Impact of Elevated Cystatin C Level on Cardiovascular Disease Risk in Predominantly High Cardiovascular Risk Populations

A Meta-Analysis

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Background—Chronic kidney disease is a growing public health problem that carries substantial risk for cardiovascular disease (CVD). Single studies have differentially examined the role of cystatin C, a novel renal index, with varying cut-point use. We undertook a meta-analysis of prospective studies to properly assess the link between cystatin C (dichotomized and continuous) versus CVD.

Methods and Results—Systematic literature search for studies reporting a multivariate-adjusted estimate, represented as relative risk (RR) with 95% confidence interval (CI), of the association between cystatin C and subsequent risk of (1) any CVD event and (2) specific CVD events. Data were collated from 14 studies, with 13 high cardiovascular risk population cohorts and 1 general population cohort, involving 23,509 subjects with 2,321 CVD events, 741 coronary heart disease events, and 828 stroke events. Highest cystatin C category versus lowest was associated with greater risk of CVD (RR, 2.62; 95% CI, 2.05 to 3.37, P<0.001), coronary heart disease (RR, 1.72; 95% CI, 1.27 to 2.34; P<0.001), and stroke (RR, 1.83; 95% CI, 1.12 to 3.00; P=0.02) after adjustment for established cardiovascular risk factors. Each standard deviation rise in cystatin C concentration boosted CVD risk (RR, 1.34; 95% CI, 1.18 to 1.51; P<0.001). Highest cystatin C category was also independently linked to greater risk of all-cause mortality and heart failure.

Conclusions—The meta-analysis, mostly derived from high cardiovascular populations, showed that cystatin C is strongly and independently associated with subsequent CVD risk. Further investigation is warranted to clarify whether measurement of cystatin C can usefully enhance CVD stratification beyond established predictors already in clinical use. (Circ Cardiovasc Qual Outcomes. 2010;3:675-683.)

Key Words: cystatin C ■ cardiovascular diseases ■ coronary artery disease ■ stroke ■ meta-analyses ■ risk factors

Chronic kidney disease is a worldwide health problem that carries a substantial risk for cardiovascular morbidity and death.1–3 Serum creatinine concentrations or creatinine-based estimating equations have served as the primary tool for evaluation of kidney function in clinical practice and in epidemiological research studies. However, the widely used and expert recommended creatinine-based Modification of Diet and Renal Disease study formula for estimating glomerular filtration rate (GFR) still has substantial inaccuracy when applied to healthy persons and older individuals.4

Recently, the use of serum creatinine–based formulas to assess renal function has been challenged by novel markers, particularly cystatin C, which may be a more reliable index of renal function.5,6 Cystatin C is a small serine protease inhibitor that is secreted from almost all active functional cells in the body. At a molecular weight of 13 kDa, the protein is freely filtered by the renal glomerulus and then metabolized by the proximal tubule.6,7 Mounting data suggest that cystatin C is superior to serum creatinine or creatinine-based estimating equations for prediction of cardiovascular events among elderly persons8 and among persons with known coronary heart disease (CHD).9 However, no systematic review has been conducted to evaluate the totality of available evidence regarding a link between cystatin C and subsequent risk of (1) any cardiovascular disease (CVD) event or (2) specific cardiovascular events including major coronary events or strokes. We therefore undertook a meta-analysis of cohort studies to qualitatively and
quantitatively assess the association between cystatin C and risk of CVD.

**WHAT IS KNOWN**

- Chronic kidney disease is a worldwide health problem that carries a substantial risk for cardiovascular morbidity and death.
- The use of serum creatinine–based formulas to assess renal function has been challenged by novel markers, particularly cystatin C, which may be a more reliable index of renal function. However, little is known about the qualitative and quantitative impact of cystatin C on cardiovascular risk using the totality of published data.

**WHAT THE STUDY ADDS**

- This meta-analysis, derived mostly from populations at high cardiovascular risk, showed that cystatin C is strongly and independently associated with future cardiovascular events.

### Methods

#### Data Sources and Searches

The search strategy was conducted according to the recommendations of the Meta-analysis of Observational Studies in Epidemiology.10 We performed a systematic search of PUBMED (1966 to May 2010), EMBASE (1947 to May 2010), and the Cochrane Library using the search strategy: “cystatin C” crossed with “cardiovascular disease” or “myocardial ischemia” or “ischemic heart disease” or “coronary heart disease” or “angina” or “stroke” or “cerebrovascular disease” or “cerebrovascular attack” or “cerebral infarct” or “intracranial hemorrhage.” We also searched abstracts in all American Heart Association (AHA)-sponsored meetings, from January 2003 to February 2009, via the AHA Abstract Archive Tool (http://www.abstractsonline.com/arch/home.aspx?lookupkey=12345). We restricted the search to human studies. There were no language restrictions. Further information was retrieved through a manual search of references from recent reviews and relevant published original studies.

