Associations Between Cardiac Biomarkers and Cardiac Structure and Function in CKD

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Introduction: Subclinical changes to cardiac structure and function detected with echocardiography precede the development of clinical heart failure (HF) in persons with chronic kidney disease (CKD). Circulating cardiac biomarkers may reflect these pathophysiological changes. This study investigated associations between established biomarkers (N-terminal pro-B-type natriuretic peptide [NT-proBNP] and high-sensitivity troponin T [hsTnT]) and novel biomarkers (growth differentiation factor 15 [GDF-15], galectin-3 [Gal-3], and soluble ST-2 [sST-2]), using echocardiographic measurements in persons with CKD.

Methods: In cross-sectional analyses among 2101 participants with mild to moderate CKD in the Chronic Renal Insufficiency Cohort (CRIC), biomarker levels measured at baseline were evaluated with echocardiographic measurements 1 year later. These included left ventricular mass index (LVMI), left ventricular end-systolic volume (LVESV), left ventricular end-diastolic volume (LVEDV), left ventricular ejection fraction (LVEF), and left atrial diameter (LAD). Multivariable linear regression analyses tested associations of each biomarker with echocardiographic measurements, adjusting for covariates.

Results: GDF-15 was significantly associated with higher LVMI (1.0 g/m$^{2.7}$; 95% CI, 0.4–1.7), LVESV (0.4 ml/m$^{2.7}$; 95% CI, 0.0–0.7), and LVEDV (0.6 ml/m$^{2.7}$; 95% CI, 0.1–1.1), but not with LVEF or LAD. These findings were not significant when adjusting for NT-proBNP and hsTnT. Gal-3 and sST-2 had no significant associations. Higher levels of NT-proBNP and hsTnT were associated with all echocardiographic measurements.

Conclusion: In patients with CKD, the novel biomarker GDF-15, a marker of inflammation and tissue injury, and clinical biomarkers NT-proBNP and hsTnT, were associated with echocardiographic measurements of subclinical cardiovascular disease. Collectively, these biomarkers may highlight biological pathways that contribute to the development of clinical HF.

H F is one of the most common cardiovascular complications for persons with CKD,[1] and lower estimated glomerular filtration rate (eGFR) has a graded

association with HF development. The pathophysiology of HF is complex in CKD and involves multiple pathways, including inflammation, neurohormonal responses, metabolic and nutritional changes, hemodynamics and fluid status, acid–base changes, and anemia.[2,3]

Structural and functional cardiac changes can be appreciated on echocardiography[4] and may precede the development of clinical HF in persons with CKD. Echocardiographic parameters of left ventricular (LV)

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structure[5,6] (LVMI, LVEDV), LV function,[7] (LVESV, LVEF), and left atrial structure[8,9] have been linked with cardiovascular events, mortality, and HF in patients with CKD.

Both clinically available and novel cardiac biomarkers have been found to be associated with risk of HF in CKD and non-CKD populations. Among the biomarkers being investigated, GDF-15, Gal-3, and sST-2 have emerged as potentially important indicators of cardiovascular disease and outcomes, and may reveal insights into cardiac structure.[10,11] These biomarkers have been shown to have associations with major cardiovascular events and outcomes in the general population,[12–20] and more recently in CKD patients.[21] GDF-15 is a member of the transforming growth factor (TGF)-beta cytokine family,[22] plays a role in cardiomyocyte repair,[23] and is increased in inflammation.[24] Gal-3 is a biomarker approved by the US Food and Drug Administration for evaluation in patients with HF,[25] belongs to the β-galactoside-binding protein family, and is proinflammatory and profibrotic in cardiomyocytes.[26] sST-2, also approved by the US Food and Drug Administration, is a member of the interleukin-1 receptor family that promotes cardiomyocyte hypertrophy and fibrosis.[25] Clinical biomarkers, NT-proBNP, a marker of myocardial stretch and volume, and hsTnT, a marker of myocardial injury, have also been strongly associated with cardiovascular outcomes in patients with CKD.[27]

Whether widely available and novel cardiac biomarkers are associated with early structural and functional HF, as identified by echocardiography, is not known and may help identify early biological alterations that lead to clinical HF in persons with CKD. In this study, we investigate and compare associations of novel (gal-3, GDF-15, sST2) and commonly used (NT-proBNP, hsTnT) biomarkers with a broad array of echocardiographic measurements in patients with CKD without clinical HF.

