Association of Mild to Moderate Kidney Dysfunction and Coronary Calcification

Joachim H. Ix,* Ronit Katz,† Bryan Kestenbaum,‡ Linda F. Fried,¶ Holly Kramer,§ Catherine Stehman-Breen,¶¶ and Michael G. Shlipak††

*Department of Medicine, Division of Nephrology and Hypertension, University of California San Diego and Veterans Affairs San Diego Healthcare System, San Diego, California; †Collaborative Health Studies Coordinating Center, ‡Department of Medicine and Division of Nephrology, and §Department of Epidemiology, University of Washington, Seattle, Washington; ‖Renal Section, Medical Service, Veterans Affairs Pittsburgh Healthcare System, Pittsburgh, Pennsylvania; ‡Department of Medicine and Division of Nephrology, Loyola University, Maywood, Illinois; ¶Amgen, Thousand Oaks, California; and ††Departments of Medicine and Epidemiology and Biostatistics, University of California San Francisco and Veterans Affairs Medical Center, San Francisco, California

ABSTRACT

Coronary artery calcification (CAC) is prevalent and predicts mortality among patients with ESRD, but whether less severe kidney dysfunction is associated with CAC is uncertain. To address this question, 6749 participants of the Multi-Ethnic Study of Atherosclerosis, who were middle-aged and without known cardiovascular disease, were evaluated. Renal function was categorized by cystatin C quartiles and estimated GFR (eGFR; < 60 ml/min per 1.73 m²), and CAC was evaluated by computed tomography (CT). Fifty percent of participants had CAC, mean cystatin C was 0.90 mg/L, and 10% had eGFR < 60 ml/min per 1.73 m². In unadjusted analysis, kidney dysfunction by either measure was strongly associated with CAC; however, the associations were lost after adjustment for age, gender, race, hypertension, and IL-6 (relative risk 1.04 [95% confidence interval 0.97 to 1.11] for the highest cystatin C quartile compared with the lowest, and relative risk 1.03 [95% confidence interval 0.98 to 1.08] for eGFR below compared with above 60 m/min per 1.73 m²). Similarly, neither higher cystatin C nor eGFR <60 was associated with severity of CAC. These results suggest that a higher burden of CAC is unlikely to explain the association between mild to moderate kidney dysfunction and cardiovascular mortality.


Chronic kidney disease (CKD) affects approximately 8 million adults in the United States¹ and is a strong risk factor for cardiovascular mortality. This association is independent of traditional cardiovascular risk factors and inflammatory biomarkers and is observed even with modest decrements in kidney function.²,³ At each stage of kidney disease, the risk for cardiovascular mortality is several-fold higher than the risk for progression to ESRD.⁴ Despite intense investigation,⁵ the mechanisms responsible for this strong association remain unknown.

Patients with ESRD have extraordinarily high risk for cardiovascular mortality with annual event rates >15% in the United States.⁶ This population is characterized by extensive coronary artery calcification (CAC),⁷–⁹ and the prevalence and severity of CAC predict mortality.¹⁰,¹¹ Thus, vascular calcification may represent an important mediator of the association between ESRD and cardiovascular events.¹⁰

Vascular calcification could potentially repre-
RESULTS

Among the 6749 study participants, the mean age was 63 yr, and 53% were female. Thirty-eight percent were white, 28% were black, 22% were Hispanic, and 12% were Chinese. The mean cystatin C level was 0.90 ± 0.24 mg/L, and mean eGFR was 79 ± 18 ml/min per 1.73 m². A total of 667 participants (10%) had eGFR <60 ml/min per 1.73 m². Fifty percent (n = 3362) had CAC (Agatston score >0). Among participants with CAC, the median calcium score was 87 (interquartile range 22 to 298).

Participants were categorized by quartiles of cystatin C (Table 1). Compared with the lowest cystatin C quartile, participants with higher cystatin C concentrations were older, more frequently male and white, and less likely Chinese; more had a history of diabetes, hypertension, and tobacco use; and they were more likely to have an atherogenic lipid profile, higher body mass index, and higher serum concentrations of inflammatory biomarkers.

