Relation of Biomarkers of Cardiac Injury, Stress, and Fibrosis With Cardiac Mechanics in Patients ≥ 65 Years of Age

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High sensitivity cardiac troponin T (hscTnT), soluble ST2 (sST2), N-terminal B-type natriuretic peptide (NT-proBNP), and galectin-3 are biomarkers of cardiac injury, stress, myocardial stretch, and fibrosis. Elevated levels are associated with poor outcomes. However, their association with cardiac mechanics in older persons is unknown. Associations between these biomarkers and cardiac mechanics derived from speckle tracking echocardiography, including left ventricular longitudinal strain (LVLS), early diastolic strain, and left atrial reservoir strain (LARS) were evaluated using standardized beta coefficients () in a cross sectional analysis with cardiac biomarkers in older patients without cardiovascular disease, low ejection fraction, or wall motion abnormalities. Biomarker associations with strain were attenuated by demographics and risk factors. In adjusted models, LVLS was associated with continuous measures of hscTnT ($\beta^{\wedge}$−0.06, p = 0.020), sST2 ($\beta^{\wedge}$ −0.05, p = 0.024) and NT-proBNP ($\beta^{\wedge}$ −0.06, p = 0.007). "High" levels (i.e., greater than prognostic cutpoint) of hscTnT (>13 ng/ml), sST2 (>35 ng/ml), and NT-proBNP (>190 pg/ml) were also associated with worse LVLS. In risk factor adjusted models, LARS was associated with hscTnT ($\beta^{\wedge}$ −0.08, p = 0.003) and NT-proBNP ($\beta^{\wedge}$−0.18, p <0.0001). High hscTnT (>13 ng/ml) and high NT-proBNP (>190 pg/ml) were also both associated with worse LARS. Gal-3 was not associated with any strain measure. In conclusion, in persons ≥ 65 years of age, without cardiovascular disease, low ejection fraction, or wall motion abnormalities, hscTnT, sST2, and NT-proBNP are associated with worse LVLS. HscTnT and NT-proBNP are associated with worse LARS. In conclusion, these subclinical increases in blood biomarkers, and their associations with subtle diastolic and systolic dysfunction, may represent pre-clinical heart failure. **(Am J Cardiol 2020;136:156−163)**

High sensitivity cardiac troponin T (hscTnT), N-terminal B-type natriuretic peptide (NT-proBNP), soluble ST2 (sST2), and galectin-3 (gal-3) are soluble blood biomarkers of cardiac injury, myocardial stretch, strain, and fibrosis which are associated with poor outcomes[1−8] including cardiovascular death, sudden death, myocardial infarction, and incident heart failure in ambulatory older adults. It is unknown to what extent these biomarkers are linked to changes in systolic and diastolic function in older adults free of overt cardiac disease, or asymptomatic decrease in LV ejection fraction. Therefore, we evaluated the cross-sectional association of hscTnT, sST2, NT-proBNP, and gal-3 with sensitive[9,10] measures of systolic and diastolic LV and LA function measured by speckle tracking echocardiography, using digitized archived echocardiogram tapes of community-based participants in the Cardiovascular Health Study (CHS).

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Methods

CHS is a multicenter prospective observational cohort study of cardiovascular disease in adults aged 65 years or older. Participants (n = 5,888) include those initially enrolled in 1989 to 1990 (n = 5,201), and a supplemental cohort (n = 687) of African-Americans enrolled in 1992 to 1993. The methodology and design of the CHS have been reported previously.[11] Participants in the main cohort were recruited from 4 centers in the United States: Sacramento, CA; Pittsburgh, PA; Winston-Salem, NC; and Hagerstown, MD. For the supplemental cohort the PA, NC, and CA sites were used.

An extensive examination was performed at baseline, including medical history, medication inventory, venipuncture, seated blood pressure, electrocardiogram, and ultrasound of the carotid arteries. Measures of clinical and subclinical CVD were determined. Clinical cardiovascular disease included history of myocardial infarction (MI), angina pectoris, use of nitroglycerin, pacemaker, coronary artery bypass or angioplasty, claudication, congestive heart failure, stroke, transient ischemic attack, or history of carotid endarterectomy. Participants with prevalent coronary heart disease (previous MI, angina, coronary artery bypass, or angioplasty) or previous congestive heart failure at the time of echocardiography were excluded from the present analysis. Participants were also excluded if they had decreased LV ejection fraction or LV wall motion abnormalities observed on the echocardiogram.

Participants within the baseline cohort (enrolled in 1989 to 1990) were included if complete cardiac mechanics and biomarker data were both collected on the same examination day. Participants within the supplemental cohort were included if complete cardiac mechanics and biomarker data were collected during an echocardiogram completed in 1994 to1995 and from blood samples obtained in 1995 to 1996.

The CHS was approved by the institutional review boards of the University of Washington, Seattle, and the participating centers. All participants gave written informed consent. The present study was determined by the institutional review board of the University of Maryland to be exempt from human subjects review.

The design of the echocardiography protocol used in CHS has been described in detail elsewhere.[12] Between 2017 to 2018, archived echocardiograms originally recorded on videotape in analog format were digitized using the TIMS 2000 DICOM System (Foresight Imaging, Chelmsford, Massachusetts). Cine loops of 2 to 4 cardiac cycles were digitized at a frame rate of 30 to 40 frames per second (fps) and stored offline in DICOM format. Validation of speckle tracking echocardiography using digitized videotapes in comparison with prospective digital acquisition has been previously reported.[13] The measures reported in this study included LVLS, determined as average peak longitudinal strain (obtained from apical 4 chamber views), LVEDSR, and LARS. Reproducibility of these measures is reported in **Supplemental Table S1.**

The 4 biomarkers studied (hscTnT, sST2, NT-proBNP, and gal-3) were measured in serum collected in 1989 to 1990 for the main cohort, and in 1995 to 1996 for the supplemental cohort, and stored at −70C to −80C. Samples were thawed just before testing (maximum of 3 freeze-thaw cycles) and measured in April 2010 (hscTnT and NTproBNP), April through September 2013 (sST) and May through October 2013 (Gal 3) as previously reported.[1,4,14] Analyte stability has been demonstrated for at least 1 to 2 years from previously frozen samples.[15−17] As in previous studies by our group, standardized approaches to collection, aliquoting, and freezing of samples were followed with the intent of minimizing degradation.

