Title Page:

Association of Cystatin C with Left Ventricular Structure and Function: The Dallas Heart Study
Parag C. Patel, MD*, Colby R. Ayers, MS†, Sabina A. Murphy, MPH‡, Ronald Peshock, MD*†, Amit Khera, MD, MSc*†, James A. de Lemos, MD*†, Jody A. Balko, MSc*, Sachin Gupta, MD*, Pradeep PA Mammen, MD*, Mark H. Drazner, MD, MSc*†, and David W. Markham, MD*†

*Division of Cardiology, University of Texas Southwestern Medical Center, Dallas, Texas
†Donald W. Reynolds Cardiovascular Clinical Research Center, University of Texas Southwestern Medical Center, Dallas, Texas
‡Brigham and Women’s Hospital, Boston, Massachusetts.

Correspondence to Dr. David Markham, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd. HA9.133, Dallas, TX 75390-9047

Fax: 214-645-7501 Phone: 214-645-7500 Email: David.Markham@utsouthwestern.edu

Word Count - 5748

Subject Codes: 10, 15, 115
Abstract

Background: Cystatin C, a novel marker of renal function, has been associated with heart failure and cardiovascular mortality in older individuals. We tested the hypothesis that cystatin C is associated with preclinical cardiac structural and functional abnormalities in a younger population-based sample.

Methods and Results: The study included participants in the Dallas Heart Study (ages 30 to 65 years) who had measurements of cystatin C and cardiac magnetic resonance imaging. The associations of cystatin C with LV mass, LV end systolic and diastolic volumes (LVESV and LVEDV), concentricity (LV mass/LVEDV), LV wall thickness, and LVEF were evaluated.

Cystatin C levels ranged from 0.46 to 6.55 mg/l. In univariable analyses, increasing levels of cystatin C correlated with higher LV mass, concentricity, and wall thickness (p < 0.001), but not with LVESV, LVEDV, or LVEF. After adjustment with traditional covariates and estimated glomerular filtration rate (GFR) by the Modification of Diet in Renal Disease formula, log-transformed cystatin C remained independently associated with LV mass (p < 0.001), concentricity (p = 0.027), and wall thickness (p < 0.001). These associations persisted when creatinine or estimated GFR by the Cockcroft-Gault formula were included in the models.

Conclusions: Higher levels of cystatin C were associated with increased LV mass and a concentric LVH phenotype. These findings were independent of potential confounding variables including standard measurements of renal function, supporting the hypothesis that cystatin C may be useful to identify individuals with preclinical structural heart abnormalities.
Key Words: Cystatin C, Concentricity, Left Ventricular Hypertrophy, and Dallas Heart Study
Introduction

Abnormal renal function is known to be an independent risk factor for atherosclerotic cardiovascular disease, heart failure, and increased overall cardiovascular mortality.\textsuperscript{1-4} Traditionally, glomerular filtration rate (GFR) has been estimated from serum creatinine or creatinine-based equations. However, this approach has numerous limitations including a nonlinear relationship between creatinine and GFR, inability of creatinine to detect small changes in GFR, and difficulty in accounting for the confounding influences of non-renal factors (i.e. age, body weight, ethnicity, and sex).\textsuperscript{5}

Recently, cystatin C has been shown to be a biomarker of renal function that overcomes many of the above limitations.\textsuperscript{6} Concentrations of cystatin C are independent of age, sex, and muscle mass.\textsuperscript{6-9} In many studies, serum cystatin C has performed better than creatinine and is at least comparable to creatinine-based equations to estimate GFR, primarily when assessing mild to moderate renal impairment.\textsuperscript{9-11} These qualities make cystatin C measurement attractive for the detection of subtle changes in renal function.