The prevalence of CAC increased from approximately one third in the lowest cystatin C quartile to two thirds in the highest quartile, a nearly two-fold relative risk in unadjusted analysis (Table 2); however, this association was markedly attenuated in models adjusted for age, gender, and race/ethnicity. In the fully adjusted model, adjusted for age, gender, race/ethnicity, hypertension, and IL-6 concentrations, the association was

Table 1. Baseline demographics by quartiles of cystatin C

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cystatin C Quartiles</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (≤0.76 mg/L)</td>
<td>2 (0.77 to 0.86 mg/L)</td>
</tr>
<tr>
<td>n</td>
<td>1737</td>
<td>1755</td>
</tr>
<tr>
<td>Demographics</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>age (yr; mean ± SD)</td>
<td>57 ± 9</td>
<td>60 ± 9</td>
</tr>
<tr>
<td>female [n [%]]</td>
<td>1088 (63)</td>
<td>920 (52)</td>
</tr>
<tr>
<td>race/ethnicity [n [%]]</td>
<td>white</td>
<td>580 (33)</td>
</tr>
<tr>
<td></td>
<td>black</td>
<td>528 (30)</td>
</tr>
<tr>
<td></td>
<td>Hispanic</td>
<td>347 (20)</td>
</tr>
<tr>
<td></td>
<td>Chinese</td>
<td>282 (16)</td>
</tr>
<tr>
<td>Medical history [n [%]]</td>
<td>diabetes</td>
<td>242 (14)</td>
</tr>
<tr>
<td></td>
<td>hypertension</td>
<td>591 (34)</td>
</tr>
<tr>
<td></td>
<td>tobacco use</td>
<td>current</td>
</tr>
<tr>
<td></td>
<td>former</td>
<td>577 (33)</td>
</tr>
<tr>
<td></td>
<td>never</td>
<td>950 (55)</td>
</tr>
<tr>
<td>Measurements</td>
<td>systolic BP (mmHg; mean ± SD)</td>
<td>122 ± 20</td>
</tr>
<tr>
<td></td>
<td>diastolic BP (mmHg; mean ± SD)</td>
<td>72 ± 10</td>
</tr>
<tr>
<td></td>
<td>total cholesterol (mg/dl; mean ± SD)</td>
<td>196 ± 34</td>
</tr>
<tr>
<td></td>
<td>LDL (mg/dl; mean ± SD)</td>
<td>117 ± 31</td>
</tr>
<tr>
<td></td>
<td>HDL (mg/dl; mean ± SD)</td>
<td>55 ± 16</td>
</tr>
<tr>
<td></td>
<td>triglycerides (mg/dl; median [IQR])</td>
<td>98 (69 to 143)</td>
</tr>
<tr>
<td></td>
<td>body mass index (kg/m²; mean ± SD)</td>
<td>27 ± 4</td>
</tr>
<tr>
<td></td>
<td>C-reactive protein (mg/dl; median [IQR])</td>
<td>1.5 (0.7 to 3.7)</td>
</tr>
<tr>
<td></td>
<td>fibrinogen (mg/dl; mean ± SD)</td>
<td>330 ± 70</td>
</tr>
<tr>
<td></td>
<td>IL-6 (mg/dl; median [IQR])</td>
<td>1.0 (0.6 to 1.5)</td>
</tr>
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further attenuated and was no longer statistically significant (Table 2). Results were similar when participants with eGFR <60 ml/min per 1.73 m² were compared with individuals with higher eGFR (Table 3). We evaluated for effect modification of the associations of cystatin C quartiles with CAC by diabetes and race/ethnicity and observed no evidence for interaction with either variable (P = 0.76 and 0.36, respectively, for interaction). Similarly, there was no statistically significant interaction of these variables with eGFR when it was used as the measure of kidney function (P = 0.11 for diabetes and 0.22 for race/ethnicity, for interaction).