Log-transformed biomarkers were modeled as continuous variables and as binary variables utilizing cut-points employed in previous analyses.[3,8,18,19] Specifically these are: hscTnT >13 ng/ml, a partition value previously established in CHS[4]; sST2 >35 ng/ml, the FDA-approved cut-point for heart failure; NT-proBNP >190 pg/ml, the threshold previously identified indicating greater risk of heart failure in CHS, and gal-3 >17.8 ng/ml, the FDA-approved cut-point for prognostication in HF. For hscTnT, participants with levels below the limit of detection (3 ng/ml) were assigned imputed values at 2.99 ng/ml.

Demographic and clinical factors were compared across quartiles of each strain measure using ANOVA or Kruskal-Wallis tests for continuous variables, and Chi-squared test for categorical variables. Linear pairwise correlations in log-transformed cardiac biomarkers and echocardiographic strain measures were estimated using Pearson's correlation coefficients.

Cross-sectional associations of each log-transformed biomarker with cardiac mechanics were evaluated in separate hierarchal multivariable linear regression models. The baseline model was adjusted for echocardiography site, speckle tracking reader, and chamber image quality. A demographics-adjusted model was additionally adjusted for age, race and gender. Finally, a risk-factor model was adjusted for demographic variables as well as prevalent diabetes and hypertension, systolic and diastolic blood pressure, previous stroke, HDL-cholesterol, LDL-cholesterol, triglycerides, smoking, body mass index, and estimated glomerular filtration rate (eGFR). For all linear regression models, we tested the assumptions of homoskedasticity with the Cook-Weissberg test.

In an exploratory analysis, we tested the effect of addition of atrial fibrillation to the risk-factor adjusted model. Additionally, we evaluated nonlinear models through restricted cubic and basis spline models with variable knots. We also assessed the effects of interaction terms between each log-transformed and normalized biomarker with race and gender in risk-factor adjusted models. Where more than 1 biomarker was patiently associated with a strain variable in risk-factor adjusted models, we tested for simultaneous attenuation or residual patient effects in a multivariable risk-factor adjusted model.

Regression coefficients are presented as the primary measure of association. As the different strain measures are quantified by different units of measurement, we additionally present standardized regression coefficients ($\beta^{\wedge}$) after transforming each strain outcome and log-transformed biomarker on a N (0, 1) normalization to permit qualitative comparisons across different strain measures. Since the study was comprised of an initial and a supplementary

cohort, we performed analyses to assess effect modification by cohort.

To minimize the likelihood of Type II error, for reasons described by others[20–22] p values were not adjusted for multiple comparisons. Specifically, the present study focused on a limited number of comparisons, the comparisons were made on sensible scientific rationales, and all comparisons are reported.

Results

In 5,888 CHS participants 1,845 by design (Supplemental Figure S1) had 1 or more baseline exclusion criteria (1,171 coronary heart disease, 298 congestive heart failure, 753 abnormal or borderline ejection fraction, and 887 abnormal or borderline segmental wall motion). An additional 1,277 participants had 1 or more missing biomarker measurement, and 693 had 1 or more missing strain measure. Hence, 2,073 (51%) participants met study criteria for absence of prevalent coronary heart disease and/or heart failure, and complete cross-sectional biomarker and strain data. The mean (SD) age of included participants was 72[5] years and 64% were female. Participants with complete biomarker and strain data (Supplemental Figure S2) were slightly younger (av 72.4 vs 72.7 yrs), less likely to be African American (10.3 vs 13.4 y%), had lower prevalence of stroke (2.3 vs 4.0%), lower body mass index (26.2 vs 26.7 kg/m^2), but higher prevalence of diabetes (14.3 vs 11.6 %).

Patient log transformed biomarker values plotted against strain values are shown in Supplemental Figure 2. Model fit was not improved using non-linear models (Supplemental Table S3). Older age, black race, higher SBP, DBP and BMI, and higher prevalence of hypertension were associated with lower (worse) values of LVLS, LVEDSR, and LARS (Table 1). The proportion of male participants was highest in participants with lower LVLS and LVEDSR, and lowest in participants with lower LARS. Lower HDL and higher triglycerides and higher prevalence of diabetes were associated with lower values of LVLS and LVEDSR, but not LALS. Higher prevalence of stroke and chronic kidney disease, and lower eGFR were associated with lower LVEDSR only.

Higher values of hscTnT, sST2, and NT-proBNP were associated with lower values of all strain measures, LVLS, LVEDSR and LARS. Galectin-3 was not associated with any strain measure.

Correlation between cardiac biomarkers and speckle-tracking echocardiographic measures (Table 2) were low (all Pearson r $\leq$ 0.18). HscTnT was moderately correlated with NT-proBNP (r = 0.33) and sST2 (r = 0.17), but poorly correlated with all strain measures (all r $\leq$ 0.13). LVLS was moderately to strongly correlated (r = 0.70) with LVEDSR.

Associations of biomarkers with cardiac strain are presented in Table 3 for continuous measures, and Table 4 for prognostic biomarker endpoints. LV longitudinal systolic strain: In risk factor adjusted models, LVLS was significantly associated with hscTnT sST2, and NT-proBNP . Similarly high hscTnT (>13 ng/ml), high sST2 (>35 ng/ml), and high NT-proBNP (>190 pg/ml) were associated with worse systolic strain after adjustment for risk factors. Gal-3, either as a continuous measure or dichotomized based on a prognostic cut point, was not associated with LVLS.