Further, cystatin C has been shown to be a risk factor for heart failure and cardiovascular disease mortality.\textsuperscript{12-17} We hypothesized that cystatin C could be a marker for subclinical cardiac structural and functional abnormalities. We tested this hypothesis in 2548 participants in the Dallas Heart Study, a multiethnic, population-based sample of Dallas county residents between 30 and 65 years old.\textsuperscript{18}
Methods

The Dallas Heart Study

Details of the design and methods of the Dallas Heart Study have been previously reported. In summary, we conducted a multistage probability sampling of the estimated 1.43 million civilian, non-institutionalized English- or Spanish-speaking Dallas County residents based on the U. S. Postal Service 2000 Delivery Sequence File. Oversampling was performed in the black population so that they represented 50% of the final cohort. The study was conducted in three sequential visits: 1) an initial home visit, including a household survey (ages 18 – 65, n=6101), 2) a second home visit (ages 30 – 65, n= 3399) to obtain blood and urine specimens, and 3) a clinic visit at the University of Texas at Southwestern Medical Center to perform a cardiac MRI and DEXA scan (ages 30 - 67, n=3072).

The survey was completed by trained field interviewers at the first home visit. They obtained detailed information on patient demographics, medical history, and medications. Demographic variables and blood pressures were similar between subjects completing all three visits. Sampling weights reflecting the different probabilities of selection and sample attrition were created at each step so that the findings could be extrapolated back to the target population. In the present study, patients were included in this analysis if they completed cardiac MRI and cystatin C measurements (n = 2548).

Measures of renal function

Cystatin C

Venous blood was collected in standard blood collection tubes and samples were maintained at 4° C for ≤ 4 hours and then centrifuged (1430g for 15 minutes) at 4° C. Plasma was then removed and frozen at -70° C until assays were performed. Measurements of serum cystatin C were completed with a BNII nephelometer (Dade Behring, Inc., Deerfield, IL; now Siemens Healthcare Diagnostics, Inc)
with a particle enhanced immunonephelometric assay (N Latex Cystatin C, Dade Behring, Inc.). The nephelometer utilized monoclonal antibodies coated on polystyrene particles which agglutinate to increase the intensity of scattered light based on cystatin C concentrations. The inter- and intra-assay coefficient of variations (CV) were less than 3.5%. The range of detection of the assay was 0.195 – 7.330 mg/L.

**Creatinine and estimated Glomerular Filtration Rate**

A Beckman Coulter analyzer (Beckman Coulter, Fullerton, CA) was utilized for measuring serum creatinine concentrations through the alkaline picrate method. The inter- and intra-assay CVs for serum creatinine were less than 5.0%. Glomerular filtration rate (GFR, ml/min per 1.73 m² BSA) was estimated with the modified Modification of Diet in Renal Disease Study formula (MDRD): GFR = 186 x (serum creatinine⁻¹.154) x (age⁻⁰.203) x 0.742 (if female) x 1.21 (if black) and the Cockcroft-Gault formula: GFR = [(140 – age) x weight (kg)] / [72 x creatinine (mg/dl)] x 0.85 (if female).

**Cardiac MRI**

The MRI protocol has been previously described. Briefly, two comparable 1.5 Tesla MRI systems (Philips Medical Systems) were used for all of the cardiac MRIs. Short-axis breath-hold, electrocardiographic-gated cine MR images were obtained from the apex to the base of the left ventricle using the following parameters: 6mm slice, 4mm gap, field of view of 36-40, acquired pixel size at 36cm field of view 1.29 x 2.58, and temporal resolution of 40 msec. MASS software (Medis medical imaging systems, Leiden, The Netherlands) was used to analyze the data. Endocardial and epicardial borders were traced manually allowing calculation of ventricular volumes. Left ventricular ejection fraction (LVEF) was calculated in the standard fashion from the endocardial volumes:
ejection fraction = 100*(end-diastolic volume (LVEDV) – end-systolic volume (LVESV))/end-diastolic volume). Inter-observer difference for LVEDV was −3.0±9.4 mL, intra-observer difference was −2.7±3.2 mL, and inter-scan variability was −2.3±8.9 mL. Inter-observer difference for LVEF was −4±4%, intra-observer difference was 1±1%, and inter-scan variability was −1.3±2.5%. Inter-observer difference for LV mass was 9.2 ±5g (n=1), intra-observer difference was 10.5 ±8.6 grams (n=8), and inter-scan variability was 4.9 ±10.9g (n=8).21

