

Cystatin C and Mortality Risk in the Elderly: The Health, Aging, and Body Composition Study

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Kidney dysfunction is known to decrease life expectancy in the elderly. Cystatin C is a novel biomarker of kidney function that may have prognostic utility in older adults. The association of cystatin C with mortality was evaluated in a biracial cohort of black and white ambulatory elderly and compared with that of serum creatinine concentrations. The Health, Aging and Body Composition study is a cohort of well-functioning elderly that was designed to evaluate longitudinal changes in weight, body composition, and function. A total of 3075 participants who were aged 70 to 79 yr and had no disability were recruited at sites in Memphis, TN, and Pittsburgh, PA, between April 1997 and June 1998 with a follow-up of 6 yr. At entry, the mean cystatin C was 1.05 mg/L and the mean creatinine was 1.06 mg/dl. After 6 yr of follow-up, 557 participants had died. The mortality rates in each ascending cystatin C quintile were 1.7, 2.7, 2.9, 3.1, and 5.4%/yr. After adjustment for demographic risk factors, comorbid health conditions, and inflammatory biomarkers (C-reactive protein, IL-6, and TNF- α), each quintile of cystatin C was significantly associated with increased mortality risk compared with the lowest: Hazard ratios (HR; 95% confidence intervals) quintile 1, -1.0 (referent); quintile 2, -1.74 (1.21 to 2.50); quintile 3, -1.51 (1.05 to 2.18); quintile 4, -1.49 (1.04 to 2.13); and quintile 5, -2.18 (1.53 to 3.10). These associations did not differ by gender or race. Results were consistent for cardiovascular and other-cause mortality, but not cancer mortality. Creatinine quintiles were not associated with mortality after multivariate adjustment (HR: 1.0 [referent], 1.00 [0.72 to 1.39], 0.95 [0.68 to 1.32], 1.11 [0.79 to 1.57], 1.16 [0.86 to 1.58]). Cystatin C is a strong, independent risk factor for mortality in the elderly. Future studies should investigate whether cystatin C has a role in clinical medicine.

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Kidney dysfunction is a risk factor for mortality in the elderly (1-3). Serum creatinine concentrations or creatinine-based estimators of GFR have been the standard measure of kidney function in clinical practice and research and are generally thought to reflect 24-h clearance rates. However, as a breakdown product of muscle, serum creatinine concentrations are influenced by a variety of nonrenal factors, including body weight, nutritional status, race, age, and gender (4-7). Furthermore, creatinine is highly insensitive for detecting

reductions in kidney function, which may deteriorate >50% before the serum creatinine exceeds the normal range (8,9). These other factors limit the accuracy of creatinine for assessing kidney function, particularly in older people, in whom decreased muscle mass with age may further disguise a decline in kidney function. Therefore, the importance of kidney function as a prognostic factor in the elderly may be underestimated by serum creatinine concentrations.

Cystatin C is a promising new measure of kidney function that has been found to better predict GFR than creatinine. Cystatin C may have particular advantage in the elderly, as the concentrations do not seem to be influenced by age, gender, or muscle mass (10-12). Investigators from the Cardiovascular Health Study found that cystatin C had a much stronger and more linear association with mortality risk than creatinine or creatinine-based estimates of GFR (13); however, the study did not have adequate numbers of black participants to evaluate

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whether the association of cystatin C with mortality differed by race.

The Health, Aging, and Body Composition Study (Health ABC) is a cohort of well-functioning elderly people, aged 70 to 79 yr, that includes a large number of black men and women. In Health ABC, we measured serum cystatin C at baseline to determine whether cystatin C concentrations were associated with mortality risk. The specific objectives of these inquiries were (1) to determine the association of cystatin C concentrations with mortality risk and to contrast this association with that of serum creatinine concentrations, (2) to evaluate whether the association differed by race and gender, and (3) to investigate whether inflammatory factors were potential mediators for the association between cystatin C and mortality.

Materials and Methods

Design and Participants

Health ABC is a prospective study that was initiated by investigators at the National Institute on Aging to investigate the effect of age-related changes in body composition and health on subsequent health and incident functional limitation and disability. Each of the two study sites, Pittsburgh, PA, and Memphis, TN, recruited participants who were aged 70 to 79 yr from a list of Medicare beneficiaries between April 1997 and June 1998. The goal of recruitment was to have a cohort of highly functional older people at baseline that was nearly balanced among men and women and white and black individuals. Race status was obtained from the Health Care Financing Administration (now the Centers for Medicare and Medicaid Services) database; recruitment was at random among all age-eligible individuals within each stratum of race (black and white). Inclusion criteria were (1) ability to walk one quarter mile, climb 10 steps, and perform basic activities of daily living without difficulty; (2) absence of life-threatening illness; and (3) plans to remain in the geographic area for at least 3 yr. The cohort enrolled 3075 participants who completed baseline evaluations, 42% of whom were black. Adequate specimens for analysis of cystatin C were available for 3044 (99%), the sample for this analysis. All participants gave informed written consent; the protocol was approved by the Institutional Review Boards of the clinical sites and the Data Coordinating Center (University of California, San Francisco, San Francisco, CA).

