The Association Between Cystatin C and Frailty Status in Older Men

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Dedication

This thesis is dedicated to my husband, Cory, and my son, Miles.

Abstract

BACKGROUND

Declining kidney function and frailty are common with aging, but the association between these conditions remains uncertain. We hypothesize that cystatin C predicts prevalent frailty status in men age 65 and older.

METHODS

Serum cystatin C (cys C) and creatinine (Cr) were measured and frailty status ascertained in a random sample of 1602 community-dwelling men age ≥ 65 yrs participating in the MrOS study. Frailty status (comprised of shrinking, weakness, exhaustion, slowness and low physical activity) was analyzed as an ordinal outcome of robust, intermediate stage, and frail based on the number of frailty components present (0, 1-2, or ≥ 3 respectively) using a multinomial logistic regression model to simultaneously evaluate the odds of being classified as intermediate vs. robust and frail vs. robust. The base model was adjusted for age, race, and clinical site.

RESULTS

The mean age of the cohort was 73.8 yrs; 9.8% were frail and 47.2% were intermediate stage. The highest quartile of cys C was associated with more than seven times the odds of being classified as frail vs. robust when compared to the lowest quartile of cystatin C and 2.38 times the odds of being classified as intermediate status. The association was attenuated but persisted after adjusting for health status, body mass index, number of comorbidities, IADL impairments, vitamin D level, serum albumin level, CRP and IL-6 levels. In contrast, neither higher serum Cr (p-value for trend across quartiles > 0.76) nor lower Cr-based eGFR (p-value for trend across quartiles > 0.47) was associated with higher odds of frailty.

CONCLUSION

Higher cys C was associated with increased odds of frailty status in this cohort of older men whereas Cr-based measures were not. This difference may be due to lower specificity of Cr based measures compared to cys C in older adults with modest reductions in kidney function, or because cys C is associated with frailty by a mechanism that is unrelated to kidney function.

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INTRODUCTION

Decreased kidney function is common in the elderly, with more than 30% of those aged 70 years and older having an estimated glomerular filtration rate (eGFR) of less than 60 ml/min/1.73m^2 (Coresh 2007). Older adults with an eGFR of less than 60 ml/min/1.73m^2 have an increased risk of subsequent cardiovascular disease and death, but the association between mild to moderate renal dysfunction and other adverse health outcomes in the aged population is uncertain.

Clinicians caring for older adults have long recognized a syndrome of frailty manifested by decreased physiologic reserve and increased vulnerability to physical decline and death. In 2001, Fried and colleagues using data collected in the cardiovascular health study (CHS) proposed a standard definition of in which three or more of the following criteria are present: shrinking (e.g. unintentional weight loss), self-reported exhaustion or poor energy, weakness manifested by low grip strength, slow walking speed and low physical activity. Subjects with none of the criteria are considered to be robust, while those with one or two criteria are considered to be in an intermediate, or pre-frail, stage (Fried 2001). In their analysis, frailty defined by this index was predictive of increased risk of falling, hospitalization, disability and mortality. While individuals with disabilities and comorbidities were more likely to be classified as frail, 27% of subjects had neither disability nor comorbidity, and frailty status was predictive of poor outcomes independent of these other factors, suggesting a physiologic basis for the phenotype described (Fried 2001, Walston 2002). Although several other instruments have been developed to operationalize the construct of frailty, the CHS index has been most extensively studied and the predictive value of this index has been validated in multiple cohorts (Woods 2005, Boyd 2005, Bandeen 2006, Cawthon 2007, Ensrud 2009).

While frailty is common among older adults with advanced or end-stage kidney disease, how mild to moderate age-related deficits in kidney function relate to frailty is less extensively studied. The association between kidney function and frailty in older adults was previously evaluated in the cardiovascular health study (CHS) cohort using serum creatinine as the measure of kidney function (Shlipak 2004). In this cross-

sectional analysis, higher creatinine was associated with higher odds of frailty. However, serum creatinine may not be an accurate measure of measure of kidney function in older individuals with decreased muscle mass (Finney 1999, Wasen 2004. In addition, frailty status was expressed as a dichotomous outcome (frail versus not frail) in this analysis, not as a continuum ranging from robust to intermediate stage to frail.