**Covariates**

All Dallas Heart Study participants completed a computerized questionnaire during the initial home visit. The questionnaire consisted of 27 modules that were administered in either Spanish or English and queried medical history, use of medications, and use of tobacco. Race and ethnicity were self-reported. BP was measured as previously described.21 CRP measurements (Roche Diagnostics, Indianapolis, IN) and brain natriuretic peptide (BNP, Biosite, San Diego, CA) were measured as described.23 Body composition was measured by DEXA scans (Delphi W unit, Hologic, Inc, Bedford, Mass) as described.23, 24

**Variable Definitions**

Hypertension was defined as a self-reported diagnosis, utilization of antihypertensive medications, or an average systolic blood pressure from the 3 separate measurements ≥ 140 mmHg or an average diastolic blood pressure ≥ 90 mmHg. Diabetes was defined as previously.25 Concentricity was defined as left ventricular mass (LV Mass) divided by LVEDV. The criteria for left ventricular hypertrophy (LVH) were: LV mass/ body surface area (BSA) ≥ 89 g/m² (women) and ≥ 112 g/m².
(men), LV mass/height$^{2.7} \geq 39 \text{ g/m}^{2.7}$ (women) and $\geq 48 \text{ g/m}^{2.7}$ (men), and LV mass/fat-free mass $\geq 3.8$ g/kg (men and women) as before.$^{21}$

**Statistics**

Smoothed histograms of cystatin C concentrations were constructed for each sex. Differences in cystatin C concentrations between men and women were compared using Wilcoxon Rank Sum Tests because of non-normally distributed data. Cystatin C levels were categorized into gender specific quartiles and baseline characteristics were compared across quartiles using analysis of variance or the Kruskal-Wallis test for continuous variables and chi-square for categorical variables. Multivariable linear regression analysis was used to evaluate the association of the predictor variable, log-transformed cystatin C (logcystatin C), with the following outcome variables: LVEDV, LV mass, concentricity (LV mass / LVEDV), LV wall thickness, and LVEF. Multivariable logistic regression analysis was used to evaluate the association of the predictor variable, logcystatin C, with the outcome variable LVH. Cystatin C concentrations were logarithmically transformed because their distribution did not follow a Gaussian distribution. The following covariates were considered potential confounders that influence the development of LV structural abnormalities: age, sex, race, diabetes, history of hypertension, systolic blood pressure, history of coronary artery disease, height$^{2.7}$, lean mass, fat mass, C-reactive protein (CRP), and log transformed BNP (model 1). In additional models, traditional markers of renal function were added to model 1 as covariates: creatinine (model 2), estimated GFR by the MDRD (model 3), and estimated GFR by Cockcroft-Gault (model 4). For statistical analysis, SAS 9.13 (SAS Institute, Inc., Cary, NC) was used. The authors had full access to the data and take responsibility for its integrity. All authors have read and agreed on the manuscript as written.
Results

Cystatin C Baseline Characteristics in DHS

Cystatin C levels ranged from 0.46-6.55 mg/L with a median of 0.82 mg/L (interquartile range: 0.74 to 0.92 mg/L) (Table 1 and Figure 1a). The range of cystatin C concentrations for all patients by quartiles is as follows: quartile I: \( \leq 0.73 \text{ mg/L} \), quartile II: 0.74 to 0.82 mg/L, quartile III: 0.83 to 0.92 mg/L, and quartile IV: \( \geq 0.93 \text{ mg/L} \). Median and interquartile ranges of cystatin C by gender are shown in the footnotes of Table 1. Women had significantly lower levels of cystatin C compared to men (\( p < 0.001 \)) (Figure 1b). Baseline characteristics of the study sample, stratified by gender-specific quartiles of cystatin C, are shown in Table 1. Participants with higher concentrations of cystatin C were more likely to be older, hypertensive, and on antihypertensive medications. Those with higher cystatin C levels were less commonly black and were more likely to have diabetes and higher BMIs. As anticipated, increasing quartiles of cystatin C were associated with higher levels of creatinine and lower estimated glomerular filtration rates.