Kidney Function

Cystatin C was measured at the Health ABC core laboratory (University of Vermont, Burlington, VT) using a BNII nephelometer (Dade Behring Inc., Deerfield, IL) that used a particle-enhanced immunonephelometric assay (N Latex Cystatin C) (14). Among 61 healthy individuals with three cystatin C measurements over a 6-mo period, the intraindividual coefficient of variation was 7.7%, reflecting long-term stability of the measurement. 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. The assay remained stable over five cycles of freeze/thaw without change in the measurement.

Before measuring cystatin C, we compared cystatin C concentrations in serum and EDTA-citrated plasma specimens from other individuals and found a linear slope of 0.977 and R^2 of 0.958 using linear regression; compared with the serum measures, the plasma measures were slightly lower on average (mean difference 0.03 ± 0.02 mg/L; absolute range of differences 0.00 to 0.07 mg/L). We chose to use the plasma specimens, which had been stored at -70°C , for the measurement of cystatin C in the Health ABC cohort. Creatinine was used as a comparative measurement of kidney function, assayed by a colorimetric technique on a

Johnson & Johnson Vitros 950 analyzer (New Brunswick, NJ); the intraindividual coefficient of variation approximately 2%.

For our primary analyses, we categorized cystatin C into quintiles. We also repeated our analyses with cystatin C concentrations grouped into low (quintile 1, <0.84 mg/L), medium (quintiles 2 to 4, 0.84 to 1.18 mg/L), and high (quintile 5, >1.18 mg/L). These categories were presented to reflect better the association of cystatin C with mortality risk and to facilitate comparisons with other studies (13).

Secondary Predictors

Other characteristics were used in these analyses as adjustment variables to determine the independence of the association of serum cystatin C with mortality. These included sociodemographic factors (age, gender, race, clinical site, education level); lifestyle factors (current smoking defined by current *versus* former or never; alcohol use defined by ≥ 1 drink per week, with no use defined as <1 drink per week; body mass index); comorbid conditions (diabetes defined by use of hypoglycemic agents, self-report, fasting plasma glucose ≥ 126 mg/dl or an oral glucose tolerance test ≥ 200 mg/dl; hypertension by either self-report plus use of antihypertensive medications, or measured systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg; chronic heart failure, coronary heart disease, myocardial infarction, angina, coronary artery bypass, chronic obstructive pulmonary disease, and cerebrovascular disease, which all were by self-report in this study); serum chemistries (glucose, total cholesterol, HDL cholesterol, triglycerides, and albumin, all measured by a colorimetric technique on a Johnson & Johnson Vitros 950 analyzer). LDL was calculated using the Friedewald equation (15). Baseline blood draws were taken after an 8-h fast. Samples then were aliquotted and stored at -80°C until analysis; all transportation was conducted using dry ice. Medications were brought in by the participant and recorded. We were particularly interested in use of aspirin, β blockers, angiotensin-converting enzyme inhibitors, calcium antagonists, statins, and diuretics as predictors of survival.

Measures of IL-6, TNF- α , and C-reactive protein (CRP) were performed using ELISA kits from R&D Systems (Minneapolis, MN). Detectable limits were 0.10 pg/ml for IL-6, 0.18 pg/ml for TNF- α , and 0.007 mg/L for CRP. Interassay coefficients of variation were determined by duplicate analyses of 150 specimens; 10.3, 8.0, and 15.8% for IL-6, CRP, and TNF- α , respectively.

Outcomes

Follow-up occurred every 6 mo either by telephone or by annual visits to clinical centers. Deaths were ascertained by review of local obituaries, by reports to the clinical centers by family members, or by means of the semiannual contacts. Immediate and underlying causes of death were determined by a central adjudication committee on the basis of review of the death certificate, all recent hospital records, and interview with the next of kin. Causes of death in this analysis were categorized as cardiovascular, cancer, and infection/other, on the basis of the underlying cause adjudicated by committee according to specific prestated protocols. One death was attributable to unknown causes, and this individual was grouped with infection/other. These analyses include all deaths that had occurred through August 30, 2004.

