Kidney function and markers of inflammation in elderly persons without chronic kidney disease: The health, aging, and body composition study

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Inflammatory markers are elevated in persons with estimated glomerular filtration rates less than 60 ml/min/1.73 m^2. As cystatin C may detect small changes in kidney function not detected by estimated glomerular filtration rate, we evaluated the association between cystatin C and serum markers of inflammation in older adults with estimated glomerular filtration rate $\geqslant$60. This is an analysis using measures from the Health, Aging, and Body Composition Study, a cohort of well-functioning adults aged 70–79 years. Cystatin C correlated with all five inflammatory biomarkers: C-reactive protein ($r = 0.08$), interleukin-6 ($r = 0.19$), tumor necrosis factor α (TNF-α) ($r = 0.41$), soluble TNF receptor 1 (STNF-R1) ($r = 0.61$), and soluble TNF receptor 2 (STNF-R2) ($r = 0.54$); $P < 0.0005$ for all. In adjusted analyses, cystatin C concentrations appeared to have stronger associations with each biomarker compared with estimated glomerular filtration rate or serum creatinine. Participants with a cystatin C $\geqslant$1.0 mg/l had significantly higher levels of all five biomarkers compared to those with a cystatin C < 1.0 (mean differences ranging 16–29%, all $P < 0.05$). Cystatin C has a linear association with inflammatory biomarkers in an ambulatory elderly cohort with estimated glomerular filtration rates $\geqslant$60; associations are particularly strong with TNF-α and the STNF-R.

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C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), and other inflammatory markers have been shown to predict cardiovascular disease.[1–3] Dialysis-dependent patients have substantially increased levels of CRP, IL-6, and TNF-α, which predict poor outcomes in this group.[4–9] Less is known about serum levels of inflammatory markers in persons with mild to moderate chronic kidney disease. Pecoits-Filho *et al.*[10] evaluated 176 pre-dialysis subjects (estimated glomerular filtration rate (eGFR) range, 1.8–16.5 ml/min/1.73 m^2) and found higher CRP and IL-6 levels in those with the lowest eGFR levels. Similarly, Landray *et al.*[11] found strong associations of kidney function with CRP, fibrinogen, von Willebrand factor, and soluble P-selectin among persons with advanced chronic kidney disease (mean eGFR = 25 ml/min). Shlipak *et al.* and Muntner *et al.*[12,13] found persons with chronic kidney disease (eGFR < 60) to have elevated CRP and fibrinogen levels compared with persons with eGFR > 60. Above that threshold, however, eGFR was not associated with levels of these inflammatory biomarkers. This discrepancy may exist because eGFR is not a reliable marker for kidney function at levels above 60 ml/min/1.73 m^2.[14,15]

Cystatin C is a novel measure of kidney function that appears to be at least as effective as 24-h urine calculations of creatinine clearance for estimating GFR,[16,17] and more sensitive than eGFR for determining changes in GFR.[18] Cystatin C may be particularly helpful in measuring kidney function in persons with eGFR > 60 ml/min/1.73 m^2, as eGFR does not appear to be accurate in this range.[15,19] Cystatin C has also been found to be a stronger predictor of multiple outcomes, including mortality, cardiovascular disease, and heart failure, than serum creatinine or eGFR.[20–23] The relationship of cystatin C and inflammatory markers has not been well characterized, particularly in persons without

chronic kidney disease (eGFR $\geqslant$ 60). To that end, we evaluated the association of cystatin C with multiple serum inflammatory biomarkers in participants with eGFR $\geqslant$ 60 ml/min/1.73 m^2.

RESULTS

Among participants in this analysis from the Health ABC Study, the average age was 73.5 years, mean cystatin C was 0.96 mg/l, mean creatinine was 0.96 mg/dl, and mean eGFR was 78.6 ml/min/1.73 m^2. The participants with a cystatin C $\geqslant$ 1.0 mg/l were older, and more frequently white and male (Table 1). Higher cystatin C concentrations were associated with higher body mass index, lower HDL cholesterol, and lower LDL cholesterol, a greater prevalence of hypertension, coronary heart disease, congestive heart failure, and cerebrovascular disease, and greater use of antiplatelet agents.

