Supplementary Online Content

Nikolova AP, Hitzeman TC, Baum R, et al. Association of a novel diagnostic biomarker, the plasma cardiac bridging integrator 1 score, with heart failure with preserved ejection fraction and cardiovascular hospitalizations. *JAMA Cardiol*. Published online October 31, 2018. doi:10.1001/jamacardio.2018.3539

This supplementary material has been provided by the authors to give readers additional information about their work.

I. Supplemental Methods

Definition of Outcomes Studied

Cardiovascular-related hospitalization was defined as any hospitalization due to heart failure, myocardial infarction, revascularization for stable or unstable coronary or peripheral vascular symptoms, stroke or transient ischemic attack (TIA), unstable arrhythmias, ICD firing, cardiovascular death, heart transplantation or implantation of a ventricular assist device (VAD).

Sample Processing for cBIN1 Concentration measurement

All plasma samples for the HF patients were collected at the time of clinic visit(s) and subsequently processed and stored in the Cedars-Sinai Medical Center Heart Institute Biobank per protocol. Whole venous blood was collected into lavender top (EDTA) tubes and stored immediately at 4ºC for less than four hours. Plasma was separated from cellular fraction by spinning tubes in a refrigerated centrifuge at 2,250g for 20 minutes. Then, over ice, 0.5 ml volume of plasma was aliquoted into individual one milliliter de-identified, bar coded cryovials, flash frozen with dry ice and ethanol, and stored in a -80ºC freezer. Clinical data were obtained from chart review and stored in a de-identified Microsoft Access database, indexed by aliquot bar code.

The concentration of cBIN1 was determined using an assay provided by Sarcotein Diagnostics. In brief, a cBIN1 specific sandwich ELISA test was used, which employs a mouse monoclonal anti-BIN1 exon 17 as capture antibody (Sigma-Aldrich) and a HRP-conjugated detection recombinant antibody specific for exon 13 (Sarcotein Diagnostics). 96-well plates were coated with capture antibody, followed by loading with patient plasma samples. 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[1]. Bound cBIN1 was detected using the HRP-conjugated anti-BIN1 antibody, with concentration determined from known protein standards. The ELISA reagents were purchased from BD Biosciences (BD OptEIA reagents kit, catalog 550534). Using positive control plasma samples with known cBIN1 concentrations, we validated that this assay is highly precise and reproducible with an inter-plate variability of < 5%.

CS Determination

CS is the natural log of the ratio of the predetermined median cBIN1 of a separate large healthy calibration cohort (10ng/ml) to that of measured cBIN1:

$$CS = \ln(\frac{10}{[cBIN1]})$$

The numerator of 10ng/ml, was the same for all samples run in this study. We express our results as a reciprocal to be consistent with clinical convention of an elevated biomarker to correlate with worsening disease status. Natural log of the ratio is used for CS because the distribution of the ratio, like most biological parameters, is log normal.

NT-proBNP assay

NT-proBNP values were obtained from the plasma of two control and one HFpEF cohorts of 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. Similarly to CS, a natural log transformation of NT-proBNP was performed and used for analyses.

Statistical Methods

Kaplan-Meier analysis was used to compare the differences in cardiovascular hospitalization rates between patients with high and low levels of CS using the log-rank test. Cox proportional hazards regression models were used to estimate the association between high and low levels of CS and the risk of cardiovascular hospitalization, adjusted for age, sex, BMI, and NT-proBNP. Non-linear relationships between CS and cardiovascular hospitalization were examined with restricted cubic splines using 5 knots located at 0, 0.25, 0.50, 0.75 and 1.00 percentiles. P-value for significance of the fit was derived from the Cox proportional hazard model. 95% confidence intervals were reported.

II. Supplemental Table 1

eTable 1. Detailed characteristics of the HFpEF cohort.