#### Study Selection

Studies were selected if they met the following entry criteria: (1) subjects were prospectively followed within a cohort study or clinical trial; (2) cystatin C measured at baseline; (3) reported CVD, CHD, and/or stroke events as outcomes; (4) follow-up duration of at least 1 year; (5) reported quantitative estimates of the multivariate-adjusted relative risk (RR) and 95% confidence interval (CI) for at least 1 of the outcomes associated with baseline cystatin C. Studies were excluded if (1) the study design was cross-sectional or case-control studies; (2) the majority of participants had end-stage renal disease (dialysis or GFR <15 mL/min/1.73 m²)11 or kidney transplant; (3) they only reported unadjusted or age- and sex-adjusted RR; (4) they did not report 95% CI; or (5) data were duplicative. For studies that reported several multivariable-adjusted RRs, we extracted the effect estimate that was most fully adjusted for potential confounders. Cystatin C was not always measured at baseline study entry, and in certain situations it was assessed retrospectively on baseline frozen blood samples (eg, Cardiovascular Health study).8

#### Data Extraction and Quality Assessment

Data were extracted using a standardized extraction form and all data from eligible studies were extracted independently by 2 investigators (M.L. and W.H.H.). Discrepancies were resolved by discussion with a third investigator (B.O.) and by referencing the original report. We assessed the quality of all manuscripts that met the selection criteria. Quality assessment was based on guidelines developed by the United States Preventive Task Force as well as the modified checklist used in previous studies.12–14 We assessed the following 8 characteristics: (1) prospective study design; (2) maintenance of comparable groups; (3) appropriate and adequate adjustment of potential confounders (ie, at least 5 of 7 Framingham cardiovascular risk factors); (4) documented loss of follow-up rate; (5) outcomes assessed blinded to exposure status; (6) appropriate and clear definition of exposures (cystatin C) and outcomes (CVD, CHD, and stroke); (7) temporality (cystatin C measured at baseline, not at time of outcomes assessment); and (8) follow-up of at least 1 year. Studies were graded as good quality if they met 6 to 8 criteria; fair if they met 3 to 5; and poor if they met less than 3 criteria.

#### Data Synthesis and Analysis

There is currently no widely standardized cystatin C measurement, but the nephelometric or turbidimetric methods are most frequently used.15 In a study on 120 samples containing between 0.5 and 9 mg/L by the nephelometric method,16 the 2 methods correlated excellently (r=0.97), although when each was calibrated with the calibrator provided by the manufacturer, the turbidimetric method produced far higher values. Conversely, when a common calibrator was used, the slope of the Passing-Bablok line was not significantly different from 1. Various studies reported different segmentation strategies for grouping patients by cystatin C level, including dichotomous, tertiles, quartiles, or quintiles, or treated cystatin C level as a continuous variable and reported increase in risk for a given unit of increase in cystatin C level. To provide clinically relevant and easily interpretable results, our primary outcomes of interest were the association of CVD, CHD, and stroke risk and cystatin C categorical level, based on the comparison of the highest category of cystatin C versus lowest. We also analyzed cystatin C level as a continuous variable to evaluate the CVD risk, based on per standard deviation cystatin C increase. Our secondary outcomes of interest were risk of all-cause mortality and incident heart failure, based on the comparison of highest cystatin C category versus lowest.

Subgroup analyses for CVD risk, based on highest cystatin C category versus lowest, were conducted according to baseline population (elderly versus CHD or stroke versus heart failure versus asymptomatic carotid artery atherosclerosis), location (North America versus Europe versus Asia), mean age (<70 years versus ≥70 years), end point (all CVD versus fatal CVD), follow-up duration (1 to 5 years versus >5 years), CVD events number (≥100 versus <100), baseline estimated GFR interval (eGFR ≥60 mL/min/1.73 m² versus eGFR <60 mL/min/1.73 m²), cutoff point of the lowest interval (reference) of cystatin C (<0.90 mg/L versus 0.90 to 1.50 mg/L), and cutoff point of the highest interval of cystatin C (0.93 to 1.29 mg/L versus ≥1.30 mg/L).

Additional analysis was done to explore the impact of per standard deviation increase in cystatin C on the general and elderly populations.