Spearman correlation coefficients between cystatin C and all five inflammatory biomarkers were positive and statistically significant, the strongest being for TNF-α, sTNF-R1, and sTNF-R2 (all $P < 0.0005$; Table 2). The associations of cystatin C with CRP, IL-6, and TNF-α concentrations are demonstrated graphically by scatterplots and linear regression lines in Figure 1a–c. Although correlations were statistically significant for both serum creatinine and eGFR with all five biomarkers, creatinine paradoxically had a negative correlation with CRP, and eGFR had positive correlations with CRP and IL-6. In addition, cystatin C appeared to have stronger correlations with each biomarker than either serum creatinine or eGFR.

In linear regression models, higher cystatin C was significantly associated with each biomarker and had β-coefficients that were greater in magnitude than those for either serum creatinine or eGFR (Table 3). In contrast, linear regression models did not show a significant association between either serum creatinine or eGFR with CRP or IL-6. Multivariate adjustment had very little effect on these associations. We determined the mean level of each biomarker among participants with cystatin C levels < 1.0 and $\geqslant 1.0$ mg/l. All mean biomarker levels were significantly higher in participants with cystatin C $\geqslant$ 1.0 compared to those with a cystatin C < 1.0 (Figure 2, mean differences 16–29% higher for each biomarker, $P < 0.05$ for all comparisons). Each of these differences remained significant after multivariate adjustment.

Table 1 | Baseline characteristics in subjects with cystatin C above and below 1.0 mg/l

	Cystatin C $\geqslant$ 1.0 mg/l (*N*=960)	Cystatin C $<$ 1.0 mg/l (*N*=1429)	*P*-value
	Mean $\pm$ s.d. or *N* (%)		
Age (years)	74 $\pm$ 3	73 $\pm$ 3	$<$0.0005
Black	375 (39)	686 (48)	$<$0.0005
Female	391 (41)	816 (57)	$<$0.0005
Ever smoker	586 (61)	775 (54)	0.001
Ever drinker	475 (50)	743 (52)	0.220
BMI (kg/$m^{2)}$	27.9 $\pm$ 4.8	26.9 $\pm$ 4.8	$<$0.0005
HDL cholesterol (mmol/l) (mg/dl)	1.32 $\pm$ 0.41 (51 $\pm$ 16)	1.47 $\pm$ 0.44 (57 $\pm$ 17)	$<$0.0005
LDL cholesterol (mmol/l) (mg/dl)	3.08 $\pm$ 0.91 (119 $\pm$ 35)	3.19 $\pm$ 0.88 (123 $\pm$ 34)	0.011
Fasting glucose (mmol/l) (mg/dl)	5.83 $\pm$ 1.78 (105 $\pm$ 32)	5.77 $\pm$ 2.05 (104 $\pm$ 37)	0.469
Diabetes	146 (15)	196 (14)	0.329
Hypertension	510 (53)	642 (45)	$<$0.0005
Coronary heart disease	227 (24)	229 (16)	$<$0.0005
Congestive heart failure	31 (3)	22 (2)	0.006
Cerebrovascular disease	81 (8)	91 (6)	0.055
Antiplatelets/aspirin use	370 (39)	502 (35)	0.105
Statin use	104 (11)	166 (12)	0.530
Serum creatinine (μmol/l) (mg/dl)	79 $\pm$ 12 (1.03 $\pm$ 0.16)	70 $\pm$ 12 (0.92 $\pm$ 0.16)	$<$0.0005
Estimated GFR (ml/min/1.73 m^2)	74 $\pm$ 10	82 $\pm$ 14	$<$0.0005

BMI, body main index; HDL, high-density lipoprotein cholesterol; GFR, glomerular filtration rate; LDL, levels of low-density lipoprotein cholesterol.

Table 2 | Spearman rank correlation coefficients of measures of kidney function and inflammatory markers

	Correlation with cystatin C		Correlation with creatinine		Correlation with eGFR	
	R	*P*-value	*R*	*P*-value	*R*	*P*-value
CRP	0.08	$<$0.0005	−0.06	0.007	0.05	0.011
IL-6	0.19	$<$0.0005	0.05	0.024	0.07	0.0006
TNF-α	0.41	$<$0.0005	0.15	$<$0.0005	−0.20	$<$0.0005
sTNF-R1	0.61	$<$0.0005	0.24	$<$0.0005	−0.31	$<$0.0005
sTNF-R2	0.54	$<$0.0005	0.21	$<$0.0005	−0.27	$<$0.0005

CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; IL-6, interleukin-6; STNF-R1, soluble TNF receptor 1; STNF-R2, soluble TNF receptor 2; TNF-α, tumor necrosis factor α.