Among the 3362 participants with prevalent CAC, we evaluated whether cystatin C or eGFR groups were associated with increased severity of CAC. We observed strong associations in unadjusted analyses, but the associations were markedly attenuated in demographic-adjusted models and completely attenuated in fully adjusted models (Tables 4 and 5).

**DISCUSSION**

In this study, we observed a strong association of kidney dysfunction with CAC in unadjusted analysis, but the association was completely attenuated after statistical adjustment for demographic parameters and confounding risk factors previously associated with cardiovascular disease. Therefore, the association of elevated cystatin C and moderate CKD by eGFR with cardiovascular mortality reported in previous studies is unlikely to be mediated by a higher prevalence or severity of CAC.

The lack of association of mild to moderate impaired kidney function with CAC provides novel insights toward the mechanisms of cardiovascular mortality in this population. Both elevated cystatin C concentrations and moderate kidney disease have consistently predicted cardiovascular mortality in community-based cohorts, and these associations are not explained by the higher prevalence of shared risk factors such as diabetes, hypertension, or inflammatory stress.2,3,26,27 When detected by CT scan, CAC has >90% sensitivity for intimal atherosclerotic lesions with ≥50% luminal stenosis on coronary angiography.28 Because the higher prevalence of CAC was explained by demographic and cardiovascular risk factors, these data suggest that the increased burden of intimal atherosclerosis or accelerated medial calcification within the coronary arteries is unlikely to be the predominant mechanism responsible for the high cardiovascular mortality in this population.

The association of decrements of kidney function and CAC were markedly attenuated with adjustment for age, gender, and race/ethnicity, but a modest and statistically significant association remained until models were further adjusted for hypertension and IL-6. It is possible that hypertension and inflammation are consequences of kidney disease and along the causal pathway toward CAC development. Our objective, however, was to determine whether CAC might represent a mediator of the relationship of kidney disease and cardiovascular disease observed in previous studies, in which the relationship remained strong and significant despite adjustment for hypertension and inflammatory biomarkers, in addition to other cardiovascular risk factors2,26,27,29; therefore, the data presented here demonstrate that CAC is unlikely to be the dominant mechanism responsible for the association of early to moderate decrements in kidney function and cardiovascular disease.

What other mechanisms may be responsible for the higher cardiovascular risk among individuals with mild to moderate decrements in kidney function? This severity of kidney dysfunction is strongly associated with cardiac structural abnormalities,30 conduction system abnormalities,31 and incident heart failure32–34 therefore, it is possible that the association of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cystatin C Quartiles (RR [95% CI])</th>
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<tbody>
<tr>
<td></td>
<td>1 (≤0.76 mg/L)</td>
</tr>
<tr>
<td>Participants with CAC (n/N [%])</td>
<td>621/1737 (36)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Demographic adjustedab</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Fully adjustedac</td>
<td>1.0 (reference)</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Parameter</th>
<th>eGFR (RR [95% CI])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥60 ml/min per 1.73 m²</td>
</tr>
<tr>
<td>Participants with CAC (n/N [%])</td>
<td>226/3387 (7)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Demographic adjusteda</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Fully adjustedb</td>
<td>1.0 (reference)</td>
</tr>
</tbody>
</table>

a CI, confidence interval.
b Adjusted for age, gender, and race/ethnicity.
c Adjusted for age, gender, race/ethnicity, history of hypertension, and IL-6.
kidney disease and cardiovascular mortality may be due to a predisposition for arrhythmia and sudden death. Alternatively, kidney disease may increase the vulnerability for individual plaque rupture. The evaluation of these potential mechanisms is beyond the scope of this article but should be evaluated in future studies.