MATERIALS AND METHODS

Study Population

We studied men and women with mild-to-moderate CKD enrolled in the CRIC study. A total of 3939 participants were enrolled into the CRIC study between June 2003 and August 2008 at 7 clinical centers across the US (Ann Arbor/Detroit, MI; Baltimore, MD; Chicago, IL; Cleveland, OH; New Orleans, LA; Philadelphia, PA; and Oakland, CA). The CRIC study initially enrolled patients with CKD with an eGFR of 20 to 70 ml/min per 1.73 m^2 using the Modification of Diet in Renal Disease (MDRD) study equation, and excluded patients with New York Heart Association class III or

IV HF. Additional details on study design, inclusion and exclusion criteria, and baseline characteristics of the participants have been previously published.[28,29] All study participants provided written informed consent, and the study protocol was approved by institutional review boards at each of the participating sites.

We performed a cross-sectional analysis of participants in the CRIC cohort. Biomarkers were measured at the time of study enrollment, and echocardiograms were performed at the 1-year follow-up visit. We excluded participants with self-reported HF at study entry (N = 382), those without all 5 cardiac biomarkers measured concurrently (N = 243), those without all 5 echocardiographic measurements available (N = 1148), and patients who progressed from CKD to end-stage renal disease prior to the first echocardiogram (N = 65). With these exclusions, 2101 participants were included in the present study, as shown in Table 1. Overall, the included population had fewer Hispanic participants and fewer participants with cardiovascular disease compared to those who were excluded.

Cardiac Biomarkers

Gal-3, GDF-15, and sST-2 were measured in batch in 2017 from ethylenediamine tetraacetic acid plasma from baseline samples stored at 70 °C at the University of Pennsylvania Laboratory. All assays were measured in duplicate. Gal-3, GDF-15, and sST-2 were measured using enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) and had intra-assay coefficients of variation of 4.0%, 2.0%, and 2.6%, respectively.

HsTnT and NT-proBNP were measured at baseline in 2008 from ethylenediamine tetraacetic acid plasma stored at −70 °C using a chemiluminescent microparticle immunoassay (www.roche-diagnostics.us) on the ElecSys 2010 (Roche, Indianapolis, IN). HsTnT was measured using a highly sensitive assay with a range of values from 3 to 10,000 pg/ml.[30] The coefficient of variation was 6.0% at a level of 26 pg/ml and 5.4% at 2140 pg/ml. The value at the 99th percentile cutoff from a healthy reference population was 13 pg/ml for hsTnT with a 10% coefficient of variation.[30] The range of values for NT-proBNP was from 5 to 35,000 pg/ml, and the coefficient of variation was 9.3% at a level of 126 pg/ml, and 5.5% at 4319 pg/ml.

Echocardiographic Measures

Assessments of cardiac structure and function were performed using echocardiography according to American Society of Echocardiography guidelines.[31] Transthoracic echocardiograms were obtained at the individual sites in accordance with a standard imaging

Table 1. Baseline characteristics of participants in the Chronic Renal Insufficiency Cohort Study who were included versus excluded from analysis

Demographics	Excluded (N = 1838)	Included (N = 2101)	P value
Age	57.7 (11.2)	57.7 (10.8)	0.96
Male	1064 (58)	1097 (52)	<0.001
Race/ethnicity			
Non-Hispanic white	617 (34)	1021 (49)	<0.001
Non-Hispanic black	759 (41)	891 (42)	
Hispanic	403 (22)	94 (4)	
Other	59 (3)	95 (5)	
Comorbidities			
Cardiovascular disease	792 (43)	524 (25)	<0.001
MI/prior revascularization	515 (28)	347 (17)	<0.001
Peripheral vascular disease	159 (9)	103 (5)	<0.001
COPD	72 (4)	52 (2)	0.01
Atrial fibrillation	359 (20)	307 (15)	<0.001
Stroke	211 (11)	181 (9)	0.003
Diabetes	1015 (55)	893 (43)	<0.001
Medications			
ACEi/ARB	1270 (69)	1419 (68)	0.29
Diuretics	1181 (64)	1151 (55)	<0.001
Beta blockers	1000 (54)	930 (44)	<0.001
Clinical variables			
Systolic blood pressure (mm Hg)	131.2 (23.6)	126.2 (20.6)	<0.001
BMI (kg/m^2)	32.9 (8.3)	31.4 (7.2)	0.51
Current smoking	270 (15)	247 (12)	0.008
Laboratory variables			
Serum creatinine (mg/dl)	1.9 (0.7)	1.8 (0.6)	<0.001
24-h protein/creatinine ratio (mg/g; median, IQR)	225.5 (70.1–1199.9)	111.9 (51.2–504.0)	<0.001
eGFR (CKD-EPI) (ml/min per 1.73 m^2)	42.7 (15.4)	45.8 (14.5)	<0.001
<15	2 (0)	2 (0)	<0.001
15–29	408 (22)	317 (15)	
30–44	688 (37)	741 (35)	
45–59	483 (26)	705 (34)	
≥60	257 (14)	336 (16)	