Data analyses used multivariable-adjusted outcome data (expressed as RRs and 95% CIs). The statistical analysis used the inverse variance approach to combine log RRs and standard errors. We used a random-effects model (DerSimonian and Laird method) and explored for sources of inconsistency (I²) and heterogeneity. All reported probability values were 2-sided, with significance set at <0.05. Heterogeneity was assessed by probability values of χ² statistics and I², which describes the percentage of variability in the effect estimates that is due to heterogeneity rather than chance.17,18 We regarded I² of <40% as “heterogeneity might not be important” and more than 75% as “considerable heterogeneity,” based on the suggestion from the Cochrane Handbook for Systematic Review of Interventions.19 Publication bias was estimated visually by funnel plots displaying standard error as the measure of sample size and RR as the measure of treatment effect.20 If publication bias was suggested by the funnel plots, a fixed-effects model would be obtained for the comparison with the random-effects model to clarify the small-study effects.20,21 Thereafter, an analysis excluding small
The systematic literature review identified 53 full articles for detailed assessment, among which 30 were excluded for not having any CVD, CHD, or stroke estimates, 3 for not having appropriate RRs and 95% CIs for at least 1 of the primary outcomes,22–24 and 2 for having follow-up durations <1 year25,26 (Figure 1). Our final primary analysis included 18 articles derived from 14 independent prospective studies;8,9,27–42 consisting of 22,509 participants. Among the 14 studies, 13 reported CVD outcomes (2321 events),8,9,27,29,31–38,40–42 4 reported CHD outcomes (741 events),8,9,31,39 and 4 reported stroke outcomes (828 events).8,9,31,37 The study characteristics are presented in Table 1. Three studies recruited subjects from a elderly population (8816 participants),8,9,38 1 recruited persons from a general population (5067 participants),35 5 recruited persons with CHD or stroke at entry (6701 participants),9,33,34,37,39,42 3 recruited persons with heart failure at entry (932 participants),27,36,40 and 1 recruited persons with asymptomatic carotid atherosclerosis (1004 participants).41 Follow-up duration ranged from 1 to 12.8 years.35 One study included men only,38 whereas others included both men and women. Of the 14 studies, 3 were done in the United States,8,9,31 3 in Sweden,27,35,38 2 in Germany,33,34 1 in The Netherlands,39 1 in Austria,41 1 in Spain,42 2 in Japan,26,40 and 1 in China.37 Overall quality of studies was good (median; 6; range, 4 to 8) on an 8-point scale. Most of the studies included in the meta-analysis used nephelometric assay (Dade Behring, Siemens), whereas 1 study27 used tubidimetric assay. Although most studies used the same assay, the estimate of CVD risk in a study36 was substantially higher than others. The cutoff point in this study was not different from others, but a range of coefficient of variation was not provided, preventing further exploration.36

Overall, 5 of 13 studies that used the nephelometric method provided data on the range of cystatin C levels; among these, the coefficient of variation for cystatin C levels ranged from 2% to 7.7%.

Highest cystatin C category versus lowest was associated with greater overall CVD risk (RR, 2.62; 95% CI, 2.05 to 3.37; P < 0.001), CHD risk (RR, 1.72; 95% CI, 1.27 to 2.34; P < 0.001), and stroke risk (RR, 1.83; 95% CI, 1.12 to 3.00; P = 0.02) after adjustment for established cardiovascular risk factors. Each standard deviation rise in cystatin C concentration was associated with increased risk for overall CVD (RR, 1.34; 95% CI, 1.18 to 1.51; P < 0.001) (Figure 2). The highest cystatin C category was also associated with greater risk of all-cause mortality (RR, 2.22; 95% CI, 1.69 to 2.92; P < 0.001) and incident heart failure (RR, 2.23; 95% CI, 1.77 to 2.81; P < 0.001) (Figure 3).

In heterogeneity testing and sensitivity analysis for studies reporting CVD outcomes (highest cystatin C interval versus lowest), significant heterogeneity was present (P < 0.001, I² = 82%) but exclusion of any single study for the analysis did not alter the overall finding. For studies reporting CHD outcomes (highest cystatin C interval versus lowest), significant heterogeneity was present (P = 0.001, I² = 73%) but exclusion of any single study for the analysis did not alter overall finding. For studies reporting stroke outcomes (highest level versus lowest), significant heterogeneity was present (P < 0.001, I² = 89%) and exclusion of the Cardiovascular Health Study8 from the analysis resulted in a rise in relative risk that was not significant (RR, 1.93; 95% CI, 0.55 to 6.84; P = 0.31). For studies reporting CVD outcomes (per standard deviation increase), significant heterogeneity was present (P < 0.001, I² = 84%) but exclusion of any single study for the analysis did not alter the overall finding. Asymmetry was noted in CVD and CHD funnel plots (highest category versus lowest) and the underrepresentation of small studies showing neutral or unexpected protective effects suggesting some degree of publication bias (supplement Figure 1). The estimate of CVD risk from fixed-effects model (RR, 2.29; 95% CI, 2.07 to 2.54) was smaller than estimated from the
Table 1. Study Characteristics

