Cystatin C and Incident Peripheral Arterial Disease Events in the Elderly

Results From the Cardiovascular Health Study

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Background: The association of cystatin C, a novel marker of renal function, with risk for developing complications related to peripheral arterial disease (PAD) has not been examined.

Methods: We evaluated the hypothesis that a high cystatin C concentration is independently associated with future PAD events among 4025 participants in the Cardiovascular Health Study who underwent serum cystatin C measurement at the 1992-1993 visit and who did not have PAD at baseline. The association of cystatin C quintiles with time to first lower-extremity PAD procedure (bypass surgery, angioplasty, or amputation) was evaluated using multivariable proportional hazards models. Secondary analyses were conducted using quintiles of serum creatinine level and estimated glomerular filtration rate (eGFR).

Results: The annualized risk of undergoing a procedure for PAD was 0.43% per year among participants in the highest cystatin C quintile (>1.27 mg/L) compared with 0.21% per year or less in all other quintiles. After multivariable adjustment for known risk factors for PAD, elevated cystatin C levels remained associated with the outcome (hazard ratio, 2.5 for highest vs lowest quintile of cystatin C, 95% confidence interval, 1.2-5.1). The highest quintiles of serum creatinine level and eGFR were not associated with future PAD events in either unadjusted or adjusted analyses.

Conclusion: Elevated concentrations of cystatin C were independently predictive of incident PAD events among community-dwelling elderly patients.

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Peripheral Arterial Disease (PAD) is common in the elderly population and is associated with significant morbidity and mortality. Recent data suggest that medical therapies may reduce the risk of amputation and/or revascularization in defined populations. Identification of patients who are at greatest risk for requiring these procedures may allow targeting of interventions to prevent PAD or slow its progression.

A growing number of studies indicate that renal insufficiency is associated with prevalent and incident PAD. Cystatin C is a novel serum marker for renal function. It is a cysteine-protease inhibitor that is produced by most human cells, freely filtered by the glomerulus, and subsequently metabolized by the proximal tubule. Concentrations of cystatin C are believed to primarily reflect glomerular filtration rate (GFR). Thus, cystatin C concentration may be a more sensitive indicator of mild renal dysfunction than serum creatinine level. Recent studies have shown that cystatin C is more strongly associated with all-cause and cardiovascular mortality as well as with myocardial infarction, stroke, and congestive heart failure than either serum creatinine or estimated GFR (eGFR). We therefore evaluated the hypothesis that a high cystatin C concentration would be independently associated with future PAD events among elderly persons living in the community who did not have prevalent PAD at baseline. We hypothesized further that this association would be stronger than that for conventional measures of renal function such as serum creatinine level or eGFR.

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METHODS

PARTICIPANTS

The Cardiovascular Health Study (CHS) is a community-based, longitudinal study of Medicare-eligible adults 65 years or older at baseline. The objective of the study was to evaluate risk factors for the development and progression of cardiovascular disease. The main cohort consisted of 5201 study participants recruited between 1989 and 1990 from 4 US communities: Sacramento County, California; Forsyth County, North Carolina; Washington County, Maryland; and Allegheny County, Pennsylvania. A second cohort of 687 African Americans was recruited in 1992 and...
Cystatin C was measured from frozen samples collected at the 1992-1993 visit. A total of 4937 study participants returned for this visit, and serum samples were available for 4734 of these. We excluded 189 participants who had undergone an amputation or revascularization prior to the 1992-1993 visit and a further 320 participants who had an ankle-arm index (AAI) of 0.9 or lower at this visit, leaving a study sample of 4025 for the present analysis.

MEASUREMENTS

All assays were performed on plasma drawn in the morning and stored at –70°C. Cystatin C concentration was measured using a BNII nephelometer (Dade Behring Inc, Deerfield, Ill) using a particle-enhanced immunonephelometric assay (N Latex Cystatin C; Dade Behring Inc, Newark, Del). Poly styrene particles were coated with monoclonal antibodies to cystatin C that agglutinate in the presence of antigen (cystatin C) to cause an increase in the intensity of scattered light. The increase in scattered light is proportional to the amount of cystatin C in the sample. The assay range is 0.195 to 7.330 mg/L, with the reference range for young healthy individuals reported as 0.53 to 0.95 mg/L. Intra-assay coefficients of variation range from 2.0% to 2.8% and interassay coefficients of variation range from 2.3% to 3.1%. Serum creatinine was measured using the Kodak Ektachem 700 Analyzer (Eastman Kodak, Rochester, NY), a colorimetric method.