Cystatin C, a lower molecular weight protein whose concentration is mainly determined by glomerular filtration, is a measure of kidney function that is less dependent on muscle mass (Westhuyzen 2006, Baxmann 2008). Thus, cystatin C may be superior to standard creatinine-based measures in detecting mild to moderate reductions in kidney function in older adults (Fliser 2001). To test the hypothesis that higher cystatin C levels are associated with greater prevalent frailty status, we measured cystatin C and assessed frailty status in a cohort of 1602 community dwelling men aged $\geq$65 years enrolled in the Osteoporotic Fractures in Men study (MrOS). In addition, we examined whether other measures of reduced kidney function, including higher serum creatinine or lower creatinine-based estimated glomerular filtration rate, were associated with frailty status.

METHODS

Participants

The Osteoporotic Fractures in Men (MrOS) Study is a prospective multi-center observational study of a cohort of 5994 community-dwelling men aged 65 or older, able to walk without assistance and without history of bilateral hip replacements recruited between 2000 and 2002 from six clinical sites: Minneapolis, Birmingham, Palo Alto, Pittsburgh, Portland, and San Diego. Detailed descriptions of the study design and recruitment for MrOS have been previously published (Blank, 2005; Orwoll, 2005). A random sample of 1604 men were selected for measurement of cystatin C, and of these, 1602 men had adequate had sufficient sera for performance of cystatin C assay and comprised the analytical cohort. The institutional review board (IRB) at each center approved the protocol and written informed consent was obtained from all participants.

Biochemical Data

Fasting morning blood was collected at the baseline examination and stored at -70° C until thawed. Serum cystatin C assays were performed at the Fairview-University of Minnesota Medical Center. Serum cystatin C levels were determined using a BN100 nephelometer (Dade Behring Inc., Deerfield, IL) using a particle-enhanced immunonephelometric assay in 2009. The assay range is 0.30 to 10.00mg/L with intra-assay coefficients of variation (CVs) ranging from 2.0 to 2.8% and inter-assay CVs from 2.3 to 3.1%. To calculate cystatin C-based eGFR ($eGFR_{CysC}$), cystatin C measurements were first standardized using a standardizing formula created with assays traceable to a certified reference material and eGFR was then computed using a CKD-EPI equation re-expressed for the standardized cystatin C (Inker 2011).

Serum creatinine was measured using the Roche 911 analyzer (Roche diagnostic Corporation, Indianapolis, IN) at the Portland VA Medical Center using the Jaffe rate-blanked method calibrated with materials assayd by isotope-dilution mass spectrometry (IDMS). Inter- and intra-assay CVs were 4.0%. Creatinine-based eGFR ($eGFR_{Cr}$) using was calculated using the chronic kidney disease epidemiology collaboration (CKD-EPI) equation (Levey 2009), which includes variables for standardized creatinine, age, gender and race. Albumin was measured using a Roche COBAS Integra 800 automated analyzer (Roche Diagnostic Corporation, Indianapolis, IN) with inter-assay CV of 1.98%. Measures of $25(OH)D_2$ and $25(OH)D_3$ were performed at the Mayo Clinic using mass spectrometry and summed for total 25(OH)D. Total intact parathyroid hormone (PTH) was measured using an immunoradiometric assay (Scantibodies Laboratory, Inc. Santee, CA) at the Columbia University Laboratory. Markers of chronic inflammatory processes were assessed by serum level of the pro-inflammatory cytokine IL-6 using a high-sensitivity ELISA from R&D systems (Minneapolis, MN) and by serum levels of high sensitivity C reactive protein (CRP) measured using the BNII nephelometer (Dade Behring Inc., Deerfield, IL) utilizing a particle-enhanced immunonephelometric assay.