Statistical Analyses

We used ANOVA to compare the mean (\pm SD) serum cystatin C concentrations by gender, by race, and across the four race/gender subgroups. We compared the distribution of each secondary predictor variable across quintiles of cystatin C, using the χ^2 test for categorical variables and ANOVA for continuous variables. Certain measures were log-transformed because of their rightward skew (glucose, triglycer-

ides, CRP, IL-6, TNF- α , and creatinine). Mortality rates (%/yr) were determined by quintile of cystatin C

Staged multivariable proportional hazards models were used to evaluate the adjusted association of cystatin C quintiles with subsequent mortality risk. Following an unadjusted model, adjustments for socio-demographic characteristics, lifestyle factors, comorbid conditions, and serum chemistry measurements were made with variables that were selected on the basis of their having an unadjusted association with mortality at $P < 0.05$. Once we had defined the optimal adjusted model, we then tested the effects of the three inflammatory biomarkers IL-6, CRP, and TNF- α . We repeated these analyses modeling cystatin C as a continuous variable.

To quantify the potential mediating effect of the inflammatory factors, we compared the parameter estimate (β coefficient) from the multivariable model before and after adjustment for each inflammatory factor. We considered the percentage change in the parameter estimate as a metric for the extent to which each biomarker mediated the association between cystatin C and mortality.

We checked the linearity of the relationship between cystatin C and all-cause mortality by the addition of a quadratic term to an unadjusted proportional hazards model. A nonlinear relationship was initially detected, but upon further examination, 12 (0.50%) cystatin C outliers were responsible for the significance of the quadratic term. The quadratic term was not significant in adjusted analyses or when the 12 outliers were excluded. These 12 individuals were included in all subsequent analyses. After determining the relation between cystatin C and all-cause mortality, we proceeded with analyses of cause-specific mortality. In these analyses, we modeled cystatin C as a continuous variable and determined the relative hazard associated with a per-SD increase in cystatin C.

We determined the mortality rates across the four gender/race subgroups. We evaluated whether gender and race modified the association of cystatin C with mortality risk. First we constructed Kaplan Meier curves plotting the mortality risk of low (quintile 1), medium (quintiles 2 to 4), and high (quintile 5) cystatin C concentrations among white women, white men, black women, and black men. We separately tested for the presence of an interaction of cystatin C quintiles with race and with gender. In addition, we tested for an interaction of cystatin C and race within each gender and of cystatin C and gender within each race.

To compare creatinine as a predictor of mortality, we categorized creatinine into gender-specific quintiles, meaning that each quintile had roughly equal numbers of men and women. We repeated the staged, multivariate analyses as described above for cystatin C for the entire cohort. We tested for creatinine interactions with gender and race, separately.

Analyses were conducted using SAS v.8 (SAS Institute, Cary, NC). Testing of the proportional hazards assumption was performed using S-Plus V6.1 (Insightful Corp., Seattle, WA). The proportional hazards assumption was met for all models. Two-sided $P < 0.05$ were considered statistically significant.

Results

Baseline Comparisons

Among the 3044 participants in Health ABC with serum cystatin C and 3047 participants with creatinine measurements, the mean \pm SD cystatin C was 1.05 ± 0.34 mg/L and the mean \pm SD creatinine was 1.06 ± 0.42 mg/dl. Cystatin C concentrations were significantly higher in men than in women (1.08 ± 0.36 versus 1.01 ± 0.33 mg/L; $P < 0.0001$) and in white individuals compared with black individuals (1.06 ± 0.33 versus

1.03 ± 0.36 mg/L; $P < 0.0001$). By race and gender subgroups, cystatin C (mean \pm SD) concentrations were 1.00 ± 0.30 mg/L in black women (referent), 1.02 ± 0.35 mg/L in white women ($P = 0.19$), 1.07 ± 0.43 mg/L in black men ($P < 0.0001$), and 1.09 ± 0.31 mg/L in white men ($P < 0.0001$).

Table 1 displays baseline characteristics by quintile of cystatin C. Higher concentrations of cystatin C were associated with older age, male gender, white race, and higher body mass index (Table 1). The prevalence of diabetes, hypertension, coronary heart disease, cerebrovascular disease, and heart failure increased with ascending quintiles of cystatin C, as did higher levels of triglycerides and lower levels of LDL and HDL. Levels of the inflammatory factors CRP, IL-6, and TNF- α all increased with rising quintiles of cystatin C.

Race and Gender Subgroups

Mortality risk differed markedly across demographic subgroups: 2.0%/yr in white women, 2.8%/yr in black women, 3.3%/yr in white men, and 5.5%/yr in black men. In each subgroup, however, low, medium, and high cystatin C concentration seemed to distinguish three levels of mortality risk (Figure 1). We found no significant interaction of cystatin C and either gender ($P = 0.09$) or race ($P = 0.07$) for predicting mortality. In addition, there was no significant interaction of gender and cystatin C within white ($P = 0.18$) or black individuals ($P = 0.11$). Conversely, there were no cystatin C and race interactions within men ($P = 0.19$) or women ($P = 0.45$).