The AAI is the ratio of ankle to arm systolic blood pressure. The standard protocol for AAI measurement in the CHS has been described previously. Briefly, manual blood pressure measurements were obtained using a Doppler stethoscope (8 MHz; Huntleigh Technology Inc, Luton, Bedfordshire, England) over the right brachial artery and both posterior tibial arteries. To be consistent with prior CHS studies of the association of AAI with cardiovascular disease, the AAI was calculated by dividing the lowest leg blood pressure by the blood pressure in the right arm.

COVARIATES

The primary predictor variable for the present analysis was serum cystatin C concentration at the 1992-1993 study visit. This was categorized a priori by quintile, with the lowest quintile serving as the referent category for interquintile comparisons. The association of cystatin C concentration with future PAD events was adjusted for potential confounders, including sociodemographic factors (age, sex, race, and income), prevalent stroke and coronary heart disease (defined as history of myocardial infarction, unstable angina, or angiplasty or coronary artery bypass procedure), and known cardiovascular and PAD risk factors: high body mass index (BMI) (calculated as weight in kilograms divided by the square of height in meters), smoking (current vs past or never), diabetes (history of diabetes, use of hypoglycemic agents or insulin, or fasting glucose ≥126 mg/dL, ≥7.0 mmol/L), hypertension (systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medications), and abnormal levels of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, C-reactive protein, and fibrinogen. In secondary analyses, we also measured the unadjusted and adjusted associations with future PAD events of serum creatinine level and eGFR, categorized into quintiles. Estimated GFR was determined using the Modification of Diet in Renal Disease formula.

STUDY OUTCOME

The current analysis examines incident lower-extremity bypass surgery, angioplasty, and amputations for PAD between the 1992-1993 visit and June 30, 2001. Incident events were identified by International Classification of Diseases, Ninth Revision, Clinical Modification procedure codes for lower-extremity bypass surgery (39.25 or 39.29), lower-extremity amputation (84.11-84.19), and peripheral angioplasty (39.30 and 39.39, excluding concurrent 39.93 or 39.61). The study outcome was defined as time from the 1992-1993 visit to the first PAD procedure as defined. We selected this severe PAD outcome both to minimize misclassification and because of the clear clinical significance of requiring a procedure for PAD relative to other candidate outcomes such as the development of intermittent claudication or of an AAI less than 0.9.

RESULTS

Across cystatin C quintiles, mean age and serum creatinine level increased. The percentage of African Americans increased across quintiles. Most other established cardiovascular disease risk factors (male sex, hypertension, high BMI, and abnormal levels of triglycerides, C-reactive protein, and fibrinogen) and the prevalence of preexisting cardiovascular disease also increased across cystatin C quintiles (Table 1). Serum levels of LDL-C and HDL-C decreased across cystatin C quintiles. There was a U-shaped relationship between diabetes and cystatin C concentration. The distributions of AAI and smoking history did not differ significantly across cystatin C quintiles in this sample.

Of the 4025 CHS participants included in this analysis, 1192 died, 980 underwent non-PAD cardiovascular events, and 60 underwent a PAD procedure during follow-up. The annualized risk of undergoing a procedure for PAD was 0.43% per year among participants in the highest cystatin C quintile (≥1.27 mg/L) compared with 0.21% per year or less in all other quintiles (Figure and Table 2). Annualized risk did not increase appreciably across quintiles of either creatinine level or eGFR (Figure).

In both unadjusted and adjusted analyses, participants with a cystatin C concentration in the highest quintile had an increased risk of requiring a PAD procedure compared with participants in the lowest cystatin C quintile (Table 2). Cystatin C was still associated with the outcome when we excluded cohort members with prevalent cardiovascular disease at baseline (hazard ratio [HR], 1.33; 95% confidence interval [CI] 1.04-1.69 per stan-
The present analysis demonstrates that a cystatin C concentration in the highest quintile (≥1.27 mg/L) was independently predictive of future receipt of a PAD procedure among a cohort of elderly community-dwelling adults who did not have PAD at the beginning of follow-up. The association of cystatin C with PAD risk was stronger than with either serum creatinine level or eGFR.

