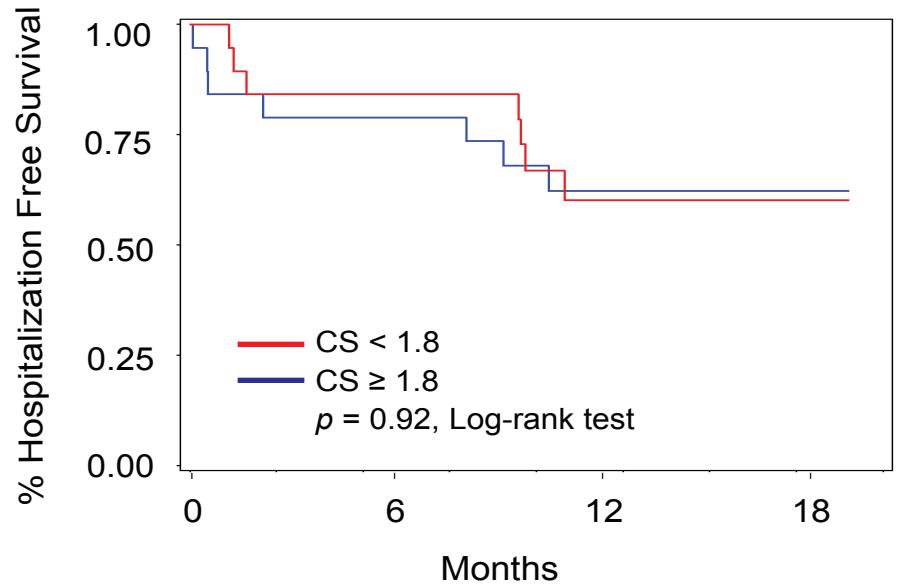


Figure 6

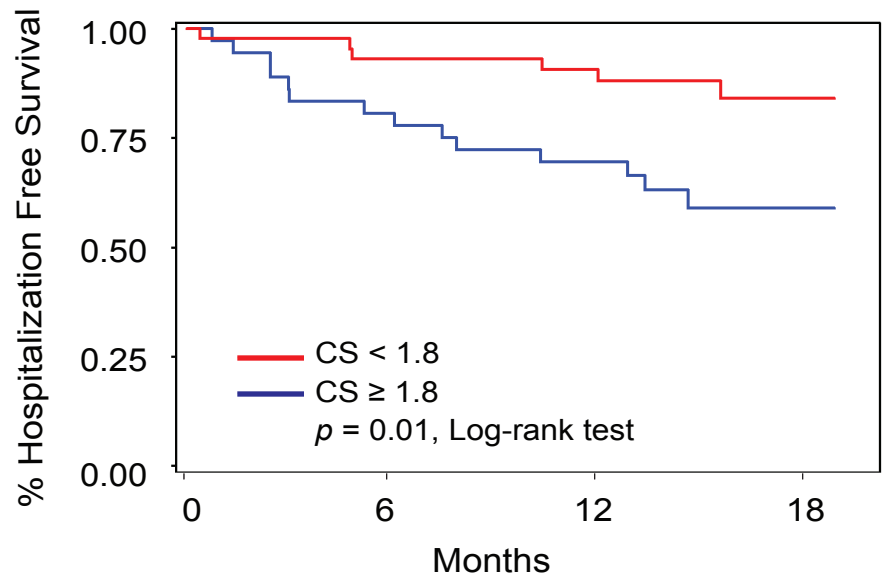
A. HFrEF



C. Ischemic HFrEF



B. Non-ischemic HFrEF



Supplemental Figure 1