Cystatin C and Mortality Risk

After 6 yr of follow-up, 557 participants had died. All-cause mortality risk increased substantially from the lowest quintile of cystatin C to the highest (Figure 2). Quintiles 2 to 4 had similar mortality risks that were 60 to 90% higher than quintile 1, whereas the risk in quintile 5 was three-fold that of quintile 1 (Table 2). The association of the highest quintile of cystatin C with mortality was attenuated somewhat by adjustment for sociodemographic factors, prevalent diseases, and laboratory measurements (adjusted model); however, risk for death in the highest quintile and in the middle quintiles remained significantly elevated compared with the lowest quintile.

Effect of Inflammation

Adjustment for the three inflammatory biomarkers (CRP, IL-6, and TNF- α) seemed to attenuate the association of each of the upper three quintiles with mortality risk, but all quintiles remained at significantly greater risk compared with the lowest quintile. We next adjusted for the inflammatory makers individually to test for which had the greatest mediating effect. Without adjustment for inflammation, the parameter estimate (β coefficient) for the high cystatin C quintile was 1.00 ± 0.18 . Adjustment for each of the biomarkers individually had the following effects on the parameter estimate: TNF- α 0.82 ± 0.17 , IL-6 0.93 ± 0.17 , and CRP 0.95 ± 0.16 . Adjustment by all three biomarkers attenuated the parameter estimate to 0.79 ± 0.18 . Thus, the modest attenuation of risk related to adjustment for inflammatory markers was largely attributable to TNF- α rather than IL-6 or CRP.

Table 1. Comparison of patient characteristics by cystatin C quintile^a

Characteristic (Baseline)	Cystatin C Quintile (mg/L)					P
	<0.84 (n = 578)	0.84 to 0.93 (n = 590)	0.94 to 1.03 (n = 605)	1.04 to 1.18 (n = 654)	≥1.19 (n = 617)	
Age (yr)	73.0 ± 2.8	73.2 ± 2.8	73.6 ± 2.9	74.0 ± 2.9	74.3 ± 2.9	<0.001
Men	194 (34)	260 (44)	304 (50)	379 (58)	340 (55)	<0.001
Black	283 (49)	262 (44)	266 (44)	231 (35)	223 (36)	<0.001
Site						
Memphis	317 (55)	309 (52)	289 (48)	311 (48)	304 (49)	0.05
Pittsburgh	261 (45)	281 (48)	316 (52)	343 (53)	313 (51)	
Education ≥ 12 yr	241 (42)	246 (42)	244 (40)	277 (42)	270 (44)	0.82
Current smoker	49 (9)	60 (10)	69 (11)	66 (10)	70 (11)	0.45
Alcohol use	304 (53)	302 (51)	301 (50)	316 (48)	283 (46)	0.15
Body mass index (kg/m ²)	26.3 ± 4.8	27.1 ± 4.8	27.5 ± 4.7	27.7 ± 4.7	28.3 ± 4.9	<0.001
Diabetes	82 (14)	74 (13)	75 (12)	100 (15)	132 (21)	<0.001
Hypertension	255 (44)	265 (45)	289 (48)	345 (53)	397 (64)	<0.001
Chronic obstructive pulmonary disease	3 (1)	4 (1)	4 (1)	7 (1)	12 (2)	0.08
Cancer	99 (17)	102 (17)	107 (18)	133 (20)	136 (22)	0.11
Coronary heart disease	87 (15)	94 (16)	111 (18)	151 (23)	176 (29)	<0.001
Cerebrovascular disease	36 (6)	39 (7)	35 (6)	61 (9)	73 (12)	<0.001
Heart failure	7 (1)	11 (2)	13 (2)	19 (3)	44 (7)	<0.001
LDL (mg/dl)	124 ± 33	122 ± 34	124 ± 35	120 ± 34	118 ± 37	0.004
HDL (mg/dl)	60 ± 18	56 ± 17	54 ± 17	51 ± 16	50 ± 16	<0.001
Triglycerides ^b (mg/dl)	120 ± 62	131 ± 78	135 ± 73	148 ± 85	156 ± 103	<0.001
Albumin (g/dl)	4.0 ± 0.3	4.0 ± 0.3	4.0 ± 0.3	4.0 ± 0.3	4.0 ± 0.3	0.12
Glucose ^b (mg/dl)	103 ± 33	104 ± 40	105 ± 37	105 ± 31	107 ± 36	0.08
CRP ^b (mg/L)	2.7 ± 4.8	2.6 ± 3.3	2.6 ± 3.2	2.9 ± 4.5	4.2 ± 6.8	<0.001
IL-6 ^b (pg/ml)	2.0 ± 1.7	2.2 ± 1.8	2.2 ± 1.7	2.5 ± 2.0	3.0 ± 2.2	<0.001
TNF-α ^b (pg/ml)	2.6 ± 1.0	3.0 ± 1.6	3.2 ± 1.1	3.7 ± 1.6	4.8 ± 2.2	<0.001
Cystatin C (mg/L)	0.75 ± 0.07	0.88 ± 0.03	0.99 ± 0.03	1.10 ± 0.04	1.48 ± 0.52	NA
Creatinine ^b (mg/dl)	0.87 ± 0.15	0.94 ± 0.15	1.01 ± 0.08	1.07 ± 0.19	1.39 ± 0.76	<0.001
Statin use	70 (12.1)	75 (12.7)	77 (12.7)	83 (12.7)	88 (14.3)	0.84
β blocker use	54 (9.3)	73 (12.4)	81 (13.4)	86 (13.2)	119 (19.3)	<0.001
Aspirin use	198 (34.3)	214 (36.3)	207 (34.2)	252 (38.5)	272 (44.1)	0.002
ACE inhibitor use	70 (12.1)	63 (10.7)	83 (13.7)	115 (17.6)	130 (21.1)	<0.001
Calcium antagonist use	122 (21.1)	127 (21.5)	132 (21.8)	153 (23.4)	166 (26.9)	0.10