LV diastolic strain rate: After adjustment for demographics and risk-factors, no biomarkers were associated with LVEDSR as either continuous measures or after dichotomization based on prognostic cutpoints.

LA reservoir strain: In risk factor adjusted models, LARS was associated with both hscTnT, and NT-proBNP. Similarly, high hscTnT (>13 ng/ml) and high NT-proBNP (>190 pg/ml) were associated with worse LARS. Neither gal-3 or sST2, either as continuous measures or dichotomized based on prognostic cut points, were associated with LARS.

Additional adjustment for atrial fibrillation (either history, or concurrent atrial fibrillation at the day of the echocardiogram) had minimal effect on associations of continuous measures of biomarkers with cardiac strain (Supplemental Tables S4a and S4b). We found a significant interaction between hscTnT and gender as predictors of LVLS (Supplemental Table S5).

When adjusted simultaneously for hscTnT, sST2, NT-proBNP, and risk-factors, no biomarker was associated with LVLS. When adjusted simultaneously for both hscTnT, NT-proBNP, and risk factors, hscTnT was no longer associated with LARS ($\beta^\wedge$ = -0.03; p-value = 0.21) but the association of NT-proBNP with LARS was essentially unchanged ($\beta^\wedge$ = -0.17; p-value <0.0001).

Evaluation of interaction effects between each biomarker and participant cohort did not suggest presence of effect modification. With exclusion of the supplementary cohort, there was slight strengthening of the association between the biomarkers and strain measures.

Discussion

The principal findings of this study are that in free-living older subjects without clinical cardiovascular disease, decreased LV ejection fraction, or regional wall motion abnormality, higher levels of cardiac-specific soluble biomarkers of atrial overload (NT-proBNP), and to a lesser extent, myocardial injury (hscTnT) are associated with worse left atrial strain. There were modest associations of hscTnT, NTproBNP and sST2 with worse LV systolic strain, and hscTnT and NTproBNP with worse LA reservoir strain. Because those associations were attenuated by adjustment for risk factors, it is possible that subclinical disease may have accounted for some of biomarker associations with worse LV systolic and LA reservoir strain. No biomarker was independently associated with LVEDSR. Galectin-3, a systemic biomarker of fibrosis and inflammation, showed no association with any measure of cardiac strain.

Importantly, the data suggest differing associations of biomarkers with LV systolic strain versus LA reservoir strain. Although both NT-proBNP and hscTnT were significantly associated with worse LARS, the association of NT-proBNP with worse LARS was independent of hscTnT (but not vice versa), and the strength of association of NT-proBNP with worse LARS was greater than that of hscTnT. It is likely that after accounting for the impact of myocardial injury (hscTnT) on elevated filling pressure (NT-

Table 1
Comparison of selected participant characteristics and biomarkers by quartiles of LV longitudinal systolic strain, LV early diastolic strain rate, and LA reservoir strain

Variable	LV Longitudinal Systolic Strain (%)				p Value
	Q1 (≤12.301) (n = 518)	Q2 (12.302−14.562) (n = 518)	Q3 (14.563−17.061) (n = 519)	Q4 (≥17.062) (n = 518)	
Age, mean (SD) (years)	73.1 ± 5.7	72.7 ± 5.5	72.1 ± 5.1	71.7 ± 5.0	<.001
Men	240 (46.3%)	204 (39.4%)	184 (35.5%)	126 (24.3%)	<.001
Black	82 (15.8%)	47 (9.1%)	46 (8.9%)	39 (7.5%)	<.001
SBP, mean (SD) (mmHg)	138.2 ± 20.8	136.8 ± 21.5	134.9 ± 20.3	132.9 ± 21.9	<.001
DBP, mean (SD) (mm Hg)	71.8 ± 11.6	70.9 ± 11.1	70.0 ± 10.4	69.1 ± 10.4	<.001
Hypertension medication*	211 (40.8%)	194 (37.6%)	192 (37.1%)	171 (33.1%)	0.08
Hypertension	322 (62.2%)	308 (59.7%)	275 (53.1%)	248 (47.9%)	<.001
Diabetes mellitus	101 (19.8%)	69 (13.5%)	65 (12.6%)	59 (11.4%)	<.001
Stroke	17 (3.3%)	12 (2.3%)	11 (2.1%)	7 (1.4%)	0.22
Atrial fibrillation	19 (4.5%)	14 (3.3%)	9 (2.1%)	11 (2.5%)	0.17
Smoker					0.91
Never	246 (47.5%)	239 (46.1%)	255 (49.2%)	258 (49.8%)	
Former	208 (40.2%)	219 (42.3%)	205 (39.6%)	202 (39.0%)	
Current	64 (12.4%)	60 (11.6%)	58 (11.2%)	58 (11.2%)	
eGFR, mean (SD) (ml/min/1.73m^2)	74.0 ± 17.1	73.4 ± 17.1	74.3 ± 16.5	75.5 ± 16.0	0.22
CKD†	112 (21.7)	121 (23.4)	102 (19.7)	102 (19.7)	0.41
BMI, mean (SD) (kg/m^2)	27.3 ± 4.6	26.3 ± 4.3	26.0 ± 4.2	25.2 ± 4.1	<.001
LDL cholesterol, mean (SD) (mg/dl)	129 ± 35	129 ± 35	131 ± 36	132 ± 35	0.52
HDL cholesterol, mean (SD) (mg/dl)	53.1 ± 15.3	54.5 ± 14.9	56.4 ± 16.0	59.1 ± 15.2	<.001
Triglycerides, median (IQR) (mg/dl)	127 (99−172)	123 (94−167)	115 (90−160)	111 (87−148)	<.0001
High-sensitivity cTnT, median (IQR) (ng/ml)	6.0 (3.0−10.2)	4.5 (3.0−8.6)	4.2 (3.0−8.2)	3.5 (3.0−7.7)	<.0001
Soluble ST2, median (IQR) (ng/ml)	24.5 (19.4−30.6)	23.1 (18.6−28.5)	22.7 (18.2−28.3)	21.2 (17.5−26.7)	<.0001
NT-proBNP, median (IQR) (pg/ml)	105.3 (50.3−218.7)	103.8 (52.7−202.4)	86.4 (50.5−167.7)	102.8 (58.2−183.5)	0.048
Galectin-3, median (IQR) (ng/ml)	15.3 (12.2−18.5)	15.6 (12.7−19.0)	15.2 (12.6−18.5)	15.1 (12.3−18.7)	0.67