Clinic Measurements

At the baseline examination, height was measured with Harpenden stadiometers and weight was measured on balance beam or digital scales. Body mass index (BMI) was calculated as kg/m^2. Appendicular skeletal mass was measured using dual energy x-ray absorptiometry (DXA) on Hologic QDR4500 machines (Hologic, Inc. Bedford, MA) using standardized protocols. Walking speed was measured on a six meter walking course with participants asked to walk at their usual pace, and expressed as meters/second. The fastest pace of two trials was recorded. Grip strength (kg) was measured using a Jamar hand dynamometer (Lafayette Instrument Co. Lafayette IN).

Questionnaire Data

Demographic information, social support, smoking status, and alcohol use were ascertained through self-administered questionnaires. Men were asked whether they had received a physician diagnosis of the following medical: hypo- or hyperthyroidism, transient ischemic attack or stroke, diabetes mellitus, hypertension, coronary heart disease or myocardial infarction, angina, congestive heart failure, chronic obstructive pulmonary disease, intermittent claudication, liver disease, Parkinsonism, or cancer (except non-melanoma skin cancer). Perceived health status was self reported as "excellent," "good," "fair," "poor," or "very poor" in response to the question "Compared to other people your own age, how would you rate your overall health?" Physical activity was assessed using the Physical Activity Scale for the Elderly (PASE) score (Washburn 1993).

Frailty Status Assessment

Frailty status was defined using a modified CHS frailty index (Cawthon 2007) comprised of the following five components: first, shrinking, defined by the lowest quintile of appendicular lean mass at the baseline examination, adjusted for height and body fat. Second, weakness, identified by grip strength in the lowest quintile stratified by body mass index quartiles. Third, exhaustion, identified by an answer of "little or none" to the question "How much of the time during the past 4 weeks did you have a lot of energy" from the Medical Outcomes Survey Short Form, a 12-item questionnaire at the

baseline examination. Fourth, slowness, identified by a walk speed at the baseline examination in the lowest quintile stratified by for standing height quartile. Fifth, low physical activity level, identified by a PASE score in the lowest quintile. Men with none of the five components were classified as robust, those with 1 or 2 components were classified as intermediate stage, and those with ≥ 3 components classified as frail. Frailty status as defined by this index predicted increased risks of falling, disability, fracture and mortality in the MrOS cohort (Cawthon 2007, Ensrud 2009).

Statistical Analysis

Differences in baseline characteristics by quartile of cystatin C were compared using chi-square (or categorical data) and ANOVA (for normally-distributed continuous data) tests.

For the analysis, Cystatin C and creatinine-based predictors were expressed as quartiles, to ensure adequate numbers of subjects and preserve power. In secondary analyses, we expressed eGFR predictors in categories using clinically relevant cutpoints corresponding to the presence or absence of CKD of < 60 ml/min/1.73m^2 and ≥ 60 ml/min/1.73m^2. Further categorization of eGFR < 60 ml/min/1.73m^2 was not performed due to inadequate numbers of subjects in these categories.

Given the ordinal nature of the frailty status outcome variable (robust, intermediate stage or frail), multinomial logistic regression was used to simultaneously calculate the odds of being frail versus robust and intermediate status versus robust. Cystatin C was expressed as quartiles, with quartile 1 serving as the referent group. Crude models were performed, then adjusted for age, race and clinical site (base model). In forming the multivariate model, the pre-specified covariates that may be related to cystatin C level or frailty status (body mass index, self-reported health status, number of comorbid conditions, alcohol consumption, smoking status, social support, independent activities of daily living (IADL) impairments, education, use of an angiotensin blockage agent, vitamin D levels, intact PTH levels, and serum albumin) were added individually to the base model to assess for significance as independent predictors of frailty status at a significance level of alpha = 0.05, as well as potential confounding defined as changing

the frailty odds parameter estimates by at least 10%. Covariates that were neither independent predictors nor potential confounders not included in the final multivariable model. A test for linear trend was performed by expressing cystatin C quartile as an ordinal variable. We also performed analyses examining the association of the presence of CKD by eGFR (eGFR < 60 ml/min/1.73m^2), defined using both cystatin C and creatinine-based equations, and frailty status.