Category	N (%)	Mean±SD
cBIN1 score (CS)	52 (100)	1.8 (1.5–2.3)[a]
NT-proBNP (pg/mL)	50 (96)	277 (99–1264)[a]
Smoking History	44 (85)	
Yes	9 (17)	
No	35 (67)	
NYHA Class	51 (98)	
I	13 (25)	
II	19 (37)	
III	17 (33)	
IV	2 (4)	
Cardiomyopathy Type	52 (100)	
Infiltrative (sarcoid, amyloid)	5 (10)	
HOCM	5 (10)	
Familial restrictive	3 (6)	
Valvular	6 (12)	
Other	33 (63)	
History of PAD/MI/PCI	13 (29)	
History of arrhythmias	29 (60)	
Presence of AICD	15 (31)	
Echocardiography parameters		
LVEF (%)	52 (100)	58±7
LVIDd (mm)	48 (92)	48±8.5
LV Mass (g)	48 (92)	220±92
Diastolic Class		
Normal	15 (38)	
Class I	11 (28)	
Class II	5 (13)	
Class III	8 (21)	
PASP (mmHg)		
< 30	20 (38)	
≥ 30	18 (35)	
Laboratory Results		
Serum K (mEg/L)	47 (90)	4.4±0.4
eGFR (Cockcroft-Gault equation, mL/min)	52 (100)	90±35
LDL (mg/dL)	36 (69)	100±38
Hemoglobin A1c (%)	30 (58)	6±0.9
Hemoglobin (g/dL)	41 (79)	13.4±1.9
Hemodynamic parameters		
Systolic BP (mmHg)	46 (69)	117±16
Diastolic BP (mmHg)	46 (69)	69±10
Heart rate (bpm)	45 (68)	68±11
Medication Therapy		
ACEI/ARB	43 (89)	
Diuretics	33 (70)	
Aldosterone antagonists	33 (69)	
Nitrates	20 (45)	
Statin	10 (23)	
Anticoagulants	17 (36)	
Antiplatelet agents	34 (72)	

a. Median and interquartile range.

AICD – automated implantable cardioverter defibrillator, BMI – body mass index, HOCM – hypertrophic cardiomyopathy, PAD – peripheral arterial disease, MI – myocardial infarction, PCI – percutaneous coronary intervention, LVIDd – LV internal diameter in diastole, PASP – pulmonary arterial systolic pressure, K – potassium, eGFR – estimated glomerular filtration rate, LDL – low density lipoprotein, BP – blood pressure, ACEI – angiotensin converting enzyme inhibitor, ARB – angiotensin receptor blocker, STD – standard deviation

