Association of cystatin C and estimated GFR with inflammatory biomarkers: the Heart and Soul Study

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Abstract

Background. Cystatin C is a marker of kidney function that may also be associated with inflammation. In this study, we compared the relative strengths of association of cystatin C and estimated glomerular filtration rate (eGFR) with inflammatory biomarkers.

Methods. We measured serum cystatin C and creatinine in 990 outpatients with coronary artery disease enrolled in the Heart and Soul Study. GFR was estimated (eGFR) by the abbreviated Modification of Diet in Renal Disease (MDRD) equation. We compared the associations of serum cystatin C and eGFR with C-reactive protein (CRP) and fibrinogen, after adjustment for 24 h creatinine clearance.

Results. Cystatin C concentrations had moderate correlations with CRP ($r = 0.15, P < 0.001$) and fibrinogen ($r = 0.26, P < 0.0001$); eGFR had similar correlations with CRP ($r = -0.17, P = 0.01$) and fibrinogen ($r = -0.25, P < 0.001$) among persons with eGFR ≤ 60 ml/min, but had no association with either biomarker among those with eGFR > 60 ml/min ($r = 0.04, P = 0.32; r = -0.03, P = 0.38$). Quartiles of cystatin C were strongly and directly associated with CRP ($P = 0.02$) and fibrinogen ($P < 0.007$) after multivariate adjustment. However, these associations disappeared after adjustment for creatinine clearance ($P = 0.26$ and 0.23, respectively).

Conclusions. Cystatin C concentrations have moderate associations with CRP and fibrinogen that are not independent of creatinine clearance. Although a gold standard of kidney function is lacking, this analysis suggests that cystatin C captures an association of mildly impaired kidney function with increased inflammation.

Keywords: chronic kidney disease; coronary artery disease; C-reactive protein; creatinine clearance; cystatin-C; inflammation

Introduction

The use of serum creatinine as a marker of kidney function is limited by factors that influence creatinine concentrations such as age, gender, race and weight. While an improvement over serum creatinine in predicting glomerular filtration rate (GFR), formulae such as the Cockcroft–Gault or the Modification of Diet in Renal Disease (MDRD) to calculate estimated GFR (eGFR), also have limitations and may be unreliable in certain populations [1,2]. The shortcomings of creatinine and creatinine-derived equations have prompted a search for a more reliable endogenous marker of kidney function. Cystatin C is a non-glycosylated cysteine protease inhibitor with a molecular weight of 13 kDa that appears to be produced at a constant rate by all nucleated cells. It is freely filtered by the renal glomerulus and metabolized by the proximal tubule [3,4]. Some studies have suggested that cystatin C may be superior to serum creatinine or creatinine-based estimating equations as a marker of kidney function, whereas others have found no difference [3–6].

A recent study from the Prevention of Renal and Vascular End-Stage Renal Disease (PREVEND) cohort found that cystatin C was significantly associated with C-reactive protein (CRP), smoking and body mass index even after adjustment for creatinine clearance (CRCL) levels [17]. The authors concluded that cystatin C levels were influenced by these factors in addition to kidney function. Recent longitudinal studies have shown that cystatin C has a stronger and more linear association with cardiovascular disease and mortality outcomes compared with creatinine-based measures [7–10]. These findings led to the hypothesis that cystatin C’s link to inflammation could explain its advantage over creatinine as a prognostic marker [11]. In the Heart and Soul Study, we compared the relative strengths of association of cystatin C and eGFR with two inflammatory biomarkers, CRP and fibrinogen, and we determined
whether these associations were independent of measured CRCL.

Methods

Study participants

The Heart and Soul Study is a prospective cohort study that was designed to examine the influence of psychosocial factors on coronary artery disease (CAD) progression. Details of our methods have been published previously [12]. Between September 2000 and December 2002, patients with CAD were identified from administrative databases at the Department of Veterans Affairs Medical Centers in Palo Alto and San Francisco, from the University of California San Francisco Medical Center, and from nine public health clinics in San Francisco. Patients were eligible to participate if their medical history contained at least one of the following: myocardial infarction, coronary revascularization, angiographic evidence of >50% stenosis in one or more coronary vessels, evidence of exercise-induced ischaemia by treadmill or nuclear testing, or a diagnosis of CAD by their internist or cardiologist. Patients were excluded if they were unable to walk one block, had a myocardial infarction in the last 6 months, or were planning to move out of the region within 3 years. A total of 1024 participants enrolled in the study and completed a day-long examination, of whom 990 provided adequate serum samples for measurement of cystatin C. The study protocol was approved by the appropriate Institutional Review Boards, and all participants provided written informed consent.