To examine the biological mechanisms that might underlie the association observed between cystatin C and frailty status, levels of calcitropic hormones (25(OH)D, iPTH) and markers of chronic inflammation (TNF-α, IL-6, TNF-αsR1, TNF-αsR2, IL-6SR, and CRP) were added individually to the base model. To further evaluate the effect of specific inflammatory markers, (CRP, IL-6) on the association, a test of interaction between cystatin C and each of these markers for prediction of frailty status was performed. To evaluate whether the association between cystatin C and frailty status was driven by one of the five components of frailty, the association between cystatin C and each individual component of frailty (e.g. shrinking, weakness, exhaustion, slowness or low physical activity) was evaluated using a multiple logistic regression model. All analyses were conducted using SAS version 9.2 (SAS inc., Cary, NC).

RESULTS

Baseline characteristics of the cohort and by quartile of cystatin C are shown in Table 1. The mean age of the cohort was 73.8 years and 91.5% of the cohort self-identified as Caucasian. 157 (9.8%) subjects in the cohort were classified as frail while 756 (47.2%) were classified as intermediate status. 1252 (78.4%) had a cystatin C-based CKD-EPI eGFR ≥ 60 ml/min; (329) 20.4% had an eGFR 30-59 ml/min, and only 21 (1.3%) had an eGFR < 30 ml/min. Using creatinine-based CKD-EPI eGFR, 1257 (83.5%) had an eGFR > 60 ml/min/m^2, 236 (15.7%) had an eGFR of 30-59 ml/min/1.73m^2, and 12 (0.8%) had an eGFR < 30 ml/min/m^2. Higher cystatin C was associated with older age, greater number of comorbidities and greater impairments in independent activities of daily living.

After adjustment for age, site and race, higher cystatin C was associated with greater odds of being classified as intermediate stage (versus robust) and frail (versus robust). Compared to men in the first quartile of cystatin C (referent), men in quartile four had a seven-fold greater odds of frailty (OR 7.1, 95% CI 3.76 – 13.46), and a greater than two-fold greater odds of intermediate status (OR 2.4, 95% CI 1.70 – 3.32)(Table 2). The associations appeared graded in nature (p for linear trend across cystatin C quartiles < 0.001). The associations were attenuated but persisted after adjusting for multiple covariates including functional impairment and number of comorbid conditions; compared to men in the first quartile of cystatin C (referent), men in quartile four had a four-fold greater odds of frailty (OR 4.17, 95% CI 2.02 – 8.59), and a 2-fold greater odds of intermediate status (OR 2.1, 95% 1.44 – 2.98). In addition to cystatin C, age (p< 0.001), self-reported health status (p< 0.001) and number of IADL impairments (p< 0.001) were most predictive of frailty status. The association between cystatin C and frailty status also persisted when the analysis was restricted to subjects without chronic kidney disease as defined by a creatinine-based estimated GFR using the CKD-EPI equation of ≥ 60 ml/min/1.73m^2 (Table 6).

After adjustment for age, race and site, higher cystatin C was associated with greater odds of each individual frailty component, including higher odds of shrinking, weakness, exhaustion, slowness and low physical activity (Table 3). After consideration of multiple confounders, associations between higher cystatin C and a greater likelihood of shrinking, slowness and low physical activity remained significant.

The association between $eGFR_{cysC}$ and frailty status was not explained by alterations in calcitropic hormones (iPTH and 25(OH)D), albumin or inflammatory markers (IL-6 or CRP) (Table 4). Further adjustment of the base model for each of the potential biological mediators had little impact on the association, though adjustment for IL-6 reduced the magnitude of the association by more than 30%. There was no evidence for interaction between cystatin C level and IL-6 ($p > 0.8$).