For each biomarker, we tested for interactions of cystatin C with sex and race; of these 10 tests for interaction, two were statistically significant. In adjusted analyses, the β-coefficients of cystatin C with log sTNF-R1 were 0.31 in black patients compared with 0.25 in white patients ($P=0.005$); for log sTNF-R2, the β-coefficients were 0.27 in black patients and 0.19 in white patients ($P<0.0005$).

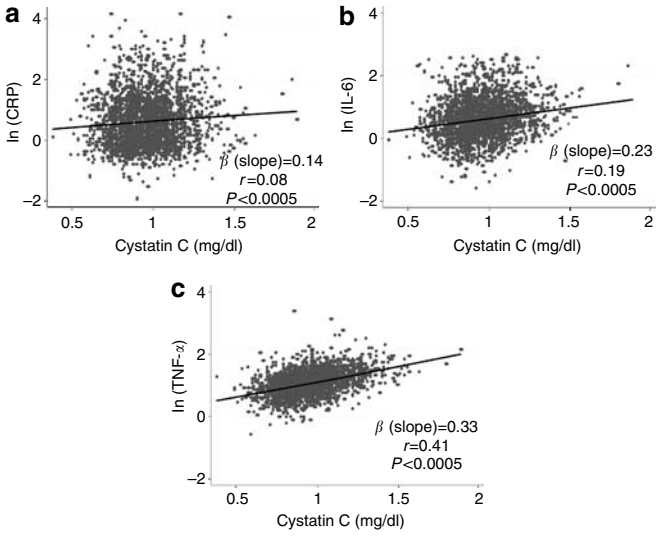

Figure 1 | Associations of Cystatin C with (a) CRP, (b) IL-6 and (c) TNF-α. (**a–c**) Plots of cystatin C versus log-transformed inflammatory markers with fitted line ($P<0.0005$ for all).

DISCUSSION

In this cross-sectional study, cystatin C had linear associations with multiple inflammatory biomarkers in an elderly cohort with eGFR $\geqslant$ 60 ml/min/1.73 m^2. Of the biomarkers studied, cystatin C had the strongest correlations with TNF-α and its soluble receptors. Overall, cystatin C had the weakest correlations with CRP, although those comparisons remained statistically significant. Both serum creatinine and estimated GFR were associated with TNF-α and its soluble receptors, but not with CRP and IL-6 after multivariate adjustment. Those participants with eGFR $\geqslant$ 60 and cystatin C $\geqslant$ 1.0 – defined as having 'preclinical kidney disease' or 'pre-CKD' in

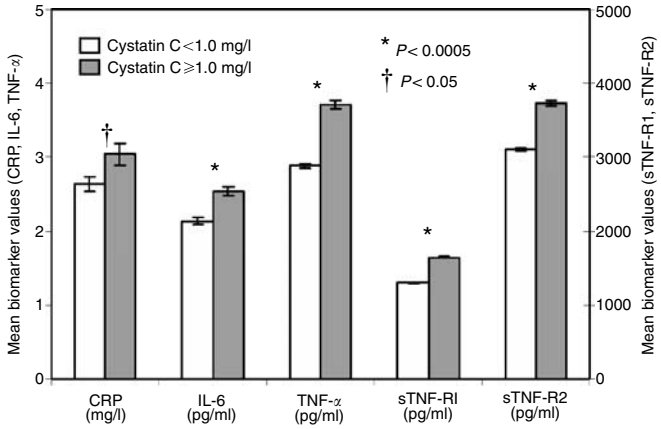

Figure 2 | Mean values of biomarkers in subjects with cystatin C < 1.0 and cystatin C $\geqslant$ 1.0 mg/l. Error bars represent s.e.m.

Table 3 | Unadjusted and adjusted associations log-transformed inflammatory markers per standard deviation of cystatin C and estimated GFR