Previous epidemiologic studies provided conflicting results regarding the association of moderate kidney disease and CAC. Fox et al., evaluated 319 participants in the Framingham Heart Study with a mean eGFR of 86 ml/min per 1.73 m$^2$. The authors observed an association between lower eGFR and high prevalence of CAC in unadjusted analysis, but the association was attenuated and of marginal statistical significance after multivariable adjustment. Kramer et al., evaluated 2660 participants in the Dallas Heart Study, among whom 41 had an eGFR <60 ml/min per 1.73 m$^2$. The authors observed a strong association between CKD and CAC among participants with diabetes but observed no association in the nondiabetic stratum. Two other studies reported positive associations of moderate kidney disease with CAC, yet both cohorts exclusively studied individuals with diabetes. Because diabetes is a strong risk factor for vascular calcification independent of kidney function, it is possible that the associations of moderate kidney disease with CAC within these studies may have represented confounding by longer duration or increased severity of diabetes, rather than an independent association of kidney disease with calcification.

This study extends these observations in several important ways. First, the MESA cohort contains substantially larger numbers of participants. In addition, the availability of cystatin C measurements among all participants provided a more accurate measure of kidney function among individuals with eGFR >60 ml/min per 1.73 m$^2$. Along with the high prevalence of CAC, these features provided unprecedented statistical power to evaluate the association of mild to moderate decrements in kidney function with CAC. Finally, the MESA cohort is ethnically and geographically diverse, thereby increasing the generalizability of the results.

Our study has several limitations. Because there were few participants with eGFR ≤45 ml/min per 1.73 m$^2$, the findings cannot be extrapolated to patients with more advanced kidney disease. MESA participants did not have clinically apparent cardiovascular disease at baseline, and the association of kidney disease with CAC may differ among individuals with established cardiovascular disease. Kidney function and CAC were determined at one point in time. Future studies are needed to evaluate whether the rate of change in kidney function over time may predict incidence or progression of CAC. Although this is the largest study of its kind, we cannot exclude the possibility that an association was missed as a result of chance.

In conclusion, in a large and ethnically diverse cohort of individuals without clinical cardiovascular disease, we did not observe an independent association of mild to moderate decrements in kidney function with CAC. This finding suggests that a higher burden of CAC is unlikely to be the dominant mediator of the association between mild to moderate kidney impairment and cardiovascular mortality.

### CONCISE METHODS

#### Participants

MESA was initiated to investigate the prevalence and progression of subclinical cardiovascular disease. Details about the study design have
been published previously. In brief, between July 2000 and August 2002, 6814 men and women who were aged 45 to 84 yr; identified themselves as white, black, Hispanic, or Chinese; and were free of clinically apparent cardiovascular disease were recruited from portions of six US communities: Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles, CA; Northern Manhattan and the Bronx, NY; and St. Paul, MN. Each field site recruited from locally available sources, which included lists of residents, dwellings, and telephone exchanges. In the last several months of the recruitment period, supplemental sources (lists of Medicare beneficiaries from the Center for Medicare and Medicaid Services and referrals by participants) were used to ensure adequate numbers of minorities and elderly individuals. The institutional review boards at each participating center approved the study, and all participants provided written informed consent. Participants for whom serum cystatin C (n = 58), creatinine (n = 7), or CAC (n = 0) measurements were missing were excluded from the analysis, resulting in an analytic sample of 6749 individuals.

**Measurements**

**Kidney Function.**

Both cystatin C and creatinine were measured among venous serum samples obtained after a 12-h overnight fast at the baseline study visit. Cystatin C concentrations were determined using a BNII nephelometer (Dade Behring, Deerfield, IL) with a particle-enhanced immunonephelometric assay (N Latex Cystatin C; Dade Behring). MAb to cystatin C were coated on polystyrene particles that agglutinate to increase the intensity of scattered light in proportion to the concentration of cystatin C. The assay range is 0.195 to 7.330 mg/L. The intra-assay coefficients of variation were ≤3%. Creatinine concentrations were determined by the amidinohydrolase method and were normalized to the reference standard at the Cleveland Clinic Foundation, to allow accurate comparison of measures across studies. The coefficients of variation were ≤2%. eGFR was calculated with the modified four-variable MDRD study formula: eGFR = 186 × serum creatinine\(^{-1.154}\) × age\(^{-0.203}\) × 0.742 (if female) × 1.21 (if black).