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CBC, complete blood count; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration equation; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; IQR, interquartile range; MI, myocardial Infarction.
Entries are mean (SD) for continuous variables or N (%) for categorical variables, except as noted. P values come from a t test assuming unequal variances for continuous variables, and from a χ^2 test for categorical variables. Included participants were those that had both cardiac biomarker measures and echocardiography.

protocol. Sonographers were initially trained in telephone conference calls and provided with a detailed scanning manual complete with a checklist. The CRIC Central Echocardiography Laboratory at the University of Pennsylvania monitored quality control and adherence to the scanning protocol and provided the sites with evaluations of the quality of the first several hundred echocardiograms. Supplemental training was provided on an as-needed basis. All echocardiograms were then quantified at the CRIC Central Echocardiography Laboratory by a single registered diagnostic cardiac sonographer who was unaware of the identity of participants whose echocardiograms were being analyzed.

Two-dimensional echocardiography had been selected by the CRIC Steering Committee to be used in all primary analyses of CRIC echocardiographic data.[32] We chose to use 5 measurements of cardiac structure and function as outcome measures: LVMI, LVEDV, LVESV, LVEF, and LAD.

Left ventricular mass (LVM) was calculated from 2-dimensional images of the left ventricular short-axis muscle area and apical left ventricular length (LVM = [5/6 area × length]). LVMI was defined using Cornell criteria and indexed to height (in meters) raised to the power of 2.7.[31] Measurements were indexed to height, as opposed to body surface area, which had previously been decided on as the metric to be used for CRIC research and publications. LVESV and LVEDV were indexed for height and reported in ml/m$^{2.7}$. LVEF was calculated using diastolic and systolic left ventricular volumes measured using the single-plane Simpson rule method: LVEF = ([diastolic volume − systolic volume]/diastolic volume) × 100. Left atrial diameter was measured in centimeters.

Covariates

Information for covariates[28,29] was obtained from the study visit at time of enrollment, including demographic characteristics, self-reported comorbid conditions, tobacco use, and medication use. Self-reported history of cardiovascular disease included coronary artery disease, myocardial infarction or revascularization, stroke, and peripheral vascular disease. Those with self-reported HF were excluded from this study. Blood pressure measurement was performed in a quiet standardized setting, and the average of 3 readings was used for the study as previously described.[33] Body mass index was derived as weight in kg divided by height in m^2. Additional covariates included eGFR, determined from serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration equation,[34] 24-hour urine protein, and medication use (angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, diuretics, and beta blockers).

Statistical Analysis

We performed multivariable linear regression analyses with robust Huber-White standard errors to estimate the associations of each novel biomarker (gal-3, GDF-15, sST-2) as a continuous predictor (per SD higher) with each of the 5 echocardiographic measurements (LVMI, LVEDV, LVESV, LVEF, left atrial volume) as continuous outcomes. We found no evidence of nonlinear associations in spline analyses. The use of robust Huber-White standard errors protects against possible heteroskedasticity, and the large sample size

Table 2. Novel and traditional biomarker associations with echocardiogram measurements in chronic kidney disease patients