III. Supplemental Table 2

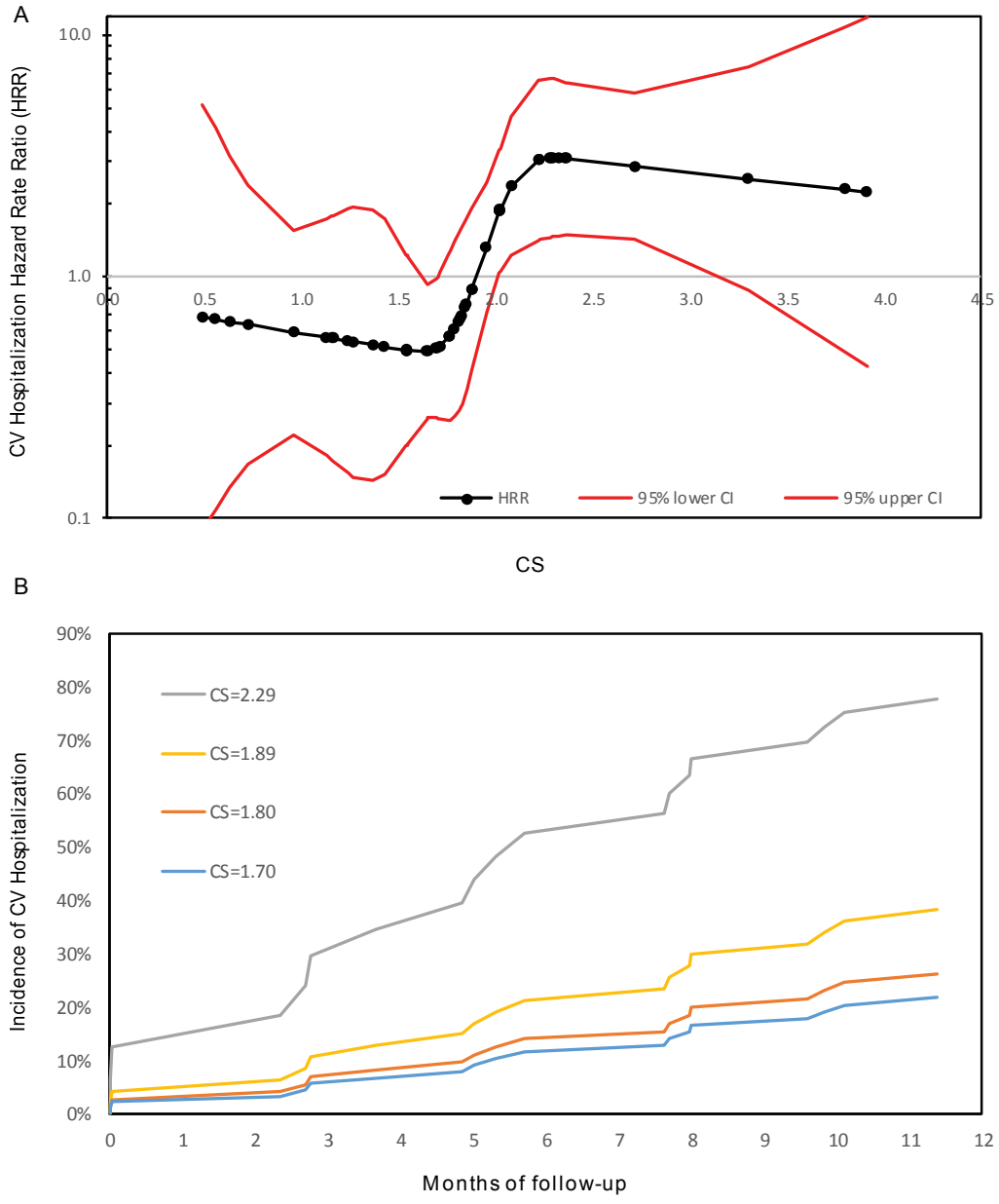
eTable2. Median cBIN1 concentration in the three studied cohorts.

Cohort	N	Median cBIN1 concentration, ng/ml (IQR)	*p*-value (compared to HFpEF
HFpEF	52	1.62 (1.05, 2.27)	
Healthy Controls	52	10.31 (5.92, 16.17)	<0.0001[a]
Comorbidity Controls	52	10.82 (6.59, 21.08)	<0.0001[a]