	Increase per s.d. cystatin C, eGFR, or serum creatinine	
	Unadjusted β-coefficient (95% CI)	Adjusted β coefficient (95%CI)
Cystatin C (mg/l)		
ln CRP	0.14 (0.07, 0.20)*	0.17 (0.11, 0.24)*
ln IL-6	0.23 (0.18, 0.29)*	0.19 (0.13, 0.24)*
ln TNF-α	0.33 (0.30, 0.36)*	0.30 (0.27, 0.33)*
ln sTNF-R1	0.30 (0.27, 0.32)*	0.28 (0.25, 0.30)*
ln sTNF-R2	0.24 (0.22, 0.26)*	0.23 (0.21, 0.25)*
Serum creatinine (mg/dl)		
ln CRP	−0.11 (−0.19, −0.03)‡	−0.03 (−0.14, 0.08)
ln IL-6	0.06 (0.00, 0.12)	−0.07 (−0.16, 0.01)
ln TNF-α	0.13 (0.09, 0.17)*	0.21 (0.16, 0.26)*
ln sTNF-R1	0.15 (0.11, 0.18)*	0.26 (0.21, 0.30)*
ln sTNF-R2	0.11 (0.08, 0.14)*	0.20 (0.16, 0.23)*
Estimated GFR (ml/min/1.73 m^2)		
ln CRP	0.06 (0.02, 0.10)‡	0.02 (−0.02, 0.06)
ln IL-6	0.06 (0.03, 0.09)†	0.03 (0.00, 0.07)
ln TNF-α	−0.09 (−0.11, −0.07)*	−0.08 (−0.10, −0.06)*
ln sTNF-R1	−0.10 (−0.12, −0.08)*	−0.10 (−0.12, −0.09)*
ln sTNF-R2	−0.08 (−0.09, −0.06)*	−0.08 (−0.09, −0.06)*

CRP, C-reactive protein; GFR, glomerular filtration rate ; STNF-R1, soluble TNF receptor 1; STNF-R2, soluble TNF receptor 2; TNF-α, tumor necrosis factor α.
*$P<0.0005$; †$P<0.005$; ‡$P<0.05$.
All biomarker models adjusted for age, sex, race, study site, smoking status, alcohol use, BMI, diabetes, hypertension, cerebrovascular disease, coronary artery disease, heart failure, use of statins and/or aspirin, LDL, HDL, albumin, and glucose. STNF-R1 and sTNF-R2 models also adjusted for sampling status.

a prior study – had significantly higher levels of each biomarker than persons with normal kidney function defined by either eGFR or cystatin C.[24]

Our findings build on the information provided from previous studies of inflammation and kidney disease. Using eGFR, Muntner *et al.*[13] and Shlipak *et al.*[12] found associations with kidney function and inflammatory biomarkers among persons with eGFR $\leqslant 60$, but not for eGFR $\geqslant 60$. Using cystatin C in subjects with advanced CKD, Landray *et al.*[11] found higher levels of CRP and fibrinogen compared with healthy controls. In a recent paper by Shlipak *et al.*,[25] both CRP and fibrinogen had linear associations with cystatin C, but U-shaped associations with eGFR, among ambulatory elderly persons. Our paper demonstrates the positive and linear association between cystatin C and an extended array of inflammatory proteins and cytokines among persons with eGFR $\geqslant 60$.

The most likely explanation for the apparently stronger association of inflammatory biomarkers with cystatin C than with eGFR or serum creatinine is that declines in kidney filtration are associated with increased levels of inflammatory biomarkers, and that cystatin C approximates actual GFR better than creatinine-based formulae, particularly when eGFR $\geqslant 60$. Although the exact mechanism of inflammatory marker elevation in kidney disease remains unclear, intact kidney function may be essential for adequate removal of inflammatory markers from the bloodstream. Biomarkers are, in general, small by weight and may be filtered at the glomerulus in subjects with normal kidney function. The approximate molecular weights for the biomarkers in our study are as follows: CRP, 115 kilodaltons (kDa); IL-6, 26 kDa; TNF-α, 17 kDa monomers, and 54 kDa trimers; sTNF-R1, 30 kDa; sTNF-R2, 33 kDa.[26–28] Although there appears to be only a moderate connection in this study between the size of the biomarker and its correlation with kidney function, those most highly associated with kidney function in our study, namely TNF-α, sTNF-R1, and sTNF-R2, are all molecules under 55 kDa. Furthermore, it has been shown that TNF-α is predominantly cleared by the kidney, wheras IL-6 and CRP are mainly cleared by the liver.[29–31] Similarly, cystatin C (13 kDa) is freely filtered at the glomerulus, which likely leads to its strong association with the inflammatory biomarkers that are renally cleared.[32]

An alternative hypothesis for the association between cystatin C and inflammatory biomarkers is that cystatin C itself may be a regulator of inflammation. Cystatin C is a potent cysteine protease inhibitor that has been demonstrated *in vitro* to regulate certain aspects of immune function.[33] Cystatin C is also upregulated in hyperthyroidism, a condition that has been associated with modified cytokine profiles.[34,35] In our study, we are unable to determine the degree to which the association of cystatin C with inflammatory biomarkers was caused by some direct link to immune regulation.