Cystatin C and LV Structure

The association of LV structural characteristics with gender-specific quartiles of cystatin C are shown (Table 2). Higher measurements of cystatin C were associated with increased LV mass, indexed LV mass (LV mass/ height\(^{2.7} \) and LV mass/BSA), concentricity, and wall thickness. Similar associations were present when cystatin C was modeled as a log transformed continuous variable. In contrast, cystatin C was not associated with LVEF, LVESV or LVEDV.
In multivariable linear regression analysis adjusting for age, sex, race, diabetes, history of hypertension, systolic blood pressure, history of coronary artery disease, fat mass, lean mass, height, CRP, and log transformed BNP (model 1), cystatin C remained independently associated with LV mass, concentricity, and LV wall thickness (Table 3). This association persisted after further adjustment for traditional measures of renal function including creatinine (model 2), estimated GFR by MDRD (model 3), and estimated GFR by Cockcroft-Gault (model 4). In subgroup multivariable analyses, there were significant associations of cystatin C with LV mass and wall thickness in hypertensives and in African Americans (Table 4). There was a strong trend for the association of cystatin C with LV concentricity in these two groups. These associations were not present in normotensives or whites. The interaction p values for both of these subgroups with logcystatin C are described in the footnotes of table 4.

Cystatin C was also significantly associated with LVH when defined by indexation to BSA in models 2-4 (model 3: OR 3.8, CI 1.7-8.5, p = 0.001). As with LV mass, this association remained present in patients with a history of hypertension (model 3: OR 3.9, CI 1.4-10.5, p = 0.007) but not in patients without a history of hypertension (model 3: OR 2.5, CI 0.5-12.5, p = 0.28) and was more robust in blacks (model 3: OR 4.0, CI 1.6-9.9, p = 0.003) than whites (model 3: OR 5.5, CI 0.4-81, p = 0.22).

Discussion and Conclusions

The Dallas Heart Study is the first study evaluating the association of cystatin C with LV structure and function in the general population. The major finding of this study is that cystatin C was independently associated with a specific cardiac phenotype of concentric hypertrophy, including
increased LV mass, concentricity, and wall thickness even after adjustment for various traditional measures of renal function. This association was particularly robust in hypertensives and blacks. Cystatin C was not associated with LV systolic function or volume. Our findings suggest that cystatin C may be a potential predictor of the presence or development of cardiac structural abnormalities.

The only prior study to our knowledge evaluating the association of cardiac structural abnormalities with cystatin C was the Heart and Soul Study (HS). This study evaluated echocardiograms in 818 participants with known coronary artery disease but without a history of clinical heart failure. Although this study did not adjust for traditional measures of renal function, after multivariable analysis higher levels of cystatin C were significantly associated with LVH and diastolic dysfunction. LVEF was not significantly associated with cystatin C after multivariable adjustment. Compared to the Heart and Soul Study, the Dallas Heart Study participants were younger and healthier with less comorbidities. As expected, the DHS cohort had lower levels of cystatin C (DHS: median 0.82 mg/L, IQ range 0.74-0.92 mg/L; Heart and Soul: median 1.05 mg/L, IQ range 0.91-1.28 mg/L). Because cystatin C maintained similar associations with measures of concentricity in this study, we postulate that the DHS participants may represent a pre-clinical population compared to the Heart and Soul study. Thus, the association of cystatin C with subclinical measures of concentricity may precede the clinical development of diastolic dysfunction.

Cystatin C: Clinical and Research Considerations

Three hypotheses potentially explain the association of cystatin C with a concentric phenotype: 1) Cystatin C may be a stronger predictor of preclinical changes in cardiovascular structure because it is a better estimate of renal function when compared to traditional measures of GFR. 2) Cystatin C may
be primarily associated with a hypertensive phenotype and, as a result, be indirectly associated with subclinical concentricity. 3) Cystatin C may be directly associated with the development of concentricity. We will discuss each of these separately.

As a more sensitive marker of renal function (hypothesis 1), cystatin C may better predict changes in LV structure compared to other biomarkers. Previous studies implicate cystatin C as a sensitive marker for early renal damage and as a predictor of renovascular disease in patients with essential hypertension, preeclampsia, and atherosclerotic hypertensive disease. Severity of renal disease has been linked to diastolic dysfunction, concentricity, and changes in LV geometry in different subsets of patients. Because cystatin C may more accurately estimate glomerular function in people with normal and mildly decreased renal function as compared to serum creatinine, cystatin C may be a better marker of subclinical cardiorenal disease.