Biomarker	LV mass index (g/m$^{2.7}$)	LV ejection fraction (%)	LV end systolic volume (ml/m$^{2.7}$)	LV end diastolic volume (ml/m$^{2.7}$)	Left atrial diameter (cm)
GDF-15					
Unadjusted	**3.7 (3.1, 4.2)**	−0.3 (−0.6, 0.0)	**0.7 (0.5, 1.0)**	**1.3 (1.0, 1.7)**	**0.10 (0.07, 0.13)**
Model 1	**1.0 (0.4, 1.7)**	−0.2 (−0.7, 0.3)	**0.4 (0.0, 0.7)**	**0.6 (0.1, 1.1)**	0.00 (−0.03, 0.04)
Model 2	0.5 (−0.1, 1.2)	−0.0 (−0.5, 0.5)	0.1 (−0.2, 0.4)	0.2 (−0.2, 0.7)	−0.02 (−0.06, 0.01)
Galectin-3					
Unadjusted	**2.4 (1.9, 2.9)**	0.0 (−0.3, 0.3)	**0.4 (0.2, 0.6)**	**0.9 (0.6, 1.2)**	**0.04 (0.01, 0.06)**
Model 1	0.3 (−0.2, 0.7)	−0.0 (−0.4, 0.3)	0.1 (−0.1, 0.3)	0.3 (−0.0, 0.6)	0.00 (−0.02, 0.03)
Model 2	0.1 (−0.3, 0.6)	−0.0 (−0.3, 0.3)	0.1 (−0.1, 0.3)	0.2 (−0.1, 0.5)	−0.00 (−0.03, 0.03)
sST-2					
Unadjusted	**1.1 (0.6, 1.7)**	−0.3 (−0.6, 0.0)	**0.5 (0.2, 0.7)**	**0.8 (0.4, 1.1)**	**0.08 (0.05, 0.10)**
Model 1	0.1 (−0.4, 0.6)	−0.1 (−0.4, 0.3)	0.1 (−0.1, 0.3)	0.2 (−0.1, 0.5)	0.02 (−0.00, 0.05)
Model 2	−0.2 (−0.7, 0.3)	0.0 (−0.3, 0.4)	−0.0 (−0.2, 0.2)	0.0 (−0.3, 0.3)	0.01 (−0.01, 0.04)
NT-proBNP					
Unadjusted	**3.7 (3.1, 4.2)**	**−0.8 (−1.2, −0.5)**	**1.1 (0.9, 1.4)**	**1.6 (1.2, 1.9)**	**0.14 (0.11, 0.17)**
Model 1	**2.2 (1.6, 2.8)**	**−1.1 (−1.6, −0.7)**	**1.2 (0.8, 1.5)**	**1.4 (1.0, 1.8)**	**0.13 (0.10, 0.16)**
Model 2	**2.0 (1.4, 2.6)**	**−1.1 (−1.5, −0.7)**	**1.1 (0.8, 1.4)**	**1.3 (0.8, 1.7)**	**0.13 (0.10, 0.16)**
hsTNT					
Unadjusted	**4.7 (4.2, 5.3)**	**−0.8 (−1.1, −0.5)**	**1.3 (1.1, 1.6)**	**2.2 (1.8, 2.5)**	**0.18 (0.15, 0.20)**
Model 1	**2.2 (1.6, 2.8)**	**−0.7 (−1.1, −0.3)**	**0.8 (0.5, 1.1)**	**1.0 (0.6, 1.4)**	**0.06 (0.02, 0.09)**
Model 2	**1.5 (0.9, 2.1)**	−0.3 (−0.8, 0.1)	**0.4 (0.1, 0.7)**	**0.6 (0.2, 1.0)**	0.02 (−0.01, 0.05)

GDF, growth differentiation factor 15; hsTNT, high-sensitivity troponin T; LV, left ventricular; NT-proBNP; N-terminal pro-B-type natriuretic peptide; sST-2, soluble ST-2.
Model 1 was adjusted for age, sex, race/ethnicity, cardiovascular disease, diabetes at baseline, smoking, estimated glomerular filtration rate, log-transformed 24-h urine protein, blood pressure, body mass index, and medication use (angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, diuretics, and beta blockers).
Model 2 was further adjusted for the alternative cardiac biomarkers.
Difference estimates are per 1-SD increment in the log-transformed biomarker.
Statistically significant values are shown in bold type.

combined with the Central Limit Theorem obviates the requirement of normally distributed errors in small sample sizes. Finally, we assumed that individual participants are independent of one another.

In our statistical models, we adjusted for age, sex, race/ethnicity, cardiovascular disease, diabetes, smoking, eGFR, 24-hour urine protein, systolic blood pressure, body mass index, and medication use (angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, diuretics, beta blockers), and this is presented in Model 1. For any associations that were statistically significant, we evaluated for interactions with history of prior cardiovascular disease, sex, and ethnicity. We repeated these analyses using the established clinical biomarkers (NT-proBNP, hsTnT, per SD higher) as predictors. We then performed a second adjusted model that included the alternative cardiac biomarkers as covariates (Model 2).