Kidney function

Cystatin C was measured from fasting serum samples collected between September 2000 and December 2002 and stored at −70°C until October 2004. Concentrations were measured using a BNII nephelometer (Dade Behring Inc., Deerfield, IL, USA) and a particle-enhanced immuno-nephelometric assay (N Latex cystatin C, Dade Behring Inc.). The detection limit of the assay is 0.05 mg/l, the analytical sensitivity is 0.005 mg/l and the reference range is 0.53–0.95 mg/l. Between and within-run coefficients of variation are both <3.6%.

We used the four-variable MDRD to calculate eGFR. The following formula was used: 
\[ eGFR = 186 \times \left[ \frac{SCR}{88.4} - 1.154 \right] \times \text{age} - 0.203 \times (0.742, \text{ if female}) \times (1.210, \text{ if African-American}) \]
Creatinine clearance was determined by 24 h urine collection, as previously described [13], using the following formula: urine creatinine (mg/dl) \times 24 h urine volume (dl)/serum creatinine (mg/dl) \times 1440 (min/day). Creatinine measurements were processed in a central lab at the San Francisco Veterans Affairs Medical Center.

Inflammatory biomarkers

We used the Roche Integra high-sensitivity assay to measure CRP in 229 participants and (due to a change at the lab) the Beckman extended range high-sensitivity CRP assay to measure CRP in the remaining 756 participants. The Roche Integra high-sensitivity CRP assay is an immuno-turbidometric assay that has been standardized against the World Health Organization reference and compared with the Dade nephelometric method with a correlation coefficient of 0.997. The interassay coefficient of variation is 3.2%, and the lowest detectable measurement of this assay is 0.025 mg/dl. The Beckman extended range CRP assay is also an immuno-turbidometric assay that is highly correlated with the Roche Integra assay (\( r = 0.99 \)). The inter-assay (between-run) coefficient of variation is ≤6.7%, and the intra-assay (within-run) coefficient of variation is ≤6.2%. Plasma fibrinogen concentrations were determined by the Clauss assay [14]. Dilutions of plasma standard (of known fibrinogen concentrations) are clotted with a high concentration of thrombin (−100 NIH U/ml), with the resultant clotting time being proportional to the fibrinogen concentration. The clotting time of the participant’s plasma was used to read the fibrinogen concentration from the standard curves. The standard assay range is from 60 mg/dl to 10 000 mg/dl and the inter- and intra-assay coefficients of variation are both <3%.

Other patient characteristics

Age, gender, race, smoking status, history of diabetes, hypertension, coronary artery bypass surgery, congestive heart failure, stroke, angina, physical activity and alcohol use were determined by self-report. We measured blood pressure, weight and height and calculated body mass index. Participants were instructed to bring their medication bottles to their study appointment, and study personnel recorded current medications. After a 12 h fast, serum samples were obtained for measurement of creatinine, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein and triglycerides. Left ventricular (LV) mass and ejection fraction were assessed using resting echocardiogram. Stress echocardiography was performed for assessment of cardiac ischaemia.

Analysis

We categorized participants into quartiles (I–IV) of cystatin C and eGFR with quartile I representing best kidney function (the lowest cystatin C and highest eGFR quartiles). Differences in baseline characteristics across cystatin C quartiles were compared using analysis of variance for continuous variables (or Kruskal-Wallis test for non-parametric variables) and chi-square tests for categorical variables. We evaluated the correlations of cystatin C and eGFR with inflammatory markers using Pearson coefficients.

We repeated the eGFR correlations after stratifying at 60 ml/min/1.73 m², because eGFR is imprecise above 60 ml/min/1.73 m² [15]. We used analysis of covariance to compare mean concentrations of CRP and fibrinogen by quartile of cystatin C, and by quartile of eGFR. These analyses were conducted in three steps, (i) unadjusted model; (ii) adjusted model with covariates from Table 1 and (iii) adjusted model plus CRCL.