Conversely, neither higher serum creatinine (p-value for trend across quartiles > 0.4) nor lower estimated GFR calculated using either the serum creatinine-based CKD-EPI or MDRD formulae ($p > 0.6$ across quartiles of $eGFR_{Cr}$) was associated with higher

odds of frailty (MDRD results not shown). When the lowest quartile of creatinine-based eGFR ($eGFR_{Cr}$ < 64.7 ml/min/1.73m^2) was compared to the highest three quartiles, the lack of an association persisted (results not shown). However, the presence of chronic kidney disease as identified by a creatinine-based eGFR < 60 ml/min/1.73m^2 (CKD-EPI formula) was associated with higher odds of both intermediate and frail status, though the associations between chronic kidney disease and frailty status appeared stronger in magnitude when a cystatin-C based equation was used to calculate eGFR (table 6).

DISCUSSION

In this cohort of older men, higher cystatin C was strongly associated with greater prevalent frailty status, and this association was independent of several potential confounders and biologic mediators. Higher cystatin C was also associated with higher odds of each of the individual components of frailty with no single component appearing to drive the association. In contrast, higher serum creatinine and lower $eGFR_{Cr}$ were not associated with higher odds of greater frailty status. The presence of CKD defined using either a cystatin-C based or creatinine-based equation was associated with a higher likelihood of greater frailty status, but this association was strongest in magnitude for CKD defined using a cystatin-C based equation.

These findings are consistent with previous studies that have found cystatin C to be predictive of other measures of unsuccessful aging, although the definitions and metrics used to define this outcome have varied. A cross-sectional study of the Health, Aging, and Body Composition (HABC) cohort reported that higher cystatin C was independently associated with poorer performance on objective tests of physical performance (Odden 2006). In contrast, lower serum creatinine-based estimated glomerular filtration rate (eGFR) was not associated with poorer performance. Longitudinal analysis of the HABC cohort showed cystatin C to be predictive of self-reported physical functional limitations (Fried 2006). In a longitudinal study of the CHS cohort, higher cystatin C was also associated with decreased aging success, using various definitions including a composite of reported comorbid conditions, cognitive impairment

and self-reported physical impairments (Sarnak 2008). To our knowledge, this is the first study to evaluate the association of kidney function as assessed by between both cystatin C and creatinine-based measures with the outcome of frailty status, a geriatric syndrome that predicts increased risks of adverse health outcomes in older adults.

The mechanism linking higher cystatin C to frailty remains unclear. Increased levels of inflammatory markers have been associated with decreased kidney function (Shlipak 2003) as well as with frailty (Hamerman 1999, Walston 2002). Inflammation may also increase the level of cystatin C (Stevens 2009). However, we found no evidence for interaction between inflammatory markers (IL-6 and CRP) and cystatin C for the prediction of frailty status. In addition, after controlling for these markers, the association between cystatin C and frailty persisted, suggesting that the association cannot be fully explained by higher levels of inflammation among those men with elevated cystatin C levels. Higher cystatin C was also associated with each of the individual components of frailty, suggesting that a single component such as shrinking or physical activity was not driving the association noted between cystatin C and frailty as a syndrome. Other potential biologic mediators such as intact PTH, vitamin D and albumin also had little or no effect on the association between cystatin C and frailty, nor did adjustment for medical comorbidities, health status and IADL impairments.

In contrast to cystatin C, we found no association of serum creatinine or eGFRCr expressed in quartiles with frailty status. A prior study using data collected in CHS reported a significant association between serum creatinine and frailty, but used a cutoff of 1.3 mg/dL in women and 1.5 mg/dL in men to define elevated creatinine (Shlipak 2004). This cutoff may represent a clinically relevant reduction in kidney function in an elderly population with decreased muscle mass. The relationship between creatinine (and $eGFR_{Cr}$) quartile and frailty status appeared J-shaped in this study, such that men in both the highest and lowest quartiles of creatinine and $eGFR_{Cr}$ had a higher odds of frailty, albeit not at the level of signficance. This J-shaped pattern between creatinine or eGFRCr and frailty may reflect the dependence of serum creatinine level and eGFRCr on muscle mass.