a. Wilcoxon rank sum test

IV. Supplemental Figure 1

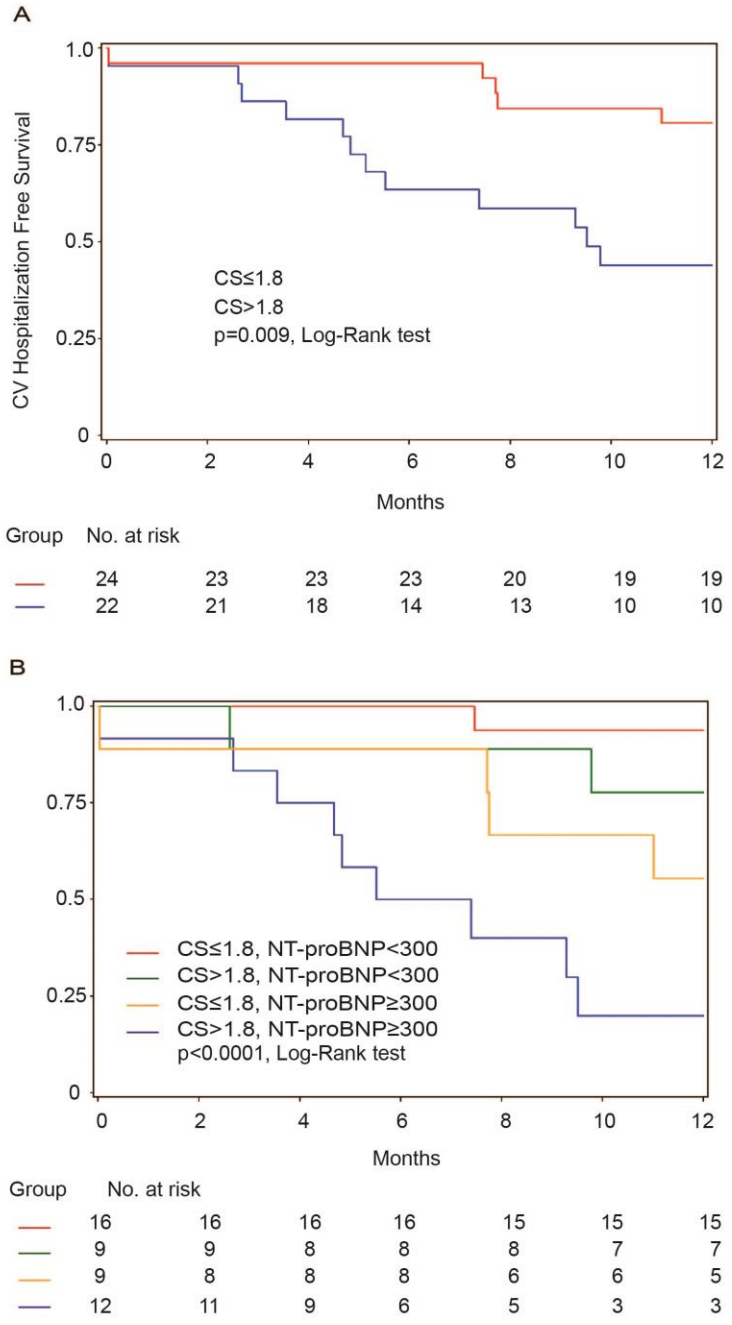
eFigure 1. Cubic spline fit and CV hospitalization incidence according to CS level



eFigure 1. Cubic spline fit and CV hospitalization incidence according to CS level. Exhibit A demonstrates a plot of restricted cubic spline fit of hazard rate ratio (HRR) for CV hospitalizations vs CS with 95% confidence intervals (CI). The HRR is defined as the CV hospitalization event rate in relation to the overall hazard rate in the cohort of 47 subjects, which was computed to be 3.98 events per 100 person-months follow-up. As illustrated, the HRR is roughly constant until approximately CS of 1.70 when it starts to increase until CS value of 2.29 when it levels off. Using the Cox proportional hazard model, the association was found to be statistically significant with a p=0404. Exhibit B demonstrates CV hospitalization incidence over one year of follow-up for four chosen CS values. This plot demonstrates the change in CV hospitalization incidence from relatively "low" CS to "high" CS chosen from Exhibit A. There is little change in the CV hospitalization cumulative incidence for CS below 1.70 or CS above 2.29.

V. Supplemental Figure 2

eFigure 2. Kaplan-Meier curves for CV hospitalization free survival



eFigure 2. Kaplan-Meier curves for cardiovascular (CV) hospitalization free survival. Panel A displays CV hospitalization free survival for the HFpEF patients using a CS cut-off value of 1.8, the median CS value in this cohort (hazard ratio (HR) 3.8, 95% CI 1.3 – 11.2, p = 0.015 for CS ≥ 1.8 versus CS < 1.8). Panel B exhibits the Kaplan-Meier curves for the HFpEF patients using a combination of the CS and NT-proBNP assays with cut-off values of 1.8 and 300 pg/mL respectively. This panel suggests that prognostication of CV hospitalizations within 1 year of follow-up in the studied cohort can be strengthened by combining the two diagnostic tests (CS ≥ 1.8 and NT-proBNP ≥ 300 pg/ml was associated with a HR of 21.4 compared to CS < 1.8 and NT-proBNP < 300 pg/ml, 95% CI 2.7 – 171.6, p = 0.004).

VI. References

1. Xu B, Fu Y, Liu Y, Agvanian S, Wirka R, Baum R, et al. The ESCRT-III pathway facilitates cardiomyocyte release of cBIN1 containing microparticles. *PLoS biology*. 2017;15:e2002354.