In secondary analyses, we also modeled each biomarker in categories: quintiles for gal-3, GDF-15, sST2, and NT-proBNP; and tertiles within the detectable range for hsTnT (undetectable group as reference). Missing covariates were multiply imputed using chained equations.[35] The multiple analyses over the imputations were combined using Rubin's rules to account for the variability in the imputation procedure.[36] All analyses were performed using the R 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria) software

environment. Please see Supplementary STROBE Statement for additional information on study design.

RESULTS

Characteristics of Study Participants

Among the 2101 participants, the mean age was 57.7 ± 10.8 years at time of enrollment; 52% were male and 48% female (Table 1). This cohort had a high prevalence of medical comorbidities, including 43% with diabetes, 25% with cardiovascular disease, 17% with prior myocardial infarction or revascularization, and 15% with atrial fibrillation. The mean eGFR was 46 ± 15 ml/min per 1.73 m^2, and the median (interquartile range) urine protein/creatinine ratio was 112 (51–504) mg/g.

Associations of Novel Biomarkers GDF-15, Gal-3, and sST-2 With Echocardiogram Measurements

GDF-15

In the unadjusted model, higher levels of GDF-15 were associated with higher LVESV, LVEDV, and left atrial volume (Table 2). In Model 1, GDF-15 remained significantly associated with higher LVMI (per 1-SD increment, 1.0 g/m$^{2.7}$; 95% CI, 0.4–1.7), LVESV (0.4 ml/m$^{2.7}$; 95% CI, 0.0–0.7), and LVEDV (0.6 ml/m$^{2.7}$; 95% CI, 0.1–1.1), but not with LVEF (−0.2%; 95% CI, −0.7 to 0.3) or LAD (0.0 cm; 95% CI, −0.03 to 0.04; Table 2). In Model 2, once adjusting for the other

Table 3. Associations of novel (Gal-3, GDF-15, sST2) and traditional (NT-proBNP, hsTnT) biomarkers (as quintiles) and echocardiogram measurements in chronic kidney disease

Predictor	Quintile	LV mass index (g/m$^{2.7}$)	LV ejection fraction (%)	LV end systolic volume (ml/m$^{2.7}$)	LV end diastolic volume (ml/m$^{2.7}$)	Left atrial diameter (cm)
GDF-15	(Reference: ≤824)					
	825–1150	–0.1 (–1.5, 1.3)	0.5 (–0.5, 1.6)	–0.6 (–1.3, 0.1)	–0.6 (–1.6, 0.3)	–0.05 (–0.13, 0.02)
	1151–1520	–0.4 (–1.9, 1.0)	–0.1 (–1.3, 1.0)	–0.1 (–0.9, 0.7)	–0.3 (–1.3, 0.8)	–0.05 (–0.13, 0.03)
	1521–2120	0.9 (–0.8, 2.6)	0.2 (–1.1, 1.5)	0.1 (–0.8, 1.0)	0.5 (–0.8, 1.7)	–0.03 (–0.13, 0.06)
	>2120	**2.6 (0.7, 4.4)**	–0.8 (–2.3, 0.6)	0.9 (–0.1, 2.0)	**1.5 (0.2, 2.9)**	0.01 (–0.09, 0.11)
Gal-3	(Reference: ≤8.96)					
	8.97–12	0.4 (–0.9, 1.6)	0.5 (–0.5, 1.4)	–0.3 (–1.0, 0.3)	–0.2 (–1.2, 0.7)	–0.02 (–0.09, 0.05)
	12.1–15.1	**1.5 (0.2, 2.8)**	–0.0 (–1.0, 1.0)	0.1 (–0.6, 0.8)	0.4 (–0.6, 1.3)	–0.03 (–0.10, 0.05)
	15.2–20	0.5 (–0.8, 1.9)	0.1 (–0.9, 1.2)	0.0 (–0.7, 0.7)	0.3 (–0.7, 1.2)	0.04 (–0.04, 0.12)
	>20	**1.7 (0.3, 3.2)**	–0.3 (–1.4, 0.8)	0.6 (–0.1, 1.3)	**1.2 (0.2, 2.3)**	0.01 (–0.07, 0.10)
SST-2	(Reference: ≤10.2)					
	10.3–13.1	–0.9 (–2.3, 0.4)	–0.3 (–1.2, 0.6)	0.1 (–0.5, 0.6)	0.0 (–0.9, 0.9)	–0.02 (–0.09, 0.05)
	13.2–16.3	0.7 (–0.7, 2.2)	–0.6 (–1.5, 0.4)	0.6 (–0.1, 1.2)	0.7 (–0.3, 1.6)	0.01 (–0.06, 0.08)
	16.4–21.6	–1.2 (–2.5, 0.2)	0.1 (–0.9, 1.1)	0.2 (–0.4, 0.8)	0.5 (–0.5, 1.4)	0.02 (–0.06, 0.09)
	>21.6	–0.2 (–1.6, 1.3)	–0.6 (–1.6, 0.5)	0.5 (–0.1, 1.2)	0.8 (–0.2, 1.8)	0.03 (–0.05, 0.11)
NT-proBNP	(Reference: ≤27.9)					
	28–69.7	**2.0 (0.8, 3.3)**	–0.2 (–1.0, 0.7)	**0.6 (0.1, 1.1)**	**1.1 (0.3, 2.0)**	**0.07 (0.00, 0.13)**
	69.8–147	**2.5 (1.2, 3.9)**	–0.7 (–1.6, 0.2)	**0.8 (0.3, 1.4)**	**1.3 (0.4, 2.2)**	**0.12 (0.05, 0.19)**
	147.1–327	**3.0 (1.5, 4.5)**	**–1.3 (–2.4, –0.3)**	**1.5 (0.8, 2.2)**	**2.1 (1.0, 3.1)**	**0.16 (0.09, 0.24)**
	>327	**6.0 (4.2, 7.8)**	**–2.9 (–4.2, –1.7)**	**2.9 (2.0, 3.9)**	**3.5 (2.2, 4.8)**	**0.42 (0.33, 0.51)**
hsTNT	(Reference: ≤10)					
	10.1–14	**1.7 (0.6, 2.9)**	–0.1 (–0.9, 0.8)	0.2 (–0.3, 0.7)	0.3 (–0.5, 1.1)	0.01 (–0.05, 0.08)
	14.1–22.9	**2.8 (1.5, 4.1)**	**–1.0 (–1.9, –0.0)**	**0.8 (0.2, 1.5)**	0.8 (–0.1, 1.8)	**0.07 (0.00, 0.15)**
	>22.9	**5.6 (4.1, 7.2)**	**–1.6 (–2.6, –0.5)**	**1.9 (1.1, 2.6)**	**2.6 (1.5, 3.6)**	**0.16 (0.08, 0.24)**