Patient Characteristics	LV Early Diastolic Strain Rate (s^{-1})				p Value
	Q1 (≤0.510) (n = 519)	Q2 (0.511−0.652) (n = 517)	Q3 (0.653−0.818) (n = 519)	Q4 (≥0.819) (n = 518)	
Age, mean (SD) (years)	73.5 ± 5.9	72.4 ± 5.1	72.0 ± 5.2	71.5 ± 5.0	<.001
Men	238 (45.9%)	199 (38.5%)	183 (35.3%)	134 (25.9%)	<.001
Black	74 (14.3%)	61 (11.8%)	43 (8.3%)	36 (6.9%)	<.001
SBP, mean (SD) (mm Hg)	139.2 ± 21.6	137.3 ± 20.7	134.9 ± 20.5	131.6 ± 21.4	<.001
DBP, mean (SD) (mm Hg)	71.9 ± 11.2	70.7 ± 11.5	70.2 ± 10.6	68.9 ± 10.2	<.001
Hypertension medication*	210 (40.6%)	205 (39.8%)	182 (35.1%)	171 (33.0%)	0.030
Hypertension	327 (63.1%)	310 (60.0%)	270 (52.2%)	246 (47.5%)	<.001
Diabetes mellitus	105 (20.5%)	74 (14.6%)	66 (12.8%)	49 (9.5%)	<.001
Stroke	21 (4.0%)	11 (2.1%)	8 (1.5%)	7 (1.4%)	0.014
Atrial fibrillation	9 (2.2%)	15 (3.4%)	11 (2.5%)	18 (4.1%)	0.34
Smoker					0.38
Never	240 (46.2%)	249 (48.3%)	242 (46.6%)	267 (51.5%)	
Former	222 (42.8%)	201 (39.0%)	221 (42.6%)	190 (36.7%)	
Current	57 (11.0%)	66 (12.8%)	56 (10.8%)	61 (11.8%)	
eGFR, mean (SD) (ml/min/1.73m^2)	72.5 ± 17.7	73.7 ± 16.4	75.1 ± 16.7	75.9 ± 15.8	0.007
CKD†	130 (25.1)	115 (22.3)	97 (18.7)	95 (18.3)	0.022
BMI, mean (SD) (kg/m^2)	27.4 ± 4.5	26.1 ± 4.2	25.9 ± 4.1	25.4 ± 4.3	<.001
LDL cholesterol, mean (SD) (mg/dl)	130 ± 36	133 ± 36	129 ± 34	130 ± 35	0.24
HDL cholesterol, mean (SD) (mg/dl)	53.1 ± 15.1	54.5 ± 14.7	56.9 ± 15.9	58.5 ± 15.8	<.001
Triglycerides, median (IQR) (mg/dl)	127 (97−173)	121 (94−169)	118 (91−160)	110 (87−147)	<.0001
High-sensitivity cTnT, median (IQR) (ng/ml)	5.6 (3.0−9.8)	4.8 (3.0−8.6)	4.3 (3.0−8.6)	3.5 (3.0−7.9)	<.0001
Soluble ST2, median (IQR) (ng/ml)	24.0 (19.5−30.2)	22.7 (18.5−27.5)	22.7 (18.0−28.5)	21.5 (17.1−27.8)	<.0001
NT-proBNP, median (IQR) (pg/ml)	109.6 (53.1−218.9)	103.5 (55.2−184.2)	88.6 (45.5−184.4)	94.4 (57.4−186.6)	0.014
Galectin-3, median (IQR) (ng/mLl)	15.5 (12.4−19.0)	15.1 (12.2−18.5)	15.4 (12.7−19.0)	15.0 (12.4−18.5)	0.24

Patient Characteristics	LA Reservoir Strain (%)				p Value
	Q1 (≤30.359) (n = 518)	Q2 (30.360−38.954) (n = 518)	Q3 (38.955−50.788) (n = 519)	Q4 (≥50.789) (n = 518)	
Age, mean (SD) (years)	73.7 ± 5.8	72.5 ± 5.5	71.6 ± 4.8	71.6 ± 4.9	<.001
Men	152 (29.3%)	176 (34.0%)	189 (36.4%)	237 (45.8%)	<.001
Black	73 (14.1%)	53 (10.2%)	43 (8.3%)	45 (8.7%)	0.008
SBP, mean (SD) (mm Hg)	137.2 ± 21.1	138.0 ± 21.9	133.6 ± 20.2	134.2 ± 21.3	0.001
DBP, mean (SD) (mm Hg)	70.5 ± 11.0	71.6 ± 10.8	69.8 ± 10.9	69.9 ± 10.7	0.030
Hypertension medication*	209 (40.5%)	194 (37.5%)	184 (35.6%)	181 (34.9%)	0.25
Hypertension	293 (56.8%)	323 (62.5%)	271 (52.2%)	266 (51.4%)	<.001