In addition to the significant association of cystatin C with all five inflammatory biomarkers, both serum creatinine and eGFR were associated with TNF-α, sTNF-R1, and sTNF-R2 in persons with eGFR $\geqslant 60$. While this seems to support the concept that serum elevations in TNF-α and its receptors are a function of their clearance by the kidney, it is also possible that TNF-associated inflammation may promote the progression of kidney disease. Limited data from other experiments support this hypothesis: in murine models, for example, TNF-α knockout mice were relatively protected from induced glomerular injury.[36] Elucidation of the pathogenic role for TNF-α in kidney-specific inflammatory pathways would help to clarify the importance and direction of the association between TNF biomarkers and kidney function.

Our study has several limitations. We are unable to determine either the direction of association or the causal pathway given the cross-sectional design of our study. For example, it is possible either that systemic inflammation leads to kidney dysfunction, or that decreased kidney filtration results in elevated inflammatory biomarkers, or that both contribute to the observed findings. Furthermore, our data were obtained from an older population of white and black subjects; the results from this group may not be generalizable to a younger population or to persons of other races and/or ethnicities. In addition, a more direct measurement of GFR, such as inulin clearance, was not used in this study as a gold standard for comparison. We also did not calibrate creatinine in this study with the Cleveland Clinic standard, as in the modification of diet in renal disease study.[37] However, arithmetic calibration of creatinine would not have affected the overall findings with eGFR and each inflammatory marker. Moreover, we lacked measures of urine albumin excretion, which may also correlate with inflammatory biomarkers in elderly persons without CKD.

Finally, the extent to which other covariates influence the relationship between cystatin C and glomerular filtration rate remains controversial in the literature. On the one hand, a recent paper by Rule *et al.*[38] found that age and sex did not influence the prediction of GFR by a cystatin C-based equation in persons with CKD. However, in a population-based study, Knight *et al.*[39] found that cystatin C concentrations were independently associated with older age, male gender, greater weight, greater height, and cigarette smoking, even after adjustment for measured creatinine clearance. Therefore, cystatin C may potentially be influenced by these covariates independent of their effects on GFR. If cystatin C concentrations are to be used as a clinical marker of kidney function, then future studies will be needed to understand comprehensively its susceptibility to bias from non-renal factors.

In summary, we found that multiple inflammatory biomarkers were highly associated with cystatin C in persons with eGFR $\geqslant 60$ ml/min/1.73 m^2. In contrast, higher creatinine levels and lower eGFR were not associated with elevations of CRP and IL-6 levels in this population. These findings suggest that serum cystatin C is a more sensitive marker than eGFR for detecting the association of

inflammation with kidney disease, especially among persons without chronic kidney disease.

MATERIALS AND METHODS

Study design and population

The Health ABC Study is a prospective cohort study designed to evaluate the relationships between body composition and weight-related health conditions with incident functional limitation among well-functioning black and white adults aged 70–79. Three thousand and seventy-five persons completed a baseline evaluation and were enrolled between April 1997 and June 1998. Serum cystatin C and creatinine levels were obtained in 3043 participants (99%). Participants were recruited from two sites: Pittsburgh, Pennsylvania, and Memphis, Tennessee. White subjects were recruited from a random sample of Medicare beneficiaries; black subjects were recruited from all age-eligible subjects within the regions. Participants were eligible if they reported no difficulty walking a quarter mile, climbing 10 steps, ambulating without assistive devices, and performing basic activities of daily living. Subjects were excluded from the study if they reported having a life-threatening illness, had a history of active cancer in the 3 years before the study, did not plan to remain in the geographic area for at least 3 years, or were participating in another study involving modification of eating or exercise behaviors. In this analysis, participants were also excluded if they had an eGFR <60 ml/min/1.73 m^2. All participants gave written informed consent that was approved by institutional review boards at both clinical sites.