Gal-3, galectin-3; GDF, growth differentiation factor 15; hsTNT, high-sensitivity troponin T; LV, left ventricular; NT-proBNP; N-terminal pro-B-type natriuretic peptide; sST-2, soluble ST-2. Values are difference (95% confidence interval). Model was adjusted for age, sex, race/ethnicity, cardiovascular disease, diabetes at baseline, smoking, estimated glomerular filtration rate, log-transformed 24-h urine protein, blood pressure, body mass index, and medication use (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics, and beta blockers).
Bold values indicate significant associations in comparison to the reference group.

biomarkers, GDF-15 did not show any significant associations with the echocardiographic measures (Table 2). When GDF-15 was analyzed by quintiles, the highest quintile of GDF-15 (when compared to the lowest) was significantly associated with higher LVMI and a higher LVEDV (Table 3). When we evaluated for possible interactions of GDF-15 with prior cardiovascular disease, sex, and ethnicity, we found a significant interaction between only LVEDV and sex ($P = 0.005$). Among men, the difference in LVEDV (per 1-SD increment in GDF-15) was 1.0 (0.0, 2.0), and among women the difference was –0.1 (95% CI, –1.0, 0.8). The remainder of the interactions were not significant.

Gal-3
In the unadjusted model, higher levels of Gal-3 were associated with higher LVMI, LVESV, LVEDV, and LAD (Table 2). None of these associations were significant in the adjusted models. When Gal-3 was analyzed by quintiles in the adjusted model, no significant associations were observed (Table 3).

sST-2
In the unadjusted model, higher levels of sST-2 were associated with higher LVMI, LVESV, LVEDV, and LAD (Table 2). However, in the adjusted models, none

of these associations remained statistically significant. When analyzed by quintiles in the adjusted model, there were no significant associations between sST2 and the echocardiographic measures (Table 2).