In contrast, we found that CKD as defined by either creatinine or cystatin C was associated with an increased odds of intermediate or frail status, though the association between CKD defined by cystatin C and frailty status was almost twice as strong when defined by cystatin C. The contrasting results when using cystatin C versus creatinine suggest that cystatin C may be a more sensitive marker of mild to moderate, but prognostically significant, decreases in kidney function in an elderly population, a hypothesis supported by the additional finding that cystatin C was predictive of frailty status even when the analysis was restricted to subjects whose eGFR calculated using serum creatinine was ≥ 60 ml/min/1.73m^2. Furthermore, the prevalence of CKD in this population was higher using a cystatin C-based formula to calculate eGFR compared to that when using a creatinine-based formula to calculate eGFR (21.6 % versus 16.5%, respectively).

This study has several limitations. Most importantly, this cross-sectional evaluation can only suggest an association between higher cystatin C and greater frailty status. Future prospective studies examining the association between baseline cystatin C among non-frail older adults and risk of incident frailty are needed. We relied on surrogate markers of kidney function rather than direct measurements. While cystatin C may be a better marker of kidney function in older adults due to its lower dependence on muscle mass, this remains an area in need of further study. Also, MrOS is a cohort of predominantly Caucasian, making the results less generalizable; however, these results are consistent with other studies in different populations. Very few men at the MrOS baseline examination had advanced CKD or proteinuria, limiting our ability to assess the association between cystatin C and frailty in those with more significant kidney function impairment. Finally, as with any observational study, the possibility for residual confounding persists.

In conclusion, higher cystatin C was associated with increased odds of greater frailty status in older men. Cystatin C (and CKD defined using a cystatin C-based equation) is a better predictor of frailty status than traditional creatinine-based measures of renal function. These findings suggest that higher cystatin C may be a promising biomarker for unsuccessful aging as defined by frailty. Further studies are needed to

establish whether cystatin C predicts the development of incident frailty, and to further elucidate whether this association is present because cystatin C is a more sensitive and specific marker of decreased kidney function in the elderly, or whether it is reflective of factors other than reduced renal function

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Appendix

Table 1. Characteristics of 1602 Participants by Quartile of Serum Cystatin C Level

Variable	Quartile of Cystatin C					
	Overall Cohort	Q1	Q2	Q3	Q4	p-value
Age, years, mean ± SD	73.8 ± 5.9	71.3 ± 4.9	72.8 ± 5.3	74.8 ± 5.6	76.4 ± 6.3	< 0.001
Caucasian race, n (%)	1465 (91.5)	358 (87.3)	369 (91.8)	375 (95.4)	363 (91.4)	< 0.001
Body mass index, kg/m2, mean ± SD	27.4 ± 3.7	26.7 ± 3.3	27.5 ± 3.6	27.5 ± 3.9	27.8 ± 4.0	< 0.001
Self-reported health status, n (%)						< 0.001
Excellent or good	1363 (85.1)	368 (89.8)	351 (87.3)	343 (87.5)	301 (75.8)	
Fair/poor or very poor	238 (14.9)	42 (10.2)	51 (12.7)	49 (12.5)	96 (24.2)	
Selected medical conditions*, n (%)						< 0.001
0-1	610 (38.1)	201 (49.0)	155 (38.6)	152 (38.7)	102 (25.7)	
2-3	777 (48.5)	182 (44.4)	202 (50.3)	193 (49.1)	200 (50.4)	
≥4	215 (13.4)	27 (6.6)	45 (11.2)	48 (12.2)	95 (23.9)	
IADL impairments, n (%)						< 0.001
none	1279 (79.8)	357 (87.3)	345 (85.8)	321 (81.9)	256 (65.0)	
1-2	240 (15.0)	42 (10.3)	47 (11.7)	53 (13.5)	98 (24.9)	
≥ 3	78 (4.9)	10 (2.4)	10 (2.5)	18 (4.6)	40 (10.2)	
Albumin, mean ± SD	4.3 ± 0.2	4.3 ± 0.2	4.3 ± 0.2	4.3 ± 0.2	4.2 ± 0.3	< 0.001
Vitamin D, mean ± SD	25.1 ± 7.9	24.7 ± 8.1	25.5 ± 7.8	24.9 ± 7.4	25.2 ± 8.4	0.5