^aData are n (%) or mean ± SD. Conventional units are displayed; to convert to Systeme International units, multiply LDL and HDL by 0.0259 for mmol/L, triglycerides by 0.0113 for mmol/L, albumin by 10 for g/L, glucose by 0.0555 for mmol/L, and creatinine by 88.4 for μmol/L. CRP, C-reactive protein; ACE, angiotensin-converting enzyme.

^bP values obtained by using the log transformation.

We repeated these analyses with cystatin C modeled as a continuous variable per SD (0.34 mg/L). In the unadjusted model, each 0.34 mg/L of cystatin C was associated with a 1.24-fold (95% confidence interval [CI], 1.20 to 1.28) risk for death. This result was unchanged by adjustment for sociodemographic characteristics, lifestyle factors, comorbid conditions, and serum chemistry measurements (hazard ratio [HR], 1.24; 95% CI, 1.19 to 1.30). However, adjustment for the inflammatory markers did moderately attenuate the association of cystatin C with all-cause mortality (HR, 1.16; 95% CI, 1.09 to 1.24).

Stratification by Race

The associations (HR; 95% CI) of ascending quintiles of cystatin C with mortality risk in white individuals were 1.0 (referent), 1.74 (1.01 to 3.01), 1.32 (0.75 to 2.32), 1.63 (0.96 to 2.76), and 1.93 (1.13 to 3.31) after adjustment for sociodemographic characteristics, lifestyle factors, comorbid conditions, serum chemistry, and inflammatory markers. These adjusted HR (95% CI) in black individuals were 1.0 (referent), 1.64 (1.00 to 2.70), 1.72 (1.06 to 2.81), 1.50 (0.90 to 2.50), and 2.39 (1.47 to 3.88). Increasing quintiles of creatinine were not associated with mortality risk in white or black individuals. The adjusted HR (95%

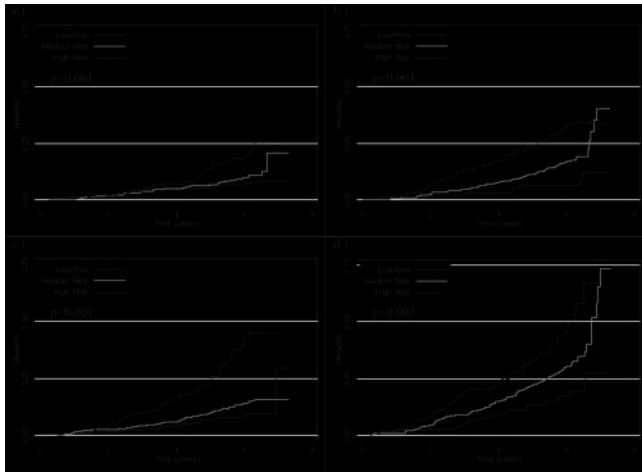


Figure 1. Nelson-Aalen cumulative hazard functions are shown in white women (a), black women (b), white men (c), and black men (d). Each curve displays mortality risks among participants with low (<0.84 mg/L), medium (0.84 to 1.18 mg/L), and high (≥ 1.19 mg/L) cystatin C concentrations, stratified by gender/race subgroup: White women, white men, black women, and black men. Each figure shows significant differences by cystatin C category ($P < 0.001$).