Cystatin C may be directly associated with a hypertensive phenotype and indirectly associated with LV concentricity (hypothesis 2). Prior studies have suggested an association of cystatin C with hypertension. In the Physician’s Health Study, the association of heart failure with cystatin C was limited to hypertensive individuals during subgroup analysis. In patients with coronary artery disease, cystatin C was linearly associated with systolic blood pressure even in people with normal renal function. In the Dallas Heart Study, blacks had 2-3 times increased prevalence of hypertension and left ventricular hypertrophy when compared to the whites. The robust association of cystatin C with LV mass in the black subgroup may be explained by the significantly increased prevalence of hypertension in this population. Thus, cystatin C could be a surrogate of a hypertensive phenotype that influences chronic changes in LV structure.
Cystatin C may directly be associated with the development of concentricity (hypothesis 3). Because the association of cystatin C with measures of LV concentricity is independent of traditional markers of renal function in our study, one could hypothesize that cystatin C is more than simply a measure of renal function and may directly influence changes in LV structure. The balance between cysteine proteases, such as cathepsin B, S, and K, with cysteine protease inhibitors, such as cystatin C, has been implicated in the pathogenesis of cardiac remodeling in hypertrophied and failing hearts.\(^{33-35}\)

Cathepsins are responsible for the physiological digestive turnover of cellular molecules, and abnormal levels may adversely influence cardiac remodeling.\(^{35}\) Even though the mechanistic link between cystatin C and LV concentricity is unknown, it is possible that the alteration of the balance between these two families of proteins may lead to the development of concentricity and hypertrophy.

In order to elucidate which of these three hypotheses truly define the mechanism of the association of cystatin C with LV structure, additional studies are needed. Additional insights could be garnered from cystatin C knockout or overexpressing animal models, or in other animal models of cardiac hypertrophy. Further, given the association of cystatin C with diastolic dysfunction, incident heart failure, cardiac events, and all-cause mortality in ambulatory patients with coronary disease\(^{15,16}\), longitudinal studies that assess the utility of cystatin C as a predictor for the development of cardiac structural abnormalities or future adverse outcomes are warranted.

**Study Limitations**

Strengths of this study include the large sample size and the detailed phenotyping of subjects, including MRI measurements of cardiac structure and function and DEXA assessments of lean and fat...
This allowed for a more precise determination of the associations of cystatin C with cardiac parameters. Several limitations should be considered when interpreting our results. The Dallas Heart Study is a cross-sectional study; therefore, causality between cystatin C and LV mass should not be inferred. The assessment of coronary artery disease, hypertension, and diabetes, were based upon self-report and may be subject to recall or response bias. Further, a participant’s willingness to participate may have introduced selection bias; however, multiple efforts were made to minimize this possibility.

As such, we previously have demonstrated that inferences from the DHS can be extrapolated back to its reference population (Dallas County). In addition, single measurements of cystatin C were taken for each patient. Whether daily variations exist with this biomarker is unknown. Some studies also suggest that corticosteroids, inflammation and abnormalities of thyroid function influence cystatin C concentrations. Due to the limited data that was available, adjustment for these potential confounders was not possible. Since most of the patients in the Dallas Heart Study had normal renal function, measures of estimated GFR (MDRD and Cockcroft-Gault) might not be as accurate as cystatin C for predicting subclinical changes in cardiovascular structure in this cohort. Also, since the study participants were a random sample of the general population, our conclusions may not apply to patients with cardiovascular disease who are typically older and with some degree of renal dysfunction.

Conclusions

Higher levels of serum cystatin C were independently associated with LV mass, concentricity, and LV wall thickness even after adjustment with traditional markers of renal function. This association was particularly strong in hypertensive and black subjects, suggesting that the association of cystatin C with concentric LVH may be mediated via elevated blood pressure. Further study of cystatin C may
provide insights into the mechanisms linking renal function and cardiac structural abnormalities.
Acknowledgements

None

Funding Sources

The Dallas Heart Study is funded by a center grant from the Donald W. Reynolds Foundation, Las Vegas, Nevada.