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Population</th>
<th>Participant No. (Women, %)</th>
<th>Mean Age, y</th>
<th>Follow-Up, y</th>
<th>Primary End Points, n</th>
<th>Comparison Cystatin C Level, mg/mL</th>
<th>*Adjusted Framingham Risk Factors, n</th>
<th>Other Adjusted Covariate, n</th>
<th>Study Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alehagen et al,27 2009</td>
<td>Sweden</td>
<td>Heart failure</td>
<td>464 (48)</td>
<td>73</td>
<td>10</td>
<td>Fatal CVD (113)</td>
<td>Highest quartile (≥1.66) vs lowest (&lt;1.22)</td>
<td>4</td>
<td>6</td>
<td>Good</td>
</tr>
<tr>
<td>Arimoto et al,40 2005</td>
<td>Japan</td>
<td>Heart failure</td>
<td>140 (38)</td>
<td>66</td>
<td>1.3</td>
<td>Cardiac death (32)</td>
<td>Per SD</td>
<td>1</td>
<td>3</td>
<td>Fair</td>
</tr>
<tr>
<td>Cardiovascular Health Study,29 2005 and 2006</td>
<td>USA</td>
<td>Elderly ages ≥65 y at entry</td>
<td>4637 (58)</td>
<td>75</td>
<td>7.4</td>
<td>Fatal CVD (530), all MI (370), and all stroke (405)</td>
<td>Highest quintile (≥1.29) vs lowest (≥0.89); per SD and high (≥1.0) vs low (&lt;1) in eGFR ≥60 mL/min/1.73 m² persons</td>
<td>4</td>
<td>7</td>
<td>Fair</td>
</tr>
<tr>
<td>Health, Aging, and Body Composition study,30,31 2006 and 2008</td>
<td>USA</td>
<td>Functional elders ages 70–79 y at entry</td>
<td>3044 (51)</td>
<td>74</td>
<td>6</td>
<td>Fatal CVD (242), all CHD (294), and all stroke (163)</td>
<td>High (≥1.19) vs low (&lt;0.84); per SD</td>
<td>6</td>
<td>14</td>
<td>Good</td>
</tr>
<tr>
<td>Heart and Soul Study,32 2007 and 2008</td>
<td>USA</td>
<td>CHD</td>
<td>990 (18)</td>
<td>67</td>
<td>3.1</td>
<td>All CVD (101), all CHD, and all stroke (CHD and stroke event no. not reported)</td>
<td>Highest quartile (≥1.30) vs lowest (≥0.91); per SD; highest quartile (≥1.30) vs others (≥1.3) in eGFR ≥60 mL/min/1.73 m² persons</td>
<td>5</td>
<td>7</td>
<td>Good</td>
</tr>
<tr>
<td>Hoke et al,41 2010</td>
<td>Austria</td>
<td>Asymptomatic carotid atherosclerosis</td>
<td>1004 (37)</td>
<td>69</td>
<td>3</td>
<td>All CVD (346)</td>
<td>Highest quartile (≥0.96) vs lowest (≤0.5)</td>
<td>4</td>
<td>8</td>
<td>Fair</td>
</tr>
<tr>
<td>Keller et al,33 2009</td>
<td>Germany</td>
<td>CHD, eGFR ≥60 mL/min/1.73 m²</td>
<td>1827 (19)</td>
<td>61</td>
<td>3.7</td>
<td>Fatal CVD (66)</td>
<td>Highest quartile (0.91–2.48) vs lowest (0.42–0.70)</td>
<td>4</td>
<td>5</td>
<td>Good</td>
</tr>
<tr>
<td>Koenig et al,34 2005</td>
<td>Germany</td>
<td>CHD</td>
<td>1033 (15)</td>
<td>59</td>
<td>2.8</td>
<td>All CVD (71)</td>
<td>Highest quintile (&gt;1.24) vs lowest (&lt;0.91)</td>
<td>4</td>
<td>5</td>
<td>Good</td>
</tr>
<tr>
<td>Malmö Diet and Cancer study,35 2009</td>
<td>Sweden</td>
<td>General</td>
<td>5067 (60)</td>
<td>58</td>
<td>12.8</td>
<td>All CVD (418)</td>
<td>Per SD</td>
<td>7</td>
<td>2</td>
<td>Good</td>
</tr>
<tr>
<td>Naruse et al,36 2009</td>
<td>Japan</td>
<td>Heart failure</td>
<td>328 (38)</td>
<td>73</td>
<td>2.5</td>
<td>Fatal CVD (52)</td>
<td>Highest quartile (≥1.31) vs lowest (≤0.89)</td>
<td>4</td>
<td>5</td>
<td>Good</td>
</tr>
<tr>
<td>Ni et al,37 2007</td>
<td>China</td>
<td>Stroke and controls</td>
<td>1187 (59)</td>
<td>61</td>
<td>5</td>
<td>All CVD (57) in stroke cohort; all stroke (269) in stroke + controls cohort</td>
<td>Highest quintile (≥1.42) vs lowest (≤0.87)</td>
<td>2</td>
<td>3</td>
<td>Fair</td>
</tr>
<tr>
<td>Taglieni et al,42 2010</td>
<td>Spain</td>
<td>Acute coronary syndrome</td>
<td>525 (26)</td>
<td>66</td>
<td>1</td>
<td>All CVD (157)</td>
<td>Highest 2 quartiles (≥0.93) vs lowest (&lt;0.81)</td>
<td>NR†</td>
<td>NR†</td>
<td>Fair</td>
</tr>
<tr>
<td>Uppsala Longitudinal Study of Adult Men,43 2008</td>
<td>Sweden</td>
<td>Approximately 71-year-old men in a city</td>
<td>1135 (0)</td>
<td>71</td>
<td>10</td>
<td>Fatal CVD (138)</td>
<td>High (≥1.5) vs low (&lt;1.5); per SD</td>
<td>7</td>
<td>3</td>
<td>Good</td>
</tr>
<tr>
<td>Windhauser et al,44 2009</td>
<td>Netherlands</td>
<td>Acute CHD, secondary analysis of a clinical trial</td>
<td>1128 (27)</td>
<td>62</td>
<td>3</td>
<td>All MI (77)</td>
<td>Highest tertile (≥1.01) vs lowest (&lt;0.86)</td>
<td>4</td>
<td>13</td>
<td>Fair</td>
</tr>
</tbody>
</table>