**Coronary Artery Calcification**

CAC was measured using two scans obtained on the same occasion during the baseline study visit, using either an Imatron C-150XL electron-beam CT scanner (GE-Imatron, South San Francisco, CA) or a multidetector CT scanner (four slices), as described previously. An expert committee developed a scanning protocol to standardize scan acquisition across the two slightly different technologies, and data quality was equivalent between CT scan techniques. Scans were obtained during a single breath-hold and were electrocardiogram-triggered at 50% of the RR interval. Scans were read centrally at the Harbor-UCLA Research and Education Institute (Torrance, CA). Agatston calcification scores were calculated as a product of the area of calcified plaque multiplied by a coefficient, rated 1 through 4 on the basis of the peak calcium density in the identified lesions. The agreement (κ) for presence of CAC on consecutive CT scans for the same participant was 0.92, and the interclass correlation coefficients for readings of CAC amount performed by the same or by different readers was >0.99. The average of the two CAC scores was used for this analysis.

**Other Measurements**

Standardized questionnaires determined age, gender, race/ethnicity, medical history, and medication usage. Smoking was defined as current, former, or never. Height and weight were measured with participants wearing light clothing and no shoes, and body mass index was calculated (in kg/m\(^2\)). Diabetes was defined as a fasting glucose ≥126 mg/dl or use of hypoglycemic medications. Resting BP was measured three times with participants in the seated position with a Dinamap model Pro 100 automated oscillometric sphygmomanometer (Critikon; General Electric; Madison, WI). The average of the last two measurements was used in analyses. Hypertension was defined as a systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg, or current use of antihypertensive medication. Total and HDL cholesterol and triglyceride concentrations were measured from venous samples obtained after a 12-h overnight fast. LDL was calculated from the Friedewald equation.

**Statistical Analyses**

Because age-, gender-, and race-specific normal ranges for serum cystatin C have not been established, we categorized cystatin C into quartile groups. We also categorized participants into two groups on the basis of eGFR <60 or ≥60 ml/min per 1.73 m\(^2\). There were too few participants with eGFR <45 ml/min per 1.73 m\(^2\) (n = 102; 2%) to categorize CKD severity further. Differences in baseline characteristics were compared across cystatin C groups using ANOVA or the Kruskal-Wallis test for continuous variables and the \(\chi^2\) test or Fisher Exact test for categorical variables, as appropriate.

CAC was categorized as present (Agatston score >0) or absent. Because the prevalence of CAC was 50% in the study sample, odds ratios (OR) would overestimate the relative risk (RR); therefore, RR estimates are presented from the regression model \(\gamma = \exp(X^T\beta)\), where \(\beta\) can be interpreted as RR. We assumed Gaussian error and used robust SE estimates. The prevalence of CAC was evaluated across kidney function groups. An initial multivariable model was adjusted for age, gender, and race/ethnicity. Subsequent “fully adjusted models” were adjusted for both demographic and laboratory covariates. All variables presented in Table 1 were considered candidate covariates for adjustment in the final model. We retained important confounders when their inclusion led to a change of >5% in the parameter coefficient (\(\beta\)) in the association of cystatin C (linear variable) with the presence or absence of CAC. Covariates that were retained in the final adjusted model for cystatin C were also used for the final eGFR models.

Among participants with detectable CAC, the relationship between kidney function groups and the severity of calcification ([ln]Agatston score) was assessed with multiple linear regression in companion analyses. These models were adjusted for identical covariates as those retained in the RR regression models described previously. This relationship is expressed as a percentage difference in calcification score.

Multiplicative interaction terms were created to evaluate for effect modification of the association of kidney function and CAC by diabetes and race/ethnicity. These candidate effect modifiers were selected \textit{a priori} on the basis of previously published research. Statistical analyses were performed with SPSS 13.0.1 software for...
Windows (SPSS, Chicago, IL) and STATA 8.0 for Windows (Stata Corp, College Station, TX).

ACKNOWLEDGMENTS

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DISCLOSURES

None.

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