To place cystatin C in a clinical context, we also categorized kidney function using a combination of eGFR and cystatin C as defined in a recent publication [16]: chronic kidney disease = eGFR < 60 ml/min/1.73 m²; preclinical kidney disease = eGFR > 60 ml/min/1.73 m² and cystatin C > 1.0 mg/l; and normal = eGFR > 60 ml/min/1.73 m².
Cystatin C, Creatinine Clearance, and Inflammation

Measurements of cystatin C were available for 990 participants. Of these, 984 had measures of CRP, 986 had measures of fibrinogen and 930 had measures of creatinine clearance available. Among the 990 total participants, those with elevated cystatin C concentrations were older, more likely to be white and male, and less likely to drink alcohol or to be physically active. Participants with elevated cystatin C concentrations were more likely to have a history of myocardial infarction, hypertension, diabetes, coronary artery bypass grafting and congestive heart failure. Higher concentrations of cystatin C were also associated with lower ejection fraction, higher LV mass index, higher wall motion score index (greater ischaemia) and lower HDL. Those with elevated cystatin C had higher systolic blood pressure, and were more likely to be using angiotensin system inhibitors, diuretics, hormone replacement therapy and β-blockers.

Cystatin C was moderately correlated with CRP (r = 0.15, P < 0.001) and fibrinogen (r = 0.26; P < 0.0001). The eGFR had similar correlations with CRP (r = –0.17, P = 0.01) and fibrinogen (r = –0.25, P < 0.001) among persons with eGFR ≤ 60 ml/min/1.73 m², but had no association with either biomarker among those with eGFR > 60 ml/min/1.73 m² (r = 0.04, P = 0.32; r = –0.03, P = 0.38). Increasing quartiles of cystatin C were associated with increased mean CRP and increased mean fibrinogen in unadjusted and adjusted models (Table 2). However, after adjustment for creatinine clearance, the associations of cystatin C quartiles with mean levels of CRP and fibrinogen were no longer statistically significant.

CRP and fibrinogen levels did not increase across quartiles of eGFR (Table 3). The highest levels of each biomarker were in quartile IV, but similar levels were observed across quartiles I–III. No significant trend was observed across quartiles in multivariate analysis.

We categorized participants as having chronic kidney disease (eGFR < 60 ml/min/1.73 m²), preclinical kidney disease (eGFR ≥ 60 ml/min/1.73 m²),

### Table 1. Characteristics of 990 participants by quartile of cystatin C (mg/l)

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Cystatin C (mg/l)</th>
<th>n (%)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.12-0.92 mg/l</td>
<td>258</td>
<td>61 ± 10</td>
</tr>
<tr>
<td>II</td>
<td>0.92-1.07 mg/l</td>
<td>239</td>
<td>188 (73)</td>
</tr>
<tr>
<td>III</td>
<td>1.07-1.30 mg/l</td>
<td>252</td>
<td>131 (51)</td>
</tr>
<tr>
<td>IV</td>
<td>&gt;1.30 mg/l</td>
<td>241</td>
<td>57 (22)</td>
</tr>
</tbody>
</table>

**Demographic**

- Age (years): 61 ± 10<br>- Sex (male): 188 (73)<br>- White race: 131 (51)<br>- Current smoking: 57 (22)<br>- Regular alcohol: 97 (38)<br>- Physically active: 181 (71)

**Medical history**

- Myocardial infarct: 122 (48)<br>- Hypertension: 168 (65)<br>- Diabetes: 60 (23)<br>- Angioplasty: 91 (35)<br>- Bypass graft: 68 (27)<br>- Heart failure: 31 (12)<br>- Weekly angina: 50 (19)

**Measurements**

- LV ejection fraction: 0.63 ± 0.09<br>- LV mass index: 93 ± 23<br>- Wall motion index: 1.12 ± 0.32<br>- Body mass index: 28.2 ± 5<br>- Total cholesterol: 181 ± 41<br>- LDL: 106 ± 34<br>- HDL: 49 ± 15<br>- Triglycerides: 132 ± 131<br>- Systolic BP: 131 ± 18<br>- Diastolic BP: 76 ± 10

**Medications**

- ACE/ARB: 99 (39)<br>- Diuretic: 44 (17)<br>- Hormone therapy: 21 (8)<br>- Statin: 160 (62)<br>- Aspirin: 197 (76)<br>- β-Blockers: 126 (49)

We used multivariate analysis of covariance to evaluate whether differences in CRP and fibrinogen were significant across the three groups. All analyses were performed using Statistical Analysis Software (Version 9, SAS Institute, Inc.).