(*continued*)

Table 1 (***Continued***)

Patient Characteristics	LA Reservoir Strain (%)				p Value
	Q1 (≤30.359) (n = 518)	Q2 (30.360−38.954) (n = 518)	Q3 (38.955−50.788) (n = 519)	Q4 (≥50.789) (n = 518)	
Diabetes mellitus	91 (17.8%)	72 (14.1%)	62 (12.0%)	69 (13.4%)	0.050
Stroke	18 (3.5%)	11 (2.1%)	8 (1.5%)	10 (1.9%)	0.18
Atrial Fibrillation	29 (6.9%)	8 (1.9%)	6 (1.3%)	10 (2.3%)	<.001
Smoker					0.06
Never	275 (53.1%)	252 (48.6%)	232 (44.7%)	239 (46.2%)	
Former	193 (37.3%)	214 (41.3%)	213 (41.0%)	214 (41.4%)	
Current	50 (9.7%)	52 (10.0%)	74 (14.3%)	64 (12.4%)	
eGFR, mean (SD) (ml/min/1.73m^2)	72.6 ± 17.1	75.4 ± 16.4	75.7 ± 16.3	73.4 ± 16.8	0.005
CKD[†]	123 (23.8)	91 (17.6)	105 (20.3)	118 (22.8)	0.07
BMI, mean (SD) (kg/m^2)	26.6 ± 4.3	26.9 ± 4.7	26.0 ± 4.1	25.4 ± 4.1	<.001
LDL cholesterol, mean (SD) (mg/dl)	129 ± 35	134 ± 37	133 ± 35	125 ± 33	<.001
HDL cholesterol, mean (SD) (mg/dl)	55.9 ± 15.1	55.5 ± 15.7	56.5 ± 15.4	55.2 ± 15.8	0.54
Triglycerides, median (IQR) (mg/dl)	117 (91−165)	123 (91−167)	123 (94−156)	115 (90−152)	0.19
High-sensitivity cTnT, median (IQR) (ng/ml)	5.4 (3.0−9.9)	4.4 (3.0−8.8)	4.2 (3.0−8.1)	4.3 (3.0−8.1)	0.004
Soluble ST2, median (IQR) (ng/ml)	23.6 (18.9−29.4)	23.3 (18.0−28.3)	21.9 (17.7−27.6)	22.8 (18.2−28.3)	0.014
NT-proBNP, median (IQR) (pg/ml)	134.3 (65.0−297.6)	105.3 (55.9−196.6)	89.5 (50.1−168.8)	85.4 (43.2−152.8)	<.0001
Galectin-3, median (IQR) (ng/ml)	15.3 (12.5−18.7)	15.3 (12.5−18.9)	15.4 (12.6−18.7)	14.9 (12.4−18.6)	0.58

Abbreviations: BMI=Body Mass Index; CKD = Chronic Kidney Disease; DBP = Diastolic Blood Pressure; eGFR = estimated Glomular Filtration Rate; HDL = High-Density Lipoprotein; LA = left atrial; LDL = Low-Density Lipoprotein; LV = left ventricular; SBP = Systolic Blood Pressure.

* Includes any of Angiotensin Converting Enzyme Inhibitor, Beta Blocker, Diuretic, Statin, Angiotensin II Receptor Blocker, and Calcium Channel Blocker.

[†] Chronic Kidney Disease (CKD) defined as eGFR<60 ml/min/1.73m^2.

proBNP level), myocardial injury per se is not meaningfully associated with LARS. Nonetheless it remains unknown whether the association of hscTnT with worse LARS might be a direct effect of release of hscTnT in response to injury of atrial myocytes, or an indirect effect of diastolic dysfunction secondary to ventricular injury and/or repair fibrosis in response to previous or ongoing myocardial injury.

Although hscTnT, a biomarker highly specific for myocardial myocyte injury or death, was initially introduced[23−25] to aid the diagnosis of acute myocardial infarction and other coronary syndromes, research has shown that low levels of hscTnT are detectable in almost all patients with stable coronary heart disease and preserved left ventricular function.[2] In the CHS[4] it was shown that even in older adults without previous history of heart failure or symptomatic coronary disease, hscTnT was detectable in almost two-thirds of subjects. Levels of hscTnT were associated with age and other risk factors, and both baseline levels and their change over time were independently associated with incident heart failure and cardiovascular death, and well as with new LV systolic dysfunction.[26]

The role of sST2 in the pathogenesis of cardiovascular disease is complex and may involve non-cardiac as well as cardiovascular effects.[27] Although the value of sST2 for cardiovascular prognosis has been described,[5−7] studies of association of sST2 with cardiac structural and functional changes have differed in their findings, but have included systolic and diastolic dysfunction and LV remodeling.[24] In the present study sST2 was associated with LV systolic strain, but not LA reservoir strain or early diastolic strain rate.

Galectins, including gal-3, are carbohydrate binding proteins important to cardiac fibrosis.[8] However, like sST2, gal-3 is not cardiac specific and has a role in a variety of multisystem pathophysiologic processes other than fibrosis including inflammation, liver and renal disease, and rheumatologic diseases. In our study, we found no association of gal-3 with cardiac strain.

The strengths of this study include the novel finding of association of biomarkers which represent complex

Table 2
Pearson's correlation among log transformed cardiac biomarkers and speckle-tracking echocardiography measures

	Soluble ST2*	NT-proBNP*	Galectin-3*	LV longitudinal Systolic Strain	LV Early Diastolic Strain Rate	LA Reservoir Strain
High-sensitivity cTnT*	0.25	0.33	0.11	−0.13	−0.11	−0.09
Soluble ST2*		0.17	0.05	−0.13	−0.09	−0.04
NT-proBNP*			0.19	−0.04	−0.02	−0.18
Galectin-3*				−0.02	−0.01	−0.03
LV longitudinal systolic strain					0.70	0.25
LV early diastolic strain rate						0.10

Abbreviations: LA = left atrial; LV = left ventricular.