This work was supported by an unrestricted grant from Dade Behring, Incorporated, now Siemens Healthcare Diagnostics, Incorporated.

DM is supported by the University of Texas Southwestern Clinical Scholars Program (North and Central Texas Clinical and Translational Science Initiative - KL2).

Conflict of Interest Disclosures

JD has served as a consultant for Roche and has consulting and grant funding from Biosite. The other authors do not have any pertinent disclosures related to this manuscript.
References:


Table Legend:

Table 1: Baseline Characteristics of Participants by Gender-Specific Cystatin C Quartiles

Table 2: LV Parameters Stratified by Gender-Specific Cystatin C Quartiles

Table 3: Multivariable Analysis Evaluating the Association of Logcystatin C with LV Parameters

Table 4: Subgroup Multivariable Analysis Evaluating the Association of Logcystatin C with LV Parameters as a Function of Hypertension and Race

Figure Legend:

Figure 1: Distribution of Cystatin C in the Dallas Heart Study in (A) all participants and (B) participants stratified by gender
Table 1. Baseline Characteristics of Participants by Gender Specific Cystatin C Quartiles

<table>
<thead>
<tr>
<th></th>
<th>Cystatin C Quartiles‡†</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Pts</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td></td>
<td>P_trend</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N= 2548</td>
<td>642</td>
<td>636</td>
<td>630</td>
<td>640</td>
<td></td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, (SD)</td>
<td>45 (9.3)</td>
<td>41 (8.0)</td>
<td>43 (8.7)</td>
<td>46 (8.8)</td>
<td>49 (9.4)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, %</td>
<td>44</td>
<td>27</td>
<td>43</td>
<td>52</td>
<td>52</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, % (n=809)</td>
<td>32</td>
<td>22</td>
<td>33</td>
<td>35</td>
<td>38</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black, % (n=1251)</td>
<td>49</td>
<td>53</td>
<td>48</td>
<td>47</td>
<td>48</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic, % (n=429)</td>
<td>17</td>
<td>24</td>
<td>16</td>
<td>16</td>
<td>12</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical History</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI, %</td>
<td>2.6</td>
<td>0.5</td>
<td>2.0</td>
<td>2.9</td>
<td>5.0</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD, %</td>
<td>15</td>
<td>12</td>
<td>14</td>
<td>14</td>
<td>21</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHF, %</td>
<td>2.8</td>
<td>1.6</td>
<td>2.2</td>
<td>2.5</td>
<td>5.0</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTN, %</td>
<td>29</td>
<td>21</td>
<td>24</td>
<td>32</td>
<td>41</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>11</td>
<td>11</td>
<td>7</td>
<td>10</td>
<td>15</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking history, %</td>
<td>28</td>
<td>22</td>
<td>26</td>
<td>27</td>
<td>34</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal Function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr (mg/dl), (SD)</td>
<td>0.89 (0.34)</td>
<td>0.79 (0.17)</td>
<td>0.87 (0.26)</td>
<td>0.88 (0.19)</td>
<td>1.01 (0.56)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDRD (mL/min/1.73m²), (SD)</td>
<td>100 (23)</td>
<td>114 (22)</td>
<td>102 (18)</td>
<td>98 (23)</td>
<td>86 (21)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cockcroft-Gault (mL/min), (SD)</td>
<td>122 (40)</td>
<td>130 (38)</td>
<td>121 (35)</td>
<td>124 (43)</td>
<td>114 (42)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg), (SD)</td>
<td>125 (19)</td>
<td>120 (16)</td>
<td>122 (17)</td>
<td>125 (19)</td>
<td>129 (21)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mmHg), (SD)</td>
<td>78 (10)</td>
<td>76 (10)</td>
<td>78 (10)</td>
<td>79 (11)</td>
<td>80 (10)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg), (SD)</td>
<td>84.9 (21.1)</td>
<td>79.0 (18.0)</td>
<td>82.4 (18.5)</td>
<td>87.0 (22.3)</td>
<td>91.5 (23.1)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²), (SD)</td>
<td>30.4 (7.3)</td>
<td>28.5 (6.0)</td>
<td>29.4 (6.3)</td>
<td>31.0 (7.6)</td>
<td>32.7 (8.3)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
BSA (m²) 1.93 (0.24) 1.87 (0.23) 1.91 (0.23) 1.95 (0.25) 2.00 (0.25) <0.001
Lean Mass (kg), (SD) 55.1 (12.2) 53.3 (12.0) 55.0 (12.1) 55.7 (12.6) 56.2 (11.8) <0.001
Fat Mass (kg), (SD) 27.1 (11.9) 23.9 (10.2) 25.5 (10.9) 28.0 (12.2) 31.1 (13.1) <0.001
BNP (ng/L), (SD) 11.9 (31.2) 8.1 (14.0) 10.5 (25.1) 12.0 (30.4) 16.9 (45.6) <0.001
CRP (mg/dl), (SD) 5.0 (5.5) 4.2 (5.1) 4.4 (5.1) 5.0 (5.3) 6.4 (6.1) <0.001