*Selected medical conditions include history of stroke, myocardial infarction, angina, congestive heart failure, fracture, cancer, diabetes, hypertension, COPD, Parkinson's, hypo- or hyperthyroidism

Table 2. Association between measures of kidney function and odds of frailty status

Measure of Kidney Function[c]	Multinomial Odds Ratio (95% Confidence Interval)			
	Base Model[a]		Multivariate Model[b]	
	Intermediate vs Robust	Frail vs Robust	Intermediate vs Robust	Frail vs Robust
Cystatin C				
Quartile 1	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Quartile 2	1.26 (0.94 - 1.69)	1.98 (1.01 - 3.88)	1.23 (0.90 - 1.69)	1.86 (0.88 - 3.89)
Quartile 3	1.15 (0.85 - 1.56)	1.66 (0.84 - 3.28)	1.13 (0.81 - 1.56)	1.31 (0.61 - 2.81)
Quartile 4	2.38 (1.70 - 3.32)	7.12 (3.76 - 13.46)	2.07 (1.44 - 2.98)	4.17 (2.02 - 8.59)
p-trend	<0.001	<0.001	<0.001	<0.001
Creatinine				
Quartile 1	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Quartile 2	0.81 (0.58 - 1.14)	0.61 (0.33 - 1.11)	0.89 (0.64 - 1.25)	0.70 (0.36 - 1.37)
Quartile 3	0.73 (0.54 - 0.99)	0.46 (0.26 - 0.80)	0.78 (0.57 - 1.07)	0.57 (0.31 - 1.06)
Quartile 4	1.11 (0.77 - 1.59)	1.36 (0.78 - 2.40)	1.11 (0.76 - 1.61)	1.21 (0.65 - 2.27)
p-trend	0.96	0.43	0.87	0.49
eGFR (CKD-EPI Cr)				
Quartile 4	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Quartile 3	1.10 (0.79 - 1.53)	0.61 (0.33 - 1.16)	1.27 (0.90 - 1.78)	0.99 (0.49 - 2.00)
Quartile 2	0.97 (0.70 - 1.33)	0.59 (0.32 - 1.11)	1.04 (0.75 - 1.45)	0.95 (0.48 - 1.91)
Quartile 1	1.12 (0.79 - 1.59)	1.01 (0.54 - 1.87)	1.12 (0.77 - 1.61)	1.11 (0.56 - 2.23)
p-trend	0.87	0.49	0.92	0.67

[a]Adjusted for age, race, and clinical site

[b]Adjusted for age, race, clinic site, health status, body mass index, number of comorbidities, IADL impairments, vitamin D level, and serum albumin

[c]Quartile cutpoints: Cystatin C: 0.80, 0.90, 1.03; Serum Creatinine: 0.80, 0.90, 1.10 ; eGFR: 64.7, 74.3, 84.2

Table 3. Association between cystatin C and frailty status amongst n = 1257 subjects with $eGFR_{Cr} > 60ml/min/1.73m^2$

Measure of Kidney Function	Multinomial Odds Ratio (95% Confidence Interval)			
	Base Model		Multivariate Model	
	Intermediate vs Robust	Frail vs Robust	Intermediate vs Robust	Frail vs Robust
Cystatin C				
Quartile 1	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Quartile 2	1.11 (0.81 - 1.52)	1.86 (0.88 - 3.93)	1.11 (0.80 - 1.54)	1.73 (0.73 - 3.86)
Quartile 3	1.19 (0.86 - 1.65)	2.08 (0.98 - 4.40)	1.16 (0.82 - 1.63)	1.68 (0.76 - 3.72)
Quartile 4	1.79 (1.20 - 2.67)	6.18 (2.88 - 13.27)	1.67 (1.10 - 2.53)	4.18 (1.83 - 9.56)
p-trend	0.009	<0.001	0.03	0.002