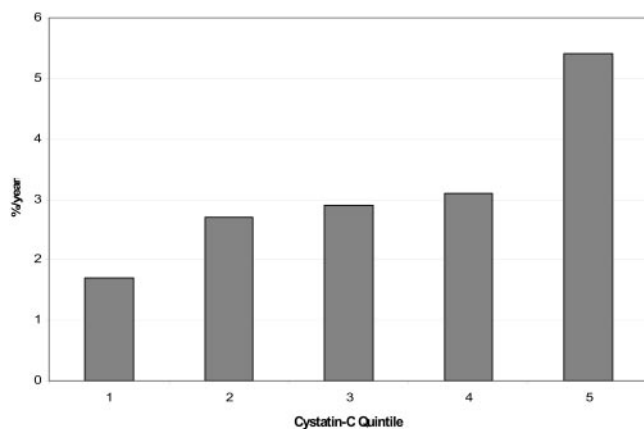


Figure 2. Cystatin C quintiles and mortality risk. This figure displays the annual all-cause mortality risk by quintile of cystatin C. Cutpoints for cystatin C quintiles are <0.84, 0.84 to 0.93, 0.94 to 1.03, 1.04 to 1.18, and ≥ 1.19 mg/L. $P < 0.001$ for comparison across quintiles.

CI) in white individuals were 1.0 (referent), 1.13 (0.74 to 1.70), 0.86 (0.55 to 1.34), 1.02 (0.64 to 1.64), and 1.10 (0.71 to 1.70). These adjusted HR (95% CI) in black individuals were 1.0 (referent), 0.78 (0.44 to 1.39), 1.02 (0.61 to 1.71), 1.12 (0.67 to 1.88), and 1.15 (0.72 to 1.84).

Reclassification of Risk Groups

We repeated the analyses after classifying participants as low (quintile 1), medium (quintiles 2 to 4), or high (quintile 5) risk on the basis of cystatin C concentrations (Table 3). The absolute mortality risk ranged three-fold from the low- to high-risk categories. After full adjustment for all covariates, the medium-

risk group had a 57% increase in risk, and the high-risk group had a 120% increase in risk compared with low-risk participants (Table 3).

Cause-Specific Mortality

The cause of death was broadly categorized as death caused by cardiovascular disease ($n = 177$); cancer ($n = 137$); or infectious, other, and unknown ($n = 242$). After multivariable analysis including adjustment for inflammation, cystatin C concentration (per SD, 0.3 mg/L) was associated with the outcomes of cardiovascular death (HR, 1.20; 95% CI 1.11 to 1.30) and death from infectious/other/unknown cause (HR 1.33; 95% CI 1.24 to 1.41) but not with cancer death (HR 1.12; 95% CI 0.96 to 1.31).

Creatinine Quintiles and Mortality

For comparison with cystatin C, we also evaluated gender-specific quintiles of creatinine as predictors of mortality (Table 4). In unadjusted analysis, the fifth quintile had a significantly higher mortality risk compared with the first quintile, but quintiles 2 to 4 had similar risk as quintile 1. After adjustment for sociodemographic factors, prevalent diseases, and laboratory measurements, the fifth quintile remained associated with a 40% higher mortality risk in both black and white participants. Further adjustment for inflammatory biomarkers substantially attenuated this association and rendered it nonsignificant. Tests for interaction in adjusted models were not significant for race ($P = 0.37$) and only marginally significant for gender ($P = 0.07$).

Discussion

Cystatin C is an alternative measure of kidney function that is attractive because of its reported independence from the influence of muscle mass. In the Health ABC cohort of well-functioning, ambulatory elderly, we found that cystatin C was a strong and independent predictor of mortality, an association that did not differ significantly by race or gender. The quintiles of cystatin C seemed to describe three distinct levels of mortality risk, low (quintile 1), medium (quintiles 2 to 4), and high (quintile 5). Although it remained highly significant, the association of high cystatin C concentrations (quintile 5) with mortality was modestly attenuated by adjustment for inflammatory biomarkers, suggesting that inflammation partially mediated the pathway from chronic kidney disease to mortality risk, although risk in both the middle and high quintiles remained statistically significant. In contrast, only the highest gender-specific quintile of creatinine predicted mortality, and this association became nonsignificant after adjustment for inflammation.