**Medications**

<table>
<thead>
<tr>
<th>Medications</th>
<th>21</th>
<th>12</th>
<th>16</th>
<th>22</th>
<th>32</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antihypertensive meds, %</td>
<td>10</td>
<td>6</td>
<td>7</td>
<td>11</td>
<td>15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Beta blockers, %</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACE-I, %</td>
<td>11</td>
<td>8</td>
<td>7</td>
<td>12</td>
<td>17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ARB, %</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>8</td>
<td>17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diuretics, %</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>7</td>
<td>13</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* All values are listed as means unless otherwise specified.

† Cystatin C concentrations (mg/L) of males by quartiles: I: ≤0.77, II: 0.78 to 0.85, III: 0.86 to 0.95, IV: ≥0.96

‡ Cystatin C concentrations (mg/L) of females by quartiles: I: ≤0.71, II: 0.72 to 0.79, III: 0.80 to 0.89, IV: ≥0.90

§ Cystatin C median (IQ Range) of all patients by quartiles: I: 0.69 (0.65-0.71), II: 0.79 (0.75-0.81), III: 0.87 (0.83-0.89), IV: 1.01 (0.96-1.10)

Cystatin C median (IQ Range) of males by quartiles: I: 0.72 (0.69-0.75), II: 0.81 (0.8-0.83), III: 0.90 (0.88-0.92), IV: 1.03 (0.99-1.12)

Cystatin C median (IQ Range) of females by quartiles: I: 0.66 (0.62-0.69), II: 0.76 (0.74-0.78), III: 0.84 (0.81-0.86), IV: 0.99 (0.93-1.09)
Table 2. LV Parameters Stratified by Gender Specific Cystatin C Quartiles

<table>
<thead>
<tr>
<th>Outcome Variables</th>
<th>Quartile I</th>
<th>Quartile II</th>
<th>Quartile III</th>
<th>Quartile IV</th>
<th>Ptrend</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF (%)</td>
<td>72.2 (6.9)</td>
<td>71.5 (8.0)</td>
<td>71.9 (8.2)</td>
<td>72.4 (7.7)</td>
<td>NS</td>
</tr>
<tr>
<td>LVESV (ml)</td>
<td>28.3 (12.2)</td>
<td>30.2 (14.4)</td>
<td>29.2 (15.6)</td>
<td>28.5 (14.47)</td>
<td>NS</td>
</tr>
<tr>
<td>LVEDV (ml)</td>
<td>101.8 (22.6)</td>
<td>101.8 (25.0)</td>
<td>102.6 (28.0)</td>
<td>101.1 (27.2)</td>
<td>NS</td>
</tr>
<tr>
<td>LV Mass (g)</td>
<td>158.7 (41.3)</td>
<td>159.8 (45.4)</td>
<td>165.2 (48.0)</td>
<td>171.2 (49.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LV Mass/HT² (g/m²⁻³)</td>
<td>39.7 (8.5)</td>
<td>39.9 (9.6)</td>
<td>40.4 (9.4)</td>
<td>42.2 (12.3)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Concentricity (g/ml)</td>
<td>1.58 (0.32)</td>
<td>1.59 (0.35)</td>
<td>1.64 (0.41)</td>
<td>1.74 (0.46)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Wall Thickness (mm)</td>
<td>11.4 (1.7)</td>
<td>11.4 (1.8)</td>
<td>11.7 (1.9)</td>
<td>12.2 (2.0)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