†Variables from 21 potential items that showed an association (P<0.1) with the study end point were entered the final multivariate model.

MI indicates myocardial infarction; NR, not reported.

*Number of factors adjusted for in the analysis among these 7 factors: age, sex, systolic blood pressure (BP), diabetes mellitus (DM), high-density lipoprotein cholesterol level, total cholesterol or low-density lipoprotein cholesterol level, and smoking.

random-effects model (RR, 2.62; 95% CI, 2.05 to 3.37), which suggested small-study effects.19,21 When we excluded small studies (CVD events <100) in our pooled analysis, the CVD risk was still substantial (RR, 2.09; 95% CI, 1.80 to 2.43; P<0.001) and the funnel plot did not suggest gross publication bias (supplement Figure 2).

Potential sources of heterogeneity in the strength of the association between cystatin C (highest category versus lowest) and subsequent risk of CVD were examined by conducting subgroup analyses (Table 2). Significant heterogeneity between pooled analyses were noted for study location (North America [RR, 2.51; 2.13 to 2.95] versus Europe [RR, 2.01; 1.62 to 2.51] versus Asia [RR, 8.00; 1.41 to 45.50], P for heterogeneity among subgroups <0.001), baseline eGFR interval (eGFR ≥60 mL/min/1.73 m² [RR, 1.90; 1.24 to 2.91] versus eGFR <60 mL/min/1.73 m² [RR, 3.24; 1.72 to 6.08], P for heterogeneity among subgroups, 0.02), CVD event number (≥100 events [RR, 2.09; 1.80 to 2.43] versus <100 events [RR, 5.02; 2.20 to 11.49], P for heterogeneity among subgroups <0.001), and end points (fatal
Figure 2. Risk ratios for A, cardiovascular disease; B, coronary heart disease; and C, stroke associated with highest interval of cystatin C level versus lowest. D, Risk ratio of cardiovascular disease associated with per standard deviation increase of cystatin C level. a, b, and c denote different cystatin C levels within the fifth quintile of the Cardiovascular Health Study.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Weight</th>
<th>Risk Ratio 4, Random, 95% CI</th>
<th>Risk Ratio 4, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alehagen 2009⁸</td>
<td>8.6%</td>
<td>1.52 [1.13, 2.04]</td>
<td></td>
</tr>
<tr>
<td>CHS 2005a⁹</td>
<td>8.3%</td>
<td>2.48 [1.78, 3.48]</td>
<td></td>
</tr>
<tr>
<td>CHS 2005b⁹</td>
<td>8.4%</td>
<td>2.73 [1.96, 3.60]</td>
<td></td>
</tr>
<tr>
<td>CHS 2005c⁹</td>
<td>8.3%</td>
<td>2.63 [2.02, 3.88]</td>
<td></td>
</tr>
<tr>
<td>HABC 2008³¹</td>
<td>7.8%</td>
<td>2.24 [1.50, 3.35]</td>
<td></td>
</tr>
<tr>
<td>Hinke 2010⁶¹</td>
<td>9.4%</td>
<td>1.94 [1.41, 2.67]</td>
<td></td>
</tr>
<tr>
<td>HSS 2007⁸</td>
<td>7.3%</td>
<td>2.00 [1.26, 3.17]</td>
<td></td>
</tr>
<tr>
<td>Keller 2009²³</td>
<td>6.7%</td>
<td>3.77 [2.18, 6.51]</td>
<td></td>
</tr>
<tr>
<td>Koenig 2006³⁴</td>
<td>6.9%</td>
<td>2.75 [1.64, 4.61]</td>
<td></td>
</tr>
<tr>
<td>Naruse 2009³⁶</td>
<td>5.9%</td>
<td>19.60 [10.30, 36.93]</td>
<td></td>
</tr>
<tr>
<td>NI 2007³⁷</td>
<td>6.3%</td>
<td>3.31 [1.62, 6.01]</td>
<td></td>
</tr>
<tr>
<td>Taglieri 2010⁴²</td>
<td>8.8%</td>
<td>1.68 [1.27, 2.17]</td>
<td></td>
</tr>
<tr>
<td>ULSAM 2008³⁸</td>
<td>8.3%</td>
<td>2.04 [1.46, 2.65]</td>
<td></td>
</tr>
</tbody>
</table>