The results of this study confirm the findings from the Cardiovascular Health Study (CHS) that previously compared the associations of cystatin C and creatinine with mortality risk (13). The CHS investigators similarly found that quintiles of cystatin C could define low, medium, and high mortality risk. Some differences in the cutpoints of cystatin C to define low, medium, and high risk were noted between the two studies. In CHS, the low-risk group was defined as the lower two quintiles (<1.0 mg/L), whereas in this study, the lowest risk was in participants in quintile 1 (<0.84 mg/L). Both CHS and Health ABC found the fifth quintile to be at markedly increased mor-

Table 2. Association of cystatin C quintiles with all-cause mortality^a

Model	Cystatin C Quintile (mg/L)				
	<0.84	0.84 to 0.93	0.94 to 1.03	1.04 to 1.18	≥1.19
All participants (N)	578	590	605	654	617
Events	58	94	103	119	183
Unadjusted	1.0	1.63 (1.17 to 2.27)	1.78 (1.29 to 2.46)	1.90 (1.38 to 2.60)	3.41 (2.53 to 4.59)
Adjusted ^b	1.0	1.71 (1.22 to 2.40)	1.64 (1.17 to 2.30)	1.71 (1.23 to 2.39)	2.69 (1.95 to 3.70)
Adjusted + inflammation ^c	1.0	1.74 (1.21 to 2.50)	1.51 (1.05 to 2.18)	1.49 (1.04 to 2.13)	2.18 (1.53 to 3.10)

^aData are hazard ratio (95% confidence interval).

^bAdjusted for race, age, gender, site, education level, current smoking status, cardiovascular disease, LDL cholesterol, HDL cholesterol, body mass index, albumin, hypertension, diabetes, coronary heart disease, cerebrovascular disease, and heart failure.

^cAdjusted for all of the above variables plus IL-6, CRP, and TNF- α ; results were similar when inflammation markers were considered individually as well.

Table 3. Low, medium, and high cystatin C concentrations association with all-cause mortality^a

	Cystatin C (mg/L)		
	Low (<0.84)	Medium (0.84 to 1.18)	High (≥1.19)
N	578	1849	617
Events	58	316	183
Absolute risk (%/yr)	1.7	2.9	5.4
Unadjusted	1.00	1.77 (1.34 to 2.35)	3.41 (2.53 to 4.59)
Adjusted ^b	1.00	1.69 (1.26 to 2.27)	2.69 (1.95 to 3.70)
Adjusted + inflammation ^c	1.00	1.57 (1.14 to 2.17)	2.20 (1.55 to 3.13)

^aData are hazard ratio (95% confidence interval).

^bAdjusted for race, age, gender, site, education level, current smoking status, cardiovascular disease, LDL cholesterol, HDL cholesterol, body mass index, albumin, hypertension, diabetes, coronary heart disease, cerebrovascular disease, and heart failure.

^cAdjusted for all of the above variables plus IL-6, CRP, and TNF- α .

Table 4. Creatinine and mortality risk^a

Model	Creatinine Quintile (mg/dl)				
	1	2	3	4	5
All participants (N)	463	687	667	517	713
Events	80	96	99	85	198
Unadjusted	1.0	0.80 (0.60 to 1.08)	0.84 (0.63 to 1.13)	0.95 (0.70 to 1.29)	1.71 (1.32 to 2.22)
Adjusted ^b	1.0	0.95 (0.69 to 1.29)	0.93 (0.68 to 1.27)	1.07 (0.77 to 1.47)	1.40 (1.05 to 1.86)
Adjusted + inflammation ^c	1.0	1.00 (0.72 to 1.39)	0.95 (0.68 to 1.32)	1.11 (0.79 to 1.57)	1.16 (0.86 to 1.58)

^aData are hazard ratio (95% confidence interval).

^bAdjusted for race, age, gender, site, education, current smoking status, cardiovascular disease, LDL cholesterol, HDL cholesterol, body mass index, albumin, hypertension, diabetes, coronary heart disease, cerebrovascular disease, and heart failure.

^cAdjusted for all of the above variables plus IL-6, CRP, and TNF- α .

tality risk, although the cutpoint for quintile 5 was ≥ 1.19 mg/L in Health ABC and ≥ 1.28 mg/L in CHS. The lower distribution of cystatin C concentrations in this study is probably due to Health ABC participants' having been selected on the basis of preserved physical function, whereas CHS recruitment was

population based. As kidney disease is associated with frailty (16), participants with kidney dysfunction would be less likely to qualify for the Health ABC study. In addition, Health ABC has a greater proportion of black participants, who seem to have lower cystatin C concentrations.