*Standard of deviations are listed in parenthesis

†See Table 1 for gender specific quartile values of cystatin C
Table 3. Multivariable Analysis Evaluating the Association of Logcystatin C with LV Parameters

<table>
<thead>
<tr>
<th>Outcome Variables</th>
<th>$\beta_{\text{Logcystatin C}}$</th>
<th>Adjusted P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVESV (ml)</td>
<td>-0.8 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>LVEDV (ml)</td>
<td>3.8 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>LV Mass (g)</td>
<td>13.8 ± 3.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Concentricity (g/ml)</td>
<td>0.09 ± 0.04</td>
<td>0.0271</td>
</tr>
<tr>
<td>Wall Thickness (mm)</td>
<td>0.62 ± 0.16</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, race, diabetes, history of hypertension, systolic blood pressure, history of CAD, fat mass, lean mass, height$^2$, CRP, logbnp, and MDRD.

Model 3 is shown. Models 1, 2, and 4 had similar p values.

†Beta coefficient of logcystatin C ± standard error are reported for each outcome variable.
Table 4. Subgroup Multivariable Analysis Evaluating the Association of Logcystatin C with LV Parameters as a Function of Hypertension and Race

<table>
<thead>
<tr>
<th></th>
<th>LV Mass</th>
<th>Wall Thickness</th>
<th>Concentricity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta_{\text{Logcystatin C}}$</td>
<td>Adjusted P</td>
<td>$\beta_{\text{Logcystatin C}}$</td>
</tr>
<tr>
<td>Hypertension, (n = 842)*$^\dagger$</td>
<td>19.7 ± 6.2</td>
<td>0.002</td>
<td>0.83 ± 0.28</td>
</tr>
<tr>
<td>No Hypertension, (n = 1670)*$^\dagger$</td>
<td>2.5 ± 3.9</td>
<td>0.532</td>
<td>0.20 ± 0.20</td>
</tr>
<tr>
<td>Black, (n = 1251)$^\dagger$§</td>
<td>21.3 ± 5.0</td>
<td>&lt;0.001</td>
<td>0.79 ± 0.23</td>
</tr>
<tr>
<td>White, (n = 809)$^\dagger$§</td>
<td>-0.1 ± 6.6</td>
<td>0.987</td>
<td>0.48 ± 0.32</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, race, diabetes, systolic blood pressure, history of CAD, fat mass, lean mass, height$^2$, CRP, logbnp, and eGFR (MDRD).

$^\dagger$ Adjusted for age, sex, diabetes, history of hypertension, systolic blood pressure, history of CAD, fat mass, lean mass, height$^2$, CRP, logbnp, and eGFR (MDRD).

§ Interaction p values between hypertension/no hypertension and logcystatin C are < 0.0001 for LV mass, 0.003 for wall thickness, and 0.007 for concentricity.

$^\|$ Interaction p values between black/white and logcystatin C are 0.001 for LV mass, 0.116 for wall thickness, and 0.581 for concentricity.

$^\|$ Beta coefficient of logcystatin C ± standard error are reported for each outcome variable.
Figure 1

A

Range: 0.46 – 6.55 mg/L
Median: 0.82 mg/L
Mean: 0.86 ± 0.28 mg/L

B

Median:
- Women: 0.8 mg/L
- Men: 0.85 mg/L
Mean:
- Women: 0.83 ± 0.24 mg/L
- Men: 0.89 ± 0.28 mg/L

p < 0.001
Association of Cystatin C with Left Ventricular Structure and Function: The Dallas Heart Study
Parag C. Patel, Colby R. Ayers, Sabina A. Murphy, Ronald Peshock, Amit Khera, James A. deLemos, Jody A. Balko, Sachin Gupta, Pradeep PA Mammen, Mark H. Drazner and David W. Markham

Circ Heart Fail. published online February 10, 2009;
Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/early/2009/02/10/CIRCHEARTFAILURE.108.807271

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Heart Failure can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Heart Failure is online at:
http://circheartfailure.ahajournals.org/subscriptions/