Table 4. Association between cystatin C level and odds of frailty components

Quartile of cystatin C[a]	Odds Ratio (95% CI)				
	Shrinking	Weakness	Exhaustion	Slowness	Low Physical Activity
Base Model[b]					
Quartile 1	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Quartile 2	1.08 (0.73 - 1.59)	1.19 (0.83 - 1.70)	1.83 (0.97 - 3.45)	1.39 (0.91 - 2.13)	1.20 (0.82 - 1.77)
Quartile 3	1.29 (0.88 - 1.89)	1.11 (0.77 - 1.60)	1.16 (0.58 - 2.33)	1.18 (0.76 - 1.82)	1.53 (1.05 - 2.25)
Quartile 4	1.81 (1.24 - 2.64)	1.82 (1.27 - 2.60)	4.05 (2.22 -7.39)	2.82 (1.88 - 4.21)	2.38 (1.64 - 3.46)
Multivariate Model[c]					
Quartile 1	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Quartile 2	1.21 (0.80 - 1.82)	1.09 (0.74 - 1.59)	2.05 (0.98 - 4.28)	1.22 (0.77 - 1.92)	1.19 (0.79 - 1.78)
Quartile 3	1.54 (1.02 - 2.32)	0.96 (0.65 - 1.43)	0.94 (0.41 - 2.13)	0.90 (0.57 - 1.45)	1.39 (0.93 - 2.09)
Quartile 4	2.31 (1.51 - 3.53)	1.32 (0.89 - 1.95)	1.91 (0.92 - 3.95)	1.65 (1.05 - 2.58)	1.89 (1.26 - 2.85)

[a]Quartile cutpoints: 0.80, 0.90, 1.03 mg/dL
[b]Adjusted for age, race, and clinic site
[c]Adjusted for age, race, clinic site, health status, body mass index, number of comorbidities, IADL impairments, vitamin D level, and serum albumin level

Table 5. Effect of biological mediators on the association between cystatin C eGFR < 60ml/min and frailty

		Odds Ratio (95% CI)[a]	
	n	Intermed vs Robust	Frail vs Robust
Base model[b]	974	2.12 (1.43 - 3.14)	5.32 (3.00 - 9.43)
Base model + IL-6	974	1.82 (1.21 - 2.73)	3.75 (2.04 - 6.88)
Base model + CRP	952	2.07 (1.39 - 3.09)	5.00 (2.80 - 8.93)
Base model + vit D	969	2.17 (1.47 - 3.22)	5.67 (3.17 - 10.13)
Base model + iPTH	969	2.06 (1.39 - 3.06)	5.02 (2.80 - 8.99)
Base model + albumin	949	2.16 (1.44 - 3.25)	4.39 (2.33 - 8.27)

[a]Reference: eGFR ≥ 60 ml/min
[b]Adjusted for age, race and clinical site

Table 6. Association between CKD (eGFR <60) and odds of frailty status, measured by cystatin C and creatinine

Measure of Renal Function	Multinomial Odds Ratio (95% Confidence Interval)			
	Base Model		Multivariate Model	
	Intermediate vs Robust	Frail vs Robust	Intermediate vs Robust	Frail vs Robust
Cystatin C eGFR (CKD-EPI)				
≥60	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
<60	2.21 (1.64 - 2.99)	4.63 (3.03 - 7.08)	1.88 (1.36 - 2.60)	2.76 (1.68 - 4.56)
Creatinine eGFR (CKD-EPI)				
≥60	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
<60	1.70 (1.21 - 2.40)	2.56 (1.59 - 4.12)	1.59 (1.11 - 2.27)	1.91 (1.11 - 3.27)