Total (95% CI) 100.0% 2.62 [2.05, 3.37]

Heterogeneity: Tau² = 0.16, Chi² = 86.14, df = 12 (P < 0.00001); I² = 62%
Test for overall effect: Z = 7.60 (P < 0.00001)

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Weight</th>
<th>Risk Ratio 4, Random, 95% CI</th>
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</tr>
</thead>
<tbody>
<tr>
<td>CHS 2005a⁹</td>
<td>15.6%</td>
<td>1.44 [1.01, 2.06]</td>
<td></td>
</tr>
<tr>
<td>CHS 2005b⁹</td>
<td>15.5%</td>
<td>1.30 [0.90, 1.89]</td>
<td></td>
</tr>
<tr>
<td>CHS 2005c⁹</td>
<td>15.7%</td>
<td>1.65 [1.15, 2.37]</td>
<td></td>
</tr>
<tr>
<td>HABC 2008³¹</td>
<td>15.7%</td>
<td>1.03 [0.72, 1.48]</td>
<td></td>
</tr>
<tr>
<td>HSS 2007 fatal⁹</td>
<td>10.1%</td>
<td>5.80 [2.93, 11.47]</td>
<td></td>
</tr>
<tr>
<td>HSS 2007 nonfatal⁹</td>
<td>13.0%</td>
<td>2.00 [1.21, 3.31]</td>
<td></td>
</tr>
<tr>
<td>Windhausen 2009⁹⁰</td>
<td>14.2%</td>
<td>1.95 [1.26, 3.02]</td>
<td></td>
</tr>
</tbody>
</table>

Total (95% CI) 100.0% 1.72 [1.27, 2.34]

Heterogeneity: Tau² = 0.12, Chi² = 22.47, df = 6 (P = 0.0010); I² = 73%
Test for overall effect: Z = 3.47 (P = 0.0005)

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Weight</th>
<th>Risk Ratio 4, Random, 95% CI</th>
<th>Risk Ratio 4, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHS 2005a⁹</td>
<td>17.3%</td>
<td>1.43 [1.01, 2.02]</td>
<td></td>
</tr>
<tr>
<td>CHS 2005b⁹</td>
<td>17.4%</td>
<td>1.97 [1.42, 2.74]</td>
<td></td>
</tr>
<tr>
<td>CHS 2005c⁹</td>
<td>17.3%</td>
<td>1.80 [1.27, 2.54]</td>
<td></td>
</tr>
<tr>
<td>HABC 2008³¹</td>
<td>16.5%</td>
<td>1.01 [0.66, 1.56]</td>
<td></td>
</tr>
<tr>
<td>HSS 2007³¹</td>
<td>14.9%</td>
<td>1.10 [0.81, 1.58]</td>
<td></td>
</tr>
<tr>
<td>NI 2007³⁷</td>
<td>16.6%</td>
<td>6.38 [4.20, 9.68]</td>
<td></td>
</tr>
</tbody>
</table>

Total (95% CI) 100.0% 1.93 [1.12, 3.00]

Heterogeneity: Tau² = 0.33, Chi² = 40.04, df = 5 (P < 0.00001); I² = 89%
Test for overall effect: Z = 2.42 (P = 0.02)