The finding of an association of cystatin C with future PAD events in participants without prevalent PAD at baseline is consistent with a growing number of studies demonstrating both cross-sectional and longitudinal associations between renal insufficiency and PAD. It is unclear whether these associations reflect a causal association or whether impaired renal function is a marker for underlying atherosclerotic disease. An AAI of 0.9 or lower was independently associated with macroalbuminuria in the Strong Heart Study and with elevated serum creatinine levels in the CHS. Indeed, the prevalence of an AAI of 0.9 or lower appears to be extremely high among those with renal insufficiency and PAD. Conversely, renal insufficiency is common among patients with advanced PAD. In addition, several studies have shown that renal insufficiency is associated with the development of claudication among the elderly population and with future ampu-
tation and revascularization events among women with coronary artery disease. However, these studies were limited somewhat in that they probably included patients with prevalent PAD.

In addition, our findings are consistent with recent studies that reported associations of cystatin C with both mortality and cardiovascular risk in the elderly population, which were stronger than those of serum creatinine level and eGFR. Most likely, cystatin C is a better predictor of PAD events and other cardiovascular outcomes than serum creatinine level because it is a more accurate measure of renal function, particularly in the elderly. In the present study, the risk of lower-extremity PAD procedures did not increase in a stepwise fashion across cystatin C quintiles, as previously reported for all-cause and cardiovascular mortality and incident heart failure, but was increased only at cystatin C levels in the highest quintile. This observation suggests that each specific cardiovascular end point may have a unique association with kidney dysfunction, as measured by cystatin C.

The reported association of cystatin C with future PAD events among those without PAD at baseline may have applications in the clinical setting because therapies are available that may reduce the incidence of PAD complications among high-risk patients. Recent studies demonstrate that statins and angiotensin-converting enzyme inhibitors reduce the incidence of lower-extremity amputation and revascularization among high-risk groups. In the MRC/BHF Heart Protection Study—a randomized, controlled, clinical trial of the effect of simvastatin on cardiovascular end points among those with high cardiovascular risk—randomization to 40-mg simvastatin was associated with a lower incidence of peripheral arterial events during follow-up. This benefit was seen among participants both with and without diabetes and in patients with PAD who had no history of other cardiovascular disease. In the Heart Outcomes Prevention

### Table 2. Risk of Lower Extremity Bypass, Angioplasty, or Amputation During Follow-up by Quintile of Cystatin C

<table>
<thead>
<tr>
<th>Cystatin C Level, mg/L</th>
<th>Subjects, No.</th>
<th>Person-Years at Risk</th>
<th>Events, No.</th>
<th>Event Rate, %/y</th>
<th>Unadjusted HR (95% CI)</th>
<th>Adjusted* HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.9</td>
<td>874</td>
<td>6807</td>
<td>10</td>
<td>0.15</td>
<td>1.0 (Referent)</td>
<td>1.0 (Referent)</td>
</tr>
<tr>
<td>0.9-1.00</td>
<td>809</td>
<td>6315</td>
<td>11</td>
<td>0.17</td>
<td>1.0 (0.5-2.0)</td>
<td>1.1 (0.5-2.4)</td>
</tr>
<tr>
<td>1.01-1.10</td>
<td>839</td>
<td>6450</td>
<td>4</td>
<td>0.05</td>
<td>1.2 (0.6-2.4)</td>
<td>1.1 (0.5-2.5)</td>
</tr>
<tr>
<td>1.11-1.27</td>
<td>785</td>
<td>5085</td>
<td>12</td>
<td>0.21</td>
<td>2.9 (1.8-5.5)</td>
<td>2.5 (1.2-5.1)</td>
</tr>
<tr>
<td>&gt;1.27</td>
<td>716</td>
<td>4600</td>
<td>20</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Risk of Lower-Extremity Bypass, Angioplasty, or Amputation During Follow-up by Quintiles of Creatinine Concentration and Estimated GFR