Associations of NT-proBNP and hsTnT With Echocardiogram Measurements
Higher levels of NT-proBNP and hsTnT were each strongly associated with all the echocardiographic measurements of interest in the unadjusted model (Table 2), and the effect size was greater than that seen with GDF-15. In Model 1, higher levels of NT-proBNP were associated with higher LVMI (2.2 g/m$^{2.7}$; 95% CI, 1.6–2.8), higher LVESV (1.2 ml/m$^{2.7}$; 95% CI, 0.8–1.5), higher LVEDV (1.4 ml/m$^{2.7}$; 95% CI, 1.0–1.8), lower LVEF (–1.1%; 95% CI, –1.6 to –0.7), and higher LAD (0.13 cm; 95% CI, 0.10–0.16). These associations were all significant when including the other biomarkers as covariates in Model 2. In Model 1, higher levels of hsTnT were associated with a higher LVMI (2.2 g/m$^{2.7}$; 95% CI, 1.6–2.8), higher LVESV (0.8 ml/m$^{2.7}$; 95% CI, 0.5–1.1), higher LVEDV (1.0 ml/m$^{2.7}$; 95% CI, 0.7–1.4), lower LVEF (–0.7%; 95% CI –1.1 to –0.3), and higher LAD (0.06 cm; 95% CI, 0.02–0.09). When including the other biomarkers as covariates in Model 2, hsTnT

remained associated with LVMI, LVESV, and LVEDV but not with LVEF or LAD. Significant findings were also observed when hsTnT and NT-proBNP were modeled in quintiles (Table 3).

DISCUSSION

In this large cross-sectional analysis of 2101 participants with CKD, we found that GDF-15 was associated with abnormal left ventricular structure (LVMI and LVEDV) and early left ventricular function (LVESV); these associations were attenuated when adjusting for the other cardiac biomarkers. Gal-3 and sST-2 were not associated with any of the echocardiogram measurements. We also confirmed that NT-proBNP and hsTnT were strongly associated with left ventricular structure and function and left atrial structure, and the effect sizes were of greater magnitude than those observed with GDF-15. These results suggest that GDF-15, NT-proBNP, and hsTnT have a role in the development of subclinical HF in patients with CKD.

We found that GDF-15 was associated with abnormal left ventricular structure (higher LVMI and LVEDV) and early left ventricular systolic dysfunction (LVESV), even when adjusted for confounders. GDF-15 expression is increased in response to inflammation and tissue injury, by both cardiovascular and noncardiovascular cell types.[24] In non-CKD patients, GDF-15 has also been associated with higher LVMI.[37,38] In a study of 299 non-CKD patients with hypertension, levels of GDF-15 were higher in persons with left ventricular hypertrophy than in persons without it.[37] More recent data from 5275 patients with atrial fibrillation in the RE-LY trial showed a significant association of GDF-15 with left ventricular hypertrophy (by electrocardiogram criteria), as well as adverse outcomes.[39] Another study of 219 participants with HF found a positive correlation between GDF-15 and LVMI, and a significant difference in levels of GDF-15 in patients with preclinical HF (American College of Cardiology Stage B) compared with controls.[38] Our findings that GDF-15 was associated with LV mass and ventricular volumes but not systolic function (LVEF) may suggest a role of GDF-15 in the pathogenesis of HF with preserved ejection fraction, which results from stiffer and less-compliant ventricles. This association has previously been shown in non-CKD patients.[40–42] Indeed, HF with preserved ejection fraction is more common than HF with reduced ejection fraction in CKD. We do acknowledge that this association was only present in participants within the highest quartile of GDF-15 level.

Alternatively, elevated GDF-15 levels may reflect underlying comorbidities.[43] In the recent PARADIGM-HF study, GDF-15 was an independent marker of risk of hospitalization and mortality in patients with HF with reduced ejection fraction, but it was not modified by sacubitril/valsartan.[44] These findings suggest that current HF therapies may not target global markers of stress and inflammation that are risk factors for morbidity and mortality. CKD itself is an inflammatory condition[45] that leads to HF, and its progression occurs faster among those with higher levels of GDF-15.[22] The directions of causality among inflammation, CKD, and HF remain unclear, but GDF-15 is plausibly an important component of this relationship.

When we included the other biomarkers as covariates in the model, GDF-15 was no longer associated with the echocardiogram measurements of interest. This could suggest some overlap in the pathways between GDF-15 and the other circulating biomarkers. Although the effect of GDF-15 was attenuated after adjusting for the established biomarkers, it is possible that NT-proBNP and hsTnT are more markers of disease than they are causally related to increased LVMI or lower LVEF, and that GDF-15 could be highlighting separate biological pathways. Additionally, hsTnT and NT-proBNP are cardiac-pecific biomarkers, whereas GDF-15 may reflect more systemic rather than organ-specific inflammation. In previous research, levels of GDF-15 plus NT-proBNP better correlated with an HF diagnosis than NT-proBNP alone.[38] Therefore, GDF-15 may provide additional insight into the mechanisms of subclinical HF in CKD.