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Weight</th>
<th>Risk Ratio 4, Random, 95% CI</th>
<th>Risk Ratio 4, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arinoto 2005⁴⁶</td>
<td>4.5%</td>
<td>1.94 [1.16, 3.25]</td>
<td></td>
</tr>
<tr>
<td>CHS 2006³⁹</td>
<td>20.6%</td>
<td>1.42 [1.31, 1.54]</td>
<td></td>
</tr>
<tr>
<td>HABC 2008³¹</td>
<td>19.3%</td>
<td>1.15 [1.05, 1.26]</td>
<td></td>
</tr>
<tr>
<td>HSS 2008³⁹</td>
<td>17.3%</td>
<td>1.54 [1.33, 1.78]</td>
<td></td>
</tr>
<tr>
<td>MDC 2009³⁹</td>
<td>20.4%</td>
<td>1.13 [1.04, 1.23]</td>
<td></td>
</tr>
<tr>
<td>ULSAM 2008³⁸</td>
<td>17.3%</td>
<td>1.43 [1.24, 1.65]</td>
<td></td>
</tr>
</tbody>
</table>

Total (95% CI) 100.0% 1.34 [1.18, 1.51]

Heterogeneity: Tau² = 0.02, Chi² = 30.67, df = 5 (P < 0.0001); I² = 84%
Test for overall effect: Z = 4.66 (P < 0.00001)
CVD [RR, 2.98; 2.05 to 4.33] versus all CVD [RR, 2.07; 1.66 to 2.58], P for heterogeneity among subgroups, 0.03). There was no evidence that the strength of the association differed according to baseline population (elderly versus CHD or stroke versus heart failure versus asymptomatic carotid atherosclerosis), mean age (<70 years versus ≥70 years), follow-up duration (1 to 5 years versus >5 years), cutoff point of reference of cystatin C levels, and cutoff point of highest cystatin C interval (1 to 1.3 mg/L versus >1.3 mg/L). However, the impact of per standard deviation increase in cystatin C was larger in the elderly population versus the general population (RR, 1.32; 95% CI, 1.25 to 1.40 versus RR, 1.13; 95% CI, 1.04 to 23; P for heterogeneity among subgroups, 0.003).

**Discussion**

Our systematic review of over 22,000 participants from 14 prospective studies found that, compared with individuals with the lowest baseline cystatin C levels, those with the highest levels have a 162% increase in the risk of CVD, 72% increase in the risk of CHD, and 83% increase in the risk of stroke. When cystatin C level was analyzed as a continuous variable, each standard deviation increase was associated with 34% rise in overall CVD risk. Persons in the highest levels of cystatin C had a 122% increase in the risk of all-cause mortality and a 123% increase in incident heart failure. The inclusion of only longitudinal studies strengthened the robustness of our findings because issues of selection bias, recall bias, and reverse causality were minimized. In addition, all studies included in our meta-analysis reported a multivariate-adjusted relative risk, mitigating the possibility of known confounding influencing our results.

A major cystatin C study that did not meet the inclusion criteria of the current meta-analysis bears mention because this is the only study that compared cystatin C with measured GFR (not eGFR) for cardiovascular risk prediction.22 In this nondiabetic chronic kidney disease cohort, the association of cystatin C level with CVD mortality was as strong as that of iothalamate GFR with these outcomes, raising the possibility that cystatin C may not just be a marker of chronic kidney disease but also a CVD risk factor perhaps reflecting proinflammatory and proatherogenic states.15,22,43 Indeed, the potential role of cystatin C beyond just being a chronic kidney disease marker may also provide an explanation for why high cystatin C was associated with a substantial increase in CVD risk among persons with eGFR ≥60 mL/min/1.73 m², who do not meet the conventional definition of chronic kidney disease.44 Still, there is a possibility that eGFR is not sensitive enough to reflect the real association between mild renal insufficiency and CVD.

We used subgroup analyses to assess the varying influence of several factors on the association between cystatin C and CVD risk. Asians with high cystatin C levels appear to be at higher CVD risk than their European and American counterparts. It has been suggested that hypertension tends to develop in Asians at earlier ages than in other races,45 and it is conceivable that a longer history of hypertension may cause more profound damage of end organs and vessels, thereby leading to a higher likelihood of vascular events within a given study period. However, the results should be interpreted with caution because the only 2 Asian studies were based on relatively small populations, and there was substantial heterogeneity within these 2 studies.