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Subjects, No.</th>
<th>Person-Years at Risk</th>
<th>Events, No.</th>
<th>Event Rate, %/y</th>
<th>Unadjusted HR (95% CI)</th>
<th>Adjusted HR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine, mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.75 (Women)</td>
<td>502</td>
<td>3676</td>
<td>7</td>
<td>0.19</td>
<td>1.00 (Referent)</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>&lt;0.95 (Men)</td>
<td>1162</td>
<td>8863</td>
<td>17</td>
<td>0.19</td>
<td>0.9 (0.4-2.2)</td>
<td>0.8 (0.3-2.1)</td>
</tr>
<tr>
<td>0.75-0.84 (Women)</td>
<td>858</td>
<td>6644</td>
<td>11</td>
<td>0.17</td>
<td>0.9 (0.3-2.2)</td>
<td>1.0 (0.4-2.6)</td>
</tr>
<tr>
<td>0.95-1.14 (Men)</td>
<td>627</td>
<td>4741</td>
<td>8</td>
<td>0.17</td>
<td>0.9 (0.3-2.4)</td>
<td>0.9 (0.3-2.6)</td>
</tr>
<tr>
<td>0.85-0.94 (Women)</td>
<td>876</td>
<td>6054</td>
<td>17</td>
<td>0.28</td>
<td>1.3 (0.5-3.2)</td>
<td>1.3 (0.5-3.2)</td>
</tr>
<tr>
<td>1.15-1.24 (Men)</td>
<td>837</td>
<td>6261</td>
<td>12</td>
<td>0.19</td>
<td>1.00 (Referent)</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>0.95-1.04 (Women)</td>
<td>811</td>
<td>6250</td>
<td>15</td>
<td>0.24</td>
<td>1.2 (0.5-2.5)</td>
<td>1.5 (0.7-3.4)</td>
</tr>
<tr>
<td>1.25-1.34 (Men)</td>
<td>840</td>
<td>6495</td>
<td>8</td>
<td>0.12</td>
<td>0.6 (0.2-1.4)</td>
<td>0.7 (0.3-1.9)</td>
</tr>
<tr>
<td>&gt;0.82.84</td>
<td>780</td>
<td>5833</td>
<td>9</td>
<td>0.15</td>
<td>0.8 (0.3-1.9)</td>
<td>1.0 (0.1-2.5)</td>
</tr>
<tr>
<td>73.8-82.83</td>
<td>757</td>
<td>5138</td>
<td>16</td>
<td>0.31</td>
<td>1.5 (0.7-3.2)</td>
<td>1.7 (0.7-3.9)</td>
</tr>
</tbody>
</table>

### Abbreviations:
- CI: confidence interval
- GFR: glomerular filtration rate
- HR: hazard ratio
- BMI: Body Mass Index

*Adjusted for age, race, sex, income, hypertension, diabetes, smoking, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, C-reactive protein, myocardial infarction, stroke, triglyceride level, fibrinogen measure, and body mass index (calculated as weight in kilograms divided by the square of height in meters).
Evaluation (HOPE) Study,\(^1\) randomization to ramipril (vs placebo) was associated with a decreased incidence of lower-extremity revascularization or amputation. The PAD events studied herein are much less common than other cardiovascular outcomes; therefore, methods to target potential preventive therapies to those at greatest risk are particularly important. Further studies are needed to determine whether cystatin C is a useful clinical measure to identify these high-risk patients.

Although our results suggest that cystatin C concentration may be a useful clinical measure for identifying patients at greatest risk for future PAD events, these findings have not yet been validated in other cohorts. Furthermore, CHS cohort members were elderly community-dwelling adults, so our findings may not be generalizable to younger populations. A gold standard measure of renal function is not available for the CHS. Thus, while our results may suggest that cystatin C is a more sensitive marker of renal insufficiency than either serum creatinine level or eGFR, it is also possible that the association of cystatin C with PAD events may reflect its association with factors other than level of renal function.\(^23\)

In conclusion, cystatin C is independently associated with the development of PAD complications requiring either amputation or revascularization among elderly patients without prevalent PAD at baseline. Further study is needed to determine whether cystatin C may have clinical utility in identifying those at increased risk for requiring a procedure for PAD.

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