In our study, Gal-3 was not significantly associated with LV structure or function, or left atrial structure, after adjusting for possible confounders. Thought to represent a link between inflammation and fibrosis,[11] Gal-3 received approval from the US Food and Drug Administration in 2010 to aid in prognosis in patients with chronic heart failure and received a class II recommendation in the American College of Cardiology/American Heart Association/Heart Failure Society of America HF guidelines for risk prediction in HF.[46] In CKD, the association of Gal-3 with cardiovascular events is less certain—some studies have found a significant association,[15] whereas others have not.[25] Our findings add to the literature by studying the association of Gal-3 with subclinical HF.

In our study, there were no associations between sST-2 and echocardiogram measurements when adjusting for confounders. sST-2 is stimulated by myocardial strain and has been associated with ventricular remodeling. In the Framingham Offspring Study, elevated sST-2 was associated with increased risk for HF, major cardiovascular events and death.[12] sST2 may provide prognostic information and be

useful for serial chronic HF monitoring.[47] In CKD patients, sST-2 has been associated with mortality, but a previous study reported no independent association between sST-2 and HF or atherosclerotic cardiovascular disease.[25] Given the findings of our study and others, further research is needed to determine the utility of sST-2 in CKD patients.

Although previous studies in CKD patients have shown associations between cardiac biomarkers and clinical outcomes, in this study, we were able to identify early subclinical echocardiographic changes, captured by LVMI, LVEDV, LVESV, and LAV, which precede development of clinical cardiovascular disease.[8,48] Previous research in CKD patients using the CRIC cohort has shown associations of NT-proBNP and hsTnT with abnormalities of left ventricular structure (increased LVMI) and function (reduced ejection fraction).[27] Here, we expand on these previous studies by showing an association between NT-proBNP and hsTnT with a more comprehensive panel of echocardiographic measurements of left ventricular structure and function, as well as LAV. These associations of NT-proBNP and hsTnT with echocardiographic abnormalities were qualitatively larger than the newer cardiac biomarkers, although these established biomarkers may be reflections of disease status rather than physiological mediators of the progression to heart failure.

Prior studies also had not evaluated the newer biomarkers (Gal-3, GDF-15, sST2) and echocardiographic parameters. Our findings suggest that there are measurable and significant changes to cardiac structure occurring prior to the onset of clinical HF in CKD patients that can be measured in part by select serum biomarker measurements. This may provide an important window of opportunity in cases in which intervening on risk factors such as subclinical ischemia or microvascular disease (which elevate NT-proBNP and hsTnT) may mitigate progression to adverse clinical events.

Our study had numerous strengths. We evaluated a large, diverse, well-characterized population of patients with CKD. The study was well powered to identify even small associations between our predictors and outcomes of interest. We adjusted for an extensive list of possible confounding variables. We found strong associations with NT-proBNP and hsTnT and the echocardiogram measurements of interest, which suggests that the outcome variables were appropriately measured and the lack of associations with Gal-3 and sST-2 were not due to a failure of study design or power. We recognize a few limitations. There were a significant number of patients that were excluded due to missing data, which could bias the analyses. We did

not quantify right-sided pressures, or inferior vena cava diameter. Additionally, because there was a 1-year time lag between biomarker and echocardiographic measurements, it is possible that we missed associations that would have been observed had they been measured simultaneously. Previous studies have reported possible associations with GDF-15 and anemia[49–55]; however, we did not have detailed information on iron stores or other measures of anemia to fully evaluate this association in the present study. This was an observational study, so we cannot determine causality. Finally, this was a study of research volunteers, so findings may not be applicable to the general CKD population.

In conclusion, among patients with CKD, GDF-15 was associated with abnormal left ventricular structure and early changes in left ventricular function, whereas no associations were seen with Gal-3 and sST-2. However, these findings were attenuated when adjusting for NT-proBNP and HsTNT. NT-proBNP and hsTnT were strongly associated with measures of left ventricular structure and function as well as left atrial structure. Collectively, these biomarkers may help identify biological pathways that can contribute to development of subclinical cardiac disease in patients with CKD.

APPENDIX

CRIC Study Investigators

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DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)
STROBE Statement.

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