We observed that the impact of elevated cystatin C was more profound on the risk of fatal CVD versus all CVD,
which probably points to the association of compromised kidney function with risk factors for generally poor clinical outcomes such as oxidative stress, widespread inflammation, electrolyte derangements, procoagulation, and presence of uremic toxins.\(^1\) The finding of a larger impact of elevated cystatin C in the elderly versus the general population is interesting. It may signify an increasing impact of kidney disease on CVD risk with rising age, as the effect of some classic risk factors (eg, total cholesterol) diminishes. We included studies\(^35,38\) adjusted all 7 Framingham risk factors (ie, age, sex, systolic blood pressure, diabetes mellitus, high-density lipoprotein cholesterol level, total cholesterol or low-density lipoprotein cholesterol level, and smoking), and pooling data from these 2 studies showed per standard decrease in cystatin C was associated with increased risk of cardiovascular disease. Furthermore, 1 study showed that addition of cystatin C to the model with established risk factors showed the trend of improvement of the C statistic for predicting cardiovascular death and that the C statistic increased significantly for the prediction of cardiovascular death when cystatin C and other 3 biomarkers were incorporated into a model with the established risk factor.\(^38\) Because kidney disease is not generally regarded as a classic risk factor, it was still substantial and highly significant.

Therefore, the positive association between elevated cystatin C and CVD risk probably is not completely attributable to publication bias.

There are concerns about whether novel biomarkers, including cystatin C, can actually provide additive value in the prediction of CVD on top of classic risk factors. Two included studies\(^35,38\) adjusted all 7 Framingham risk factors (ie, age, sex, systolic blood pressure, diabetes mellitus, high-density lipoprotein cholesterol level, total cholesterol or low-density lipoprotein cholesterol level, and smoking), and pooling data from these 2 studies showed per standard deviation increase in cystatin C was associated with increased risk of cardiovascular disease. Furthermore, 1 study showed that addition of cystatin C to the model with established risk factors showed the trend of improvement of the C statistic for predicting cardiovascular death and that the C statistic increased significantly for the prediction of cardiovascular death when cystatin C and other 3 biomarkers were incorporated into a model with the established risk factor.\(^38\) Because kidney disease is not generally regarded as a classic risk factor, it was still substantial and highly significant.
factor, yet has been consistently proven to be an independent predictor of cardiovascular mortality, and cystatin C is a reliable renal index, it may not be unreasonable to consider incorporating cystatin C in cardiovascular risk assessment to further delineate risk.

This study has limitations. First, meta-analyses may be biased if the literature search fails to identify all relevant studies or the selection criteria for including a study are applied in a subjective manner. To minimize these risks, we developed the study protocol a priori and performed thorough searches across different data bases using explicit criteria for study selection, data abstraction, and data analysis. Second, the studies varied with respect to the characteristics of participants and the definitions of CVD, CHD, and stroke in outcome assessment and follow-up duration, and indeed heterogeneity was found by formal analysis. To explore the potential heterogeneity, we did subgroup analyses to identify plausible biological and selection sources of the variation. Third, each study has its own adjustment for variables; therefore some studies adjusted more than others. Because this study was a study-level meta-analysis and not an individual-level meta-analysis, it is impossible for us to apply a uniform adjustment for variables to all studies. Fourth, cystatin C is a novel biomarker, and the best cutoff point is still under debate. Our current meta-analysis does not solve this issue. In general, cystatin C 1.3 mg/L or greater was strongly associated with subsequent CVD risk. However, when cystatin C level was analyzed as a continuous variable, the meta-analysis found a 34% increase of CVD risk in each standard deviation increase (eg, 0.18 mg/L of cystatin C level increase; eg. 1.3 mg/L standard deviation). This implies that CVD risk may increase steadily from very low cystatin C levels. Further studies using more statistically sophisticated methods may be able to more precisely explore the effect of the cutoff values.

In summary, although limited by varying cystatin C cutoff points in different studies, this meta-analysis, mostly derived from high cardiovascular risk populations, which analyzed cystatin C in highest versus lowest category as well as per standard deviation increase, found that cystatin C is strongly and independently associated with subsequent risk of CVD, CHD, and stroke. Further and more detailed investigation is warranted to clarify whether measurement of cystatin C can usefully enhance CVD stratification beyond established predictors already in clinical use.

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Disclosures
None.

References


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Meng Lee, Jeffrey L. Saver, Wen-Hung Huang, Jessica Chow, Kuo-Hsuan Chang and Bruce Ovbiagele

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SUPPLEMENTAL MATERIAL

Supplementary Figure 1: Funnel plots for studies included in primary outcomes. (A) Cardiovascular disease, (B) coronary heart disease, and (C) stroke, analyzed with highest interval of cystatin C level versus lowest. (D) Cardiovascular disease analyzed with per standard deviation increase of cystatin C level.
**Supplementary Figure 2:** A funnel plot for large studies (CVD events ≥ 100) in cardiovascular disease outcome