**Results**
cystatin C > 1.0 mg/dl) or normal kidney function (eGFR > 60 ml/min/1.73 m², cystatin C < 1.0 mg/dl) (Table 4). In both unadjusted and multivariate analyses, participants with chronic kidney disease had the highest levels of each biomarker, and participants with pre-clinical kidney disease had higher levels of inflammatory biomarkers than the normal group.

### Discussion

In this study, we compared the associations of cystatin C and eGFR with two inflammatory biomarkers, CRP and fibrinogen, and evaluated whether these associations were independent of measured CRCL. We found that quartiles of cystatin C were linearly associated with each biomarker after multivariate adjustment; however, this association was no longer significant after adjustment for CRCL. The lowest eGFR quartile (<62 ml/min/1.73 m²) was also associated with higher biomarker concentrations. However, eGFR was not correlated with inflammation among persons with eGFR > 60 ml/min/1.73 m² either before or after adjustment for creatinine clearance. When we combined eGFR and cystatin C measurements to define CKD, pre-clinical kidney disease, and normal groups, we found the highest levels of inflammatory biomarkers in persons with CKD, but...
persons with pre-clinical kidney disease were characterized by intermediate biomarker levels. These results suggest that cystatin C captures an association of pre-clinical impairments of kidney function that cannot be detected using eGFR. Our findings differ from prior work by investigators from the PREVEND study. Knight and colleagues [17] found that CRP levels, along with other characteristics, remained associated with cystatin C concentrations despite adjustment for 24 h urinary creatinine clearance. They concluded that cystatin C concentrations may be greatly influenced by inflammation and by factors related to production or catabolism. Our findings do not support an association of cystatin C with inflammation that is independent of underlying kidney function. However, our cohort differs from PREVEND in several important ways: our subjects were older, more likely male, had existing CAD, and had worse kidney function. If inflammation does in fact affect cystatin C levels, its influence could be proportionately greater in persons with lower cystatin C concentrations as in PREVEND.

Our findings are relevant for interpreting the relationship between cystatin C and cardiovascular outcomes. Several recent studies have demonstrated that cystatin C has a linear association with risk for death and cardiovascular disease [9,18]. In contrast, creatinine-based estimates of GFR predict cardiovascular disease only among persons with eGFR < 60 ml/min [19]. Few, if any, epidemiological studies have evaluated direct measures of GFR as predictors of cardiovascular disease. It is thus unclear whether cystatin C’s association with cardiovascular disease reflects a more precise measurement of kidney function or an association with non-renal factors such as inflammation. This study suggests that cystatin C’s association with cardiovascular disease is unlikely to be attributable to inflammation.

Our findings that cystatin C, but not eGFR, was associated with inflammation among participants without CKD reinforce the findings of previous studies. Investigators from the Cardiovascular Health Study recently demonstrated that pre-clinical kidney disease was independently predictive of death, cardiovascular disease and incident chronic kidney disease in elderly persons [16]. A study from the Health, Aging and Body Composition Study found that cystatin C captured associations of pre-clinical kidney disease with impaired physical function and performance in elderly persons [20]. Together, these results suggest that cystatin C can be a useful epidemiological tool for evaluating the health consequences of mild kidney impairment.

Our study has several strengths including its large sample size, availability of rigorously collected 24 h urine collections for creatinine clearance and comprehensive measurement of potential confounding variables. However, there are several limitations that must be considered. The most important limitation is that we lacked a gold standard measure of GFR. Second, the cross-sectional design of our study does not allow for causal inference. Third, thyroid disorders, which may influence concentrations of cystatin C, were not screened for in our analysis. However, given the low prevalence of thyroid abnormalities in the general population, it is unlikely to have influenced our results. Fourth, our study population was largely male and limited to patients with known CAD. Although this patient population is relevant to many of the questions raised about cystatin C, caution should be used in applying these results to other populations.

In summary, we found that cystatin C concentrations had linear associations with two inflammatory markers, CRP and fibrinogen, that did not appear to be independent of CRCL. Pre-clinical declines in kidney function appear to be characterized by elevations of inflammatory biomarkers.

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Conflict of interest statement. None declared.

References


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