* Cardiac biomarkers are log-transformed.

Table 3
. Association of continuous measures of cardiac biomarkers with cardiac strain among 2073 CHS participants

Outcome	Predictor	Beta (95% CI), per 1-SD ln(unit)*					
		Baseline† n = 2,073	p-value	Demographic Adjusted‡ n = 2,073	p-value	Risk-Factor Adjusted§ n = 2,006	p-value
LV longitudinal systolic strain	High-sensitivity cTnT	−0.14 (−0.18, −0.10)	<.0001	−0.08 (−0.13, −0.03)	0.0007	−0.06 (−0.10, −0.01)	0.020
	Soluble ST2	−0.11 (−0.15, −0.07)	<.0001	−0.06 (−0.11, −0.02)	0.003	−0.05 (−0.09, −0.01)	0.024
	NT-proBNP	−0.05 (−0.09, −0.01)	0.026	−0.05 (−0.10, −0.01)	0.015	−0.06 (−0.11, −0.02)	0.007
	Galectin-3	−0.01 (−0.05, 0.03)	0.54	−0.03 (−0.08, 0.01)	0.11	−0.01 (−0.06, 0.03)	0.51
LV early diastolic strain rate	High-sensitivity cTnT	−0.12 (−0.17, −0.08)	<.0001	−0.04 (−0.09, 0.01)	0.08	−0.003 (−0.05, 0.05)	0.89
	Soluble ST2	−0.08 (−0.12, −0.04)	0.0002	−0.03 (−0.07, 0.02)	0.24	−0.01 (−0.05, 0.04)	0.81
	NT-proBNP	−0.03 (−0.07, 0.02)	0.22	−0.01 (−0.05, 0.03)	0.67	−0.004 (−0.05, 0.04)	0.88
	Galectin-3	−0.01 (−0.05, 0.03)	0.59	−0.03 (−0.07, 0.02)	0.24	0.001 (−0.04, 0.05)	0.96
LA reservoir strain	High-sensitivity cTnT	−0.09 (−0.13, −0.05)	<.0001	-0.08 (−0.13, −0.04)	0.0005	−0.08 (−0.12, −0.03)	0.003
	Soluble ST2	−0.03 (−0.07, 0.01)	0.19	−0.04 (−0.09, 0.001)	0.06	−0.03 (−0.07, 0.02)	0.23
	NT-proBNP	−0.18 (−0.23, −0.14)	<.0001	−0.15 (−0.19, −0.10)	<.0001	−0.18 (−0.22, −0.13)	<.0001
	Galectin-3	−0.03 (−0.07, 0.02)	0.25	0.01 (−0.03, 0.05)	0.60	0.02 (−0.02, 0.07)	0.32

Pressure, prior stroke, Low-Density Lipoprotein cholesterol, High-Density Lipoprotein cholesterol, triglycerides, smoking status, body mass index, and estimated glomular filtration rate.

abbreviations: LV = left ventricular; LA = left atrial.

* Betas are standardized. Both outcome and predictor are standardized on a N(0,1) scale for regression models.

† Adjusted for image quality (LV or LA chamber continuous quality score), reader, and CHS echocardiograph site.

‡ Added adjustment for age, gender, race (African-American vs other).

§ Added adjustment for demographic variables and diabetes, hypertensive medications, Systolic Blood Pressure, Diastolic Blood.

biologic processes and which have prognostic value for heart failure and cardiovascular death, with sensitive measures of cardiac systolic and diastolic function. Notably, these associations were present in free living older patients without overt cardiovascular disease or subclinical abnormality of LV contraction on echocardiography. The findings have mechanistic implications for associations of pathobiology with sensitive measures of cardiac function that are clinically relevant.

Limitations of the study include it's restriction to participants ≥ 65 years of age, and may not be applicable to younger patients. The study is cross-sectional and measurements of cardiac strain and biomarkers were obtained at only 1 point in time. Hence, it was not possible to discern the temporal relation or sequence between changes in biomarker levels and altered cardiac strain. This, in combination with selection of relatively healthy participants without subclinical visually evident abnormality of LV contraction on echocardiography may in part account for the relatively low strength of association of biomarkers with cardiac strain measures. However, even low strengths of association shown at a single point of time may have implications for the effects of prolonged exposures on clinical outcomes.

Table 4
Association of cardiac biomarkers elevated over prognostic cutpoints with cardiac strain