Serum measurements

Serum and EDTA plasma were drawn in the morning after an overnight fast during the initial visit and stored at -70°C. Cystatin C was measured using a particle-enhanced immunonepholometric assay (N Latex Cystatin C) with a BNII nephelometer on plasma specimens (Dade Behring Inc., Deerfield, IL).[40] Polystyrene particles coated with monoclonal antibodies that agglutinate in the presence of antigen (cystatin C) cause an increase in the intensity of scattered light in proportion to the amount of cystatin C in the sample. The assay range is 0.195–7.330 mg/l, with the reference range for young, healthy individuals reported as 0.53–0.95 mg/l. Intra-assay coefficients of variation range from 2.0–2.8% and inter-assay coefficients of variation range from 2.3 to 3.1%. Serum creatinine levels were measured at the time of the 1997–1998 annual visit using colorimetry with a Johnson & Johnson Vitros 950 analyzer (New Brunswick, NJ). Estimated GFR was calculated using the creatinine-based abbreviated modification of diet in renal disease (MDRD) equation.[41]

All biomarkers were measured by enzyme-linked immunosorbent assay (CRP: Calbiochem, San Diego, CA; all others: R&D Systems, Minneapolis, MN). The detectable limits for each inflammatory marker are reported as follows: 0.08 μg/ml for C-reactive protein (using the World Health Organization's First International Reference Standard), 0.18 pg/ml for tumor necrosis factor alpha (using the HSTA50 kit), 0.10 pg/ml for interleukin-6 (using the HS600 Quantikine kit), 3 pg/ml for soluble tumor necrosis factor receptor-1 (sTNF-R1, using the DRT100 kit), and 1 pg/ml for soluble tumor necrosis factor receptor-2 (sTNF-R2, using the DRT200 kit). Blind duplicate analyses on 150 serum samples for CRP, TNF-α, and IL-6 showed an average interassay coefficient of variation of 8.0, 15.8, and 10.3%, respectively. Biomarkers were measured in varying subsamples of the cohort: CRP, $N=2383$; TNF-α, $N=2249$; IL-6, $N=2283$; sTNF-R1, $N=1147$; sTNF-R2, $N=1144$. Data for the STNF-R1 AND -R2 were measured in a total of 722 controls and randomly selected subjects, as well as 425 cases who developed severe functional impairment and were included in a separate case–control study.

Other baseline variables that were evaluated as potential confounders included demographic characteristics (age, sex, race, study site), lifestyle parameters (smoking history, defined as ever (current or former) or never; alcohol use, defined as $\geqslant 1$ drink per week; body mass index, measured at the baseline visit); comorbidities (diabetes, defined by the use of hypoglycemic medications, by self-report, or by a fasting plasma glucose $\geqslant 126$ mg/dl; hypertension, defined by the use of antihypertensive medications or by self-report; cerebrovascular disease, coronary artery disease, heart failure, all determined by self-report); medications (statins and aspirin, all brought by the patient at the baseline visit and recorded); and serum chemistries (levels of low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), albumin, and glucose, all measured using colorimetry with a Johnson & Johnson Vitros 950 analyzer (New Brunswick, NJ)). LDL was calculated using the Friedewald equation.[42]

Statistical analysis

We compared baseline characteristics among participants with cystatin C concentrations $\geqslant 1.0$. with those <1.0 mg/l using either t-tests or χ^2 tests, where appropriate. This cutpoint was chosen based on a recent study that defined elderly persons with eGFR $\geqslant 60$ ml/min and cystatin C $\geqslant 1.0$ mg/l as having 'preclinical kidney disease'.[24] We calculated Spearman correlation coefficients for cystatin C, serum creatinine, and eGFR with each of the biomarkers, and we plotted the distribution of cystatin C against each of the log-transformed inflammatory markers and fitted a linear regression line. We checked for departure from linearity by plotting the standardized residuals from the linear model against log-transformed fitted values of each biomarker.

To control for the influence of potential confounding variables, we used multivariate linear regression to determine if cystatin C, serum creatinine, and eGFR were independently associated with each of the log-transformed biomarkers. All covariates listed in Table 1 were entered into the model based on their hypothesized role as potential confounders owing to their known associations with either kidney disease or inflammation. In addition, models for sTNF-R1 and sTNF-R2 were adjusted for sampling by adding case status as a variable in the regression analyses.

The cystatin C cutpoint of 1.0 mg/l was used to define two groups, both with eGFR $\geqslant 60$ ml/min.[24] The mean levels of each biomarker in each group were presented, and a two-sample t-test was used to test for significance between groups.

We tested for a significant interaction between race and sex with cystatin C in all adjusted regression models. We used STATA 8.0 (Statacorp LP, College Station, TX) for all analyses.

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