A somewhat surprising finding from this study was the absence of association across creatinine quintiles and mortality risk, as risk was elevated only in the highest quintile. Because Health ABC participants were selected for their preserved functionality, we had hypothesized that elevated creatinine concentrations would be a strong and independent prognostic factor. However, unlike the results for cystatin C, creatinine concentrations in the highest quintile were no longer associated with increased risk for death after adjustment for inflammation. Furthermore, the uniform mortality risk across the lower four quintiles contrasts sharply with the three distinct levels of risk defined by cystatin C. These observations suggest that the multiple determinants of creatinine generation in the elderly limit its utility as a prognostic marker and perhaps as a diagnostic test. The reliance on serum creatinine in clinical research up to this time may have obscured the pathophysiologic importance of kidney function in the elderly.

An important and novel aspect of this study is its ability to compare creatinine and cystatin C as mortality predictors in black and white individuals. The association of higher cystatin C concentrations with higher mortality risk was roughly uniform among black and white participants. However, we found that cystatin C was a much stronger predictor of mortality than creatinine in black and white participants. The observation that cystatin C concentrations were lower in black than in white individuals is puzzling, however, particularly as the same was found in the CHS. A possible explanation is that the black participants had better kidney function than the white participants, but this seems unlikely as the prevalences of diabetes and elevated inflammatory markers were higher in black participants overall, and black men had higher levels of systolic BP than white men (17,18). Another explanation could be that cystatin C concentrations approximate GFR differently in black and white individuals. A comparison of cystatin C and creatinine-based estimated GFR as determinants of directly measured GFR in elderly black individuals would be an important topic for future study.

Inflammatory biomarkers are known to be elevated in people with chronic kidney disease (19,20) and to be independent predictors of mortality risk in the elderly (21). This study also suggests that inflammation may modify somewhat the strength of the association between cystatin C and mortality, as inflammatory cytokines were increased in Health ABC patients in the highest quintile of cystatin C, and the point estimate for the fifth quintile was attenuated after adjustment for CRP, IL-6, and TNF- α . Despite adjustment for CRP, IL-6, and TNF- α , however, the association of cystatin C with mortality risk remained strong and independent. This finding argues against the hypothesis that cystatin C is primarily a reflection of systemic inflammation rather than kidney function, as has been suggested (22). Furthermore, a recent study by Perkins *et al.* (23) found that cystatin C correlated tightly with serial measurements of GFR by iothalamate clearance, whereas creatinine-based Modification of Diet in Renal Disease was a much weaker reflection of GFR.

The potential role of cystatin C in clinical care warrants investigation, now that two studies have shown that cystatin C

more accurately predicts mortality risk than serum creatinine in older people. In addition to predicting death, cystatin C was an independent predictor of congestive heart failure, stroke, and myocardial infarction in the CHS (13,24). Two cohort studies of patients with acute coronary syndrome have found that cystatin C predicted subsequent cardiovascular events better than creatinine (25,26). To demonstrate clinical utility, however, studies will need to determine whether diagnostic or screening strategies that are based on cystatin C can change clinical practice compared with strategies that are based on creatinine or estimated GFR and thus lead to improvement in clinical outcomes.

Four limitations of this study should be considered. Because the Health ABC study was composed solely of ambulatory elderly people, the described associations of cystatin C with mortality in younger populations or among elderly with significant functional disability cannot necessarily be extrapolated. Second, we cannot be certain that the association of cystatin C with adverse outcomes is solely due to its approximation of kidney function. Although a study suggested that cystatin C was influenced by factors other than GFR, the study measured creatinine clearance rather than GFR, so its conclusions remain speculative (27). However, we are unaware of any direct toxic role of cystatin C or of a mediator activated by cystatin C or secreted temporally in association with cystatin C that would account for these findings. Third, as with any observational study, there is likely to be residual confounding. Severity differences in measured factors (*e.g.*, hypertension, diabetes) and a variety of unmeasured factors, such as homocysteine, lipoprotein (a), oxidative stress, and asymmetric dimethyl arginine, might modify the association between higher cystatin C concentrations and mortality. However, residual confounding is unlikely to extinguish the relatively large relative risks associated with the highest quintile of cystatin C concentration. Fourth, although we believe that the cause of death classification was rigorous, we cannot exclude the possibility of misclassification. In particular, among deaths that were classified as infection/other, we cannot exclude the possibility that cardiovascular disease was also a contributor.

In summary, we found that cystatin C was a strong and independent risk factor for all-cause and cause-specific mortality in a cohort of ambulatory elderly. Quintiles of cystatin C effectively delineated participants at low, medium, and high risk regardless of race or gender, whereas creatinine quintiles were unassociated with mortality after similar adjustment. We hypothesize that the sensitivity of cystatin C relative to creatinine for small declines in kidney function is the likely explanation for the difference in risk profiles. Future studies that measure both serum cystatin C and creatinine concentrations should address the potential clinical role for cystatin C in the diagnosis and management of kidney disease and its associated complications.

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