Outcome	Predictor*	Beta (95% CI), High vs Low					
		Baseline† n = 2,073	p-value	Demographic Adjusted‡ n = 2,073	p-value	Risk-Factor Adjusted§ n = 2,006	p-value
LV longitudinal systolic strain	High-sensitivity cTnT	−1.11 (−1.56, −0.65)	<.0001	−0.66 (−1.13, −0.18)	0.007	−0.51 (−1.01, −0.02)	0.041
	Soluble ST2	−1.10 (−1.58, −0.62)	<.0001	−0.76 (−1.24, −0.27)	0.002	−0.78 (−1.26, −0.29)	0.002
	NT-proBNP	−0.48 (−0.81, −0.14)	0.006	−0.44 (−0.79, −0.09)	0.013	−0.43 (−0.79, −0.07)	0.020
	Galectin-3	−0.03 (−0.35, 0.29)	0.84	−0.11 (−0.43, 0.21)	0.48	0.06 (−0.27, 0.39)	0.74
LV early diastolic strain rate	High-sensitivity cTnT	−0.05 (−0.08, −0.02)	0.003	−0.003 (−0.04, 0.03)	0.85	0.01 (−0.02, 0.05)	0.52
	Soluble ST2	−0.03 (−0.06, 0.01)	0.10	−0.001 (−0.04, 0.03)	0.96	0.003 (−0.03, 0.04)	0.89
	NT-proBNP	−0.02 (−0.05, −0.002)	0.048	−0.01 (−0.04, 0.01)	0.36	−0.01 (−0.03, 0.02)	0.63
	Galectin-3	−0.01 (−0.03, 0.02)	0.54	−0.01 (−0.03, 0.01)	0.45	0.005 (−0.02, 0.03)	0.70
LA reservoir strain	High-sensitivity cTnT	−3.61 (−5.68, −1.54)	0.0007	−2.86 (−5.00, −0.71)	0.009	−2.74 (−4.99, −0.49)	0.017
	Soluble ST2	−1.56 (−3.74, 0.62)	0.16	−1.94 (−4.11, 0.23)	0.08	−1.48 (−3.72, 0.77)	0.20
	NT-proBNP	−5.56 (−7.08, −4.05)	<.0001	−4.16 (−5.73, −2.59)	<.0001	−4.65 (−6.31, −3.00)	<.0001
	Galectin-3	−1.03 (−2.48, 0.42)	0.16	0.04 (−1.41, 1.48)	0.96	0.40 (−1.11, 1.91)	0.61

Pressure, prior stroke, Low-Density Lipoprotein cholesterol, High-Density Lipoprotein cholesterol, triglycerides, smoking status, body mass index, and estimated Glomular Filtration Rate.

* Predictors are dichotomized at clinical cut points comparing high values versus low. Specifically these are: hscTnT >13 ng/ml, sST2 >35 ng/ml, NT-proBNP >190 pg/ml, and gal-3 >17.8 ng/ml.

† Adjusted for image quality (LV or LA chamber continuous quality score), reader, and CHS echocardiography site.

‡ Added adjustment for age, gender, race (African-American vs other)

§ Added adjustment for demographic variables and diabetes, hypertensive medications, Systolic Blood Pressure, Diastolic Blood

It is important to note that the methods used to derive cardiac strain measures from archived analog videotapes differ substantially from contemporary clinical and research methods using direct digital acquisition and imaging protocols designed to optimize strain measurements. Specifically, the VHS videotape analog to digital conversion of the apical 4 chamber views used in this study results in a frame rate of 30 fps, as opposed to 60 fps for direct digital acquisition of 3 apical views commonly done in clinical and contemporary research imaging. The values in this study are not intended to be used for clinical diagnosis, nor compared with studies done with different methodology.

Frozen blood samples were thawed and analyzed for the biomarkers approximately 20 years after blood draw and freezing. There are no data that can confirm stability of these substances for 20 years. However, according to the Arrhenius equation[28] which describes the temperature dependence of chemical reactions, minimal degradation would occur in samples frozen at −70˚C to −80˚C. Additionally, these biomarkers have been utilized after decades of frozen storage in other studies[7] and found to be useful in prediction of clinical outcomes. Moreover, biomarker degradation would make associations with phenotypes and outcomes less, not more significant. Thus the associations of these biomarkers with functional measures are if anything underestimated rather than over-stated.

Although for reasons stated previously we chose to not adjust the analyses for multiple comparisons, the likelihood of a type 1 error may have been increased. Nonetheless, the findings are consistent with linkages between blood markers of cardiovascular disease processes and LV and LA cardiac strain measured by echocardiography in participants without overt clinical disease, and independent of risk factors.

Authors contribution

John S Gottdiener MD − conceptualization, writing original draft, edits, investigation, data acquisition, methodology.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relations that could have appeared to influence the work reported in this study.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.amjcard.2020.09.013.

1. Dieplinger B, Januzzi JL Jr., Steinmair M, Gabriel C, Poelz W, Haltmayer M, Mueller T. Analytical and clinical evaluation of a novel high-sensitivity assay for measurement of soluble ST2 in human plasma−the Presage ST2 assay. *Clin Chim Acta* 2009; 409:33–40.
2. Omland T, de Lemos JA, Sabatine MS, Christophi CA, Rice MM, Jablonski KA, Tjora S, Domanski MJ, Gersh BJ, Rouleau JL, Pfeffer MA, Braunwald E. Prevention of events with angiotensin converting enzyme inhibition Trial I. a sensitive cardiac troponin T assay in stable coronary artery disease. *N Engl J Med* 2009;361: 2538–2547.
3. deFilippi CR, Christenson RH, Gottdiener JS, Kop WJ, Seliger SL. Dynamic cardiovascular risk assessment in elderly people. The role of repeated N-terminal pro-B-type natriuretic peptide testing. *J Am Coll Cardiol* 2010;55:441–450.
4. deFilippi CR, de Lemos JA, Christenson RH, Gottdiener JS, Kop WJ, Zhan M, Seliger SL. Association of serial measures of cardiac troponin T using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. *JAMA* 2010;304:2494–2502.
5. Wang TJ, Wollert KC, Larson MG, Coglianese E, McCabe EL, Cheng S, Ho JE, Fradley MG, Ghorbani A, Xanthakis V, Kempf T, Benjamin EJ, Levy D, Vasan RS, Januzzi JL. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. *Circulation* 2012;126:1596–1604.
6. Parikh RH, Seliger SL, Christenson R, Gottdiener JS, Psaty BM, deFilippi CR. Soluble ST2 for prediction of heart failure and cardiovascular death in an elderly, community-dwelling population (abstract). *J Am Heart Assoc* 2016;5:1.
7. de Boer RA, Nayor M, deFilippi CR, Enserro D, Bhambhani V, Kizer JR, Blaha MJ, Brouwers FP, Cushman M, Lima JAC, Bahrami H, van der Harst P, Wang TJ, Gansevoort RT, Fox CS, Gaggin HK, Kop WJ, Liu K, Vasan RS, Psaty BM, Lee DS, Hillege HL, Bartz TM, Benjamin EJ, Chan C, Allison M, Gardin JM, Januzzi JL Jr., Shah SJ, Levy D, Herrington DM, Larson MG, van Gilst WH, Gottdiener JS, Bertoni AG, Ho JE. Association of cardiovascular biomarkers with incident heart failure with preserved and reduced ejection fraction. *JAMA Cardiol* 2018;3:215–224.
8. Gehlken C, Suthahar N, Meijers WC, de Boer RA. Galectin-3 in heart failure: an update of the last 3 years. *Heart Fail Clin* 2018;14:75–92.
9. Fallah-Rad N, Walker JR, Wassef A, Lytwyn M, Bohonis S, Fang T, Tian G, Kirkpatrick ID, Singal PK, Krahn M, Grenier D, Jassal DS. The utility of cardiac biomarkers, tissue velocity and strain imaging, and cardiac magnetic resonance imaging in predicting early left ventricular dysfunction in patients with human epidermal growth factor receptor II-positive breast cancer treated with adjuvant trastuzumab therapy. *J Am Coll Cardiol* 2011;57:2263–2270.
10. Shah SJ, Aistrup GL, Gupta DK, O'Toole MJ, Nahhas AF, Schuster D, Chirayil N, Bassi N, Ramakrishna S, Beussink L, Misener S, Kane B, Wang D, Randolph B, Ito A, Wu M, Akintilo L, Mongkolrattanothai T, Reddy M, Kumar M, Arora R, Ng J, Wasserstrom JA. Ultrastructural and cellular basis for the development of abnormal myocardial mechanics during the transition from hypertension to heart failure. *Am J Physiol Heart Circ Physiol* 2014;306:H88–100.
11. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, Kuller LH, Manolio TA, Mittelmark MB, Newman A, et al. The cardiovascular health study: design and rationale. *Ann Epidemiol* 1991;1:263–276.
12. Gardin JM, Wong ND, Bommer W, Klopfenstein HS, Smith VE, Tabatznik B, Siscovick D, Lobodzinski S, Anton-Culver H, Manolio TA. Echocardiographic design of a multicenter investigation of free-living elderly subjects: the cardiovascular health study. *J Am Soc Echocardiogr* 1992;5:63–72.
13. Aguilar FG, Selvaraj S, Martinez EE, Katz DH, Beussink L, Kim KY, Ping J, Rasmussen-Torvik L, Goyal A, Sha J, Irvin MR, Arnett DK, Shah SJ. Archeological echocardiography: digitization and speckle tracking analysis of archival echocardiograms in the HyperGEN study. *Echocardiography* 2016;33:386–397.

14. Christenson RH, Duh SH, Wu AH, Smith A, Abel G, deFilippi CR, Wang S, Adourian A, Adiletto C, Gardiner P. Multi-center determination of galectin-3 assay performance characteristics: Anatomy of a novel assay for use in heart failure. *Clin Biochem* 2010;43:683–690.
15. Dieplinger B, Egger M, Poelz W, Haltmayer M, Mueller T. Long-term stability of soluble ST2 in frozen plasma samples. *Clin Biochem* 2010;43:1169–1170.
16. Egger M, Dieplinger B, Mueller T. One-year in vitro stability of cardiac troponins and galectin-3 in different sample types. *Clin Chim Acta* 2018;476:117–122.
17. Cauliez B, Guignery J, Marinier S, Mariau I, Lavoinne A. Two-year stability of NT-proBNP in frozen samples using the Roche Elecsys system. *Ann Clin Biochem* 2008;45(Pt 3):318–319.
18. Gore MO, Seliger SL, Defilippi CR, Nambi V, Christenson RH, Hashim IA, Hoogeveen RC, Ayers CR, Sun W, McGuire DK, Ballantyne CM, de Lemos JA. Age- and sex-dependent upper reference limits for the high-sensitivity cardiac troponin T assay. *J Am Coll Cardiol* 2014;63:1441–1448.
19. Tang WH, Wu Y, Grodin JL, Hsu AP, Hernandez AF, Butler J, Metra M, Voors AA, Felker GM, Troughton RW, Mills RM, McMurray JJ, Armstrong PW, O'Connor CM, Starling RC. Prognostic value of baseline and changes in circulating soluble ST2 levels and the effects of nesiritide in acute decompensated heart failure. *JACC Heart Fail* 2016;4:68–77.
20. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990;1:43–46.
21. Perneger TV. What's wrong with Bonferroni adjustments. *BMJ* 1998; 316:1236–1238.
22. Althouse AD. Adjust for multiple comparisons? it's not that simple. *Ann Thorac Surg* 2016;101:1644–1645.
23. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined—a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol* 2000;36:959–969.
24. Wu AH, Jaffe AS. The clinical need for high-sensitivity cardiac troponin assays for acute coronary syndromes and the role for serial testing. *Am Heart J* 2008;155:208–214.
25. Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, Biedert S, Schaub N, Buerge C, Potocki M, Noveanu M, Breidthardt T, Twerenbold R, Winkler K, Bingisser R, Mueller C. Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. *N Engl J Med* 2009;361:858–867.
26. Glick D, deFilippi CR, Christenson R, Gottdiener JS, Seliger SL. Long-term trajectory of two unique cardiac biomarkers and subsequent left ventricular structural pathology and risk of incident heart failure in community-dwelling older adults at low baseline risk. *JACC Heart Fail* 2013;1:353–360.
27. Ho JE, Sritara P, deFilippi CR, Wang TJ. Soluble ST2 testing in the general population. *Am J Cardiol* 2015;115(7 Suppl):22B–25B.
28. Jerne NK, Perry WL. The stability of biological standards. *Bull World Health Organ* 1956;14:167–182.