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Cystatin C and Risk of Hip Fractures in Older Women

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Abstract

To test the hypothesis that older women with higher cystatin C are at increased risk of hip fracture independent of traditional risk factors including hip bone mineral density (BMD), we performed a case-cohort analysis nested in a cohort of 4709 white women attending a Year 10 (1997–1998) examination of the Study of Osteoporotic Fractures that included a random sample of 1170 women and the first 300 women with incident hip fracture occurring after Year 10 examination. Serum cystatin C and creatinine were measured in Year 10 sera. In a model adjusted for age, clinical site, body mass index and total hip BMD, higher cystatin C was associated with an increased risk of hip fracture (p for linear trend 0.008) with women in quartile 4 having a 1.9-fold higher risk (hazard ratio (HR) 1.91, 95% confidence (CI) 1.24–2.95) compared with those in quartile 1 (referent group). Further adjustment for additional risk factors only slightly attenuated the association; the risk for hip fracture was 1.7-fold (HR 1.74, 95% CI 1.11–2.72) higher in women in quartile 4 compared with those in quartile 1. In contrast, neither serum creatinine nor creatinine-based estimated glomerular filtration rate ($eGFR_{Cr}$) were associated with risk of hip fracture. Older women with higher cystatin C, but not higher serum creatinine or lower $eGFR_{Cr}$, have an increased risk of hip fracture independent of traditional risk factors. These findings

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DISCLOSURES

All authors state that they have no conflicts of interest.

Authors' roles:
Study concept and design: KEE, JAC, SRC
Data collection: KEE, JAC, TAH
Data analysis and interpretation: KEE, NP
Drafting manuscript: KEE
Critical review and final approval of manuscript content: KEE, NP, JAC, AI, YS, TAH, BCT, MS, SRC

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suggest that cystatin C may be a promising biomarker for identification of older adults at high risk of hip fracture.

Keywords

renal function; cystatin C; hip fracture; elderly; women

INTRODUCTION

Older age and female gender are associated with a higher prevalence of renal dysfunction (1) and an increased risk of osteoporosis and related fractures, including hip fractures. (2) Serum cystatin C, a low molecular weight protein whose concentration is mainly determined by glomerular filtration, has been proposed to be a promising marker of kidney function that may be superior to standard creatinine-based measures in detecting mild to moderate renal dysfunction in older adults. (3) Previous prospective studies have reported an association between higher serum cystatin C and an increased risk of hip fracture among postmenopausal women. (4;5) However, it is uncertain whether this association persists after adjustment for hip bone mineral density (BMD) and how this association compares with that between conventional measures of renal function and hip fracture.

To test the hypothesis that older women with higher cystatin C are at increased risk of hip fracture independent of traditional risk factors, we performed a case-cohort analysis nested in the cohort of 4709 white women attending a Year 10 examination of the Study of Osteoporotic Fractures (SOF) that included a random sample of 1170 women from the cohort and the first 300 women with incident hip fracture after the Year 10 examination. In addition, we examined the association between standard measures of renal function (serum creatinine and creatinine-based estimated glomerular filtration rate ($eGFR_{Cr}$) and risk of hip fracture.

METHODS

Study Population

From 1986 to 1988, a total of 9704 women who were at least 65 years old were recruited for participation in the initial examination of the prospective Study of Osteoporotic Fractures (SOF). Women were recruited from population-based listings in four areas of the United States. We excluded from the original cohort black women (due to their low incidence of hip fracture), women who had undergone bilateral hip replacement and those who were unable to walk without assistance. (6) Of the original cohort, 7008 surviving women provided at least questionnaire data for the Year 10 examination conducted between 1997 and 1998; 1148 women provided questionnaire data only, 552 completed a home or nursing home visit, and 4808 completed an in-clinic examination including 4709 women who provided serum specimens. The protocol and consent form were approved by the Institutional Review Boards at all participating institutions. All participants provided written informed consent.

Case-Cohort Study Design

This study is a case-cohort analysis (7) nested within the cohort of 4709 white women providing serum specimens at the Year 10 examination. We randomly selected 1170 women out of the 4709 women to serve as the random subcohort. We also selected the first 300 women with incident hip fracture (146 cases of femoral neck fractures and 150 cases of trochanteric fractures) occurring during an average of 5.4 years of follow-up after the Year 10 examination. The sample size of 300 women with hip fractures was based on a priori

power calculations that indicated that this number of cases provided power of 90% to detect a hazards ratio per 1 SD decrease in cystatin C of 1.30. Among these 300 women, 77 women were also sampled within the random subcohort and analyzed as cases in the analyses. Thus, the number of women without incident hip fracture after the Year 10 exam was 1093 among the 1170 women in the random subcohort.

Serum Cystatin C and Creatinine Assays

Fasting morning blood was collected at the Year 10 examination and processed for serum which was stored at −70°C until thawed. Serum cystatin C and creatinine assays were performed at the University of Minnesota Medical Center in 2010. Serum cystatin C concentrations were determined using a BN100 nephelometer (Dade Behring Inc., Deerfield, IL) using a particle-enhanced immunonepholometric assay (8) (assay range 0.23–8.00 mg/L with inter-assay coefficient of variation (CV) of 4.0% at a level of 0.71 mg/L and 3.1% at a level of 1.75 mg/L [mean inter-assay CV 3.7%]) and then converted to standardized values traceable to a certified reference material. (9) Cystatin C-based eGFR ($eGFR_{cysC}$), was computed using a CKD-EPI equation re-expressed for standardized cystatin C. (9;10)Serum creatinine was measured with a Modular P Chemistry Analyzer (Roche Diagnostics, Indianapolis IN) using an enzymatic method equation calibrated with materials traceable to an isotope-dilution mass spectrometry (IDMS) reference measurement procedure. Inter-assay CV is 4.0%. Creatinine-based eGFR ($eGFR_{Cr}$) was calculated using the CKD-EPI equation (11) which includes variables for standardized creatinine, age, gender and race.

Ascertainment of Hip Fractures

Participants were contacted every four months after the Year 10 examination to ask whether they had sustained a fracture; more than 95% of these follow-up contacts were completed through 6 years of follow-up. Hip fractures (including the location) were confirmed by review of radiographic reports.

Other Measurements

All covariates were measured at the Year 10 examination. Participants were asked about smoking status, health status, prevalent medical conditions including diabetes mellitus and cardiovascular disease (history myocardial infarction or stroke), previous fractures since age 50, and falls in the past year. Physical activity was assessed using a modified version of the Harvard Alumni Questionnaire (12;13) and expressed as a weighted score of kilocalories expended per week from walking. Depressive symptoms were evaluated using the 15-item Geriatric Depression Scale. (14) Weight was recorded with a balance beam scale and height was measured with a Harpenden stadiometer; weight and height were used to calculate body mass index (BMI). Tests of physical function included grip strength (using a handheld Jamar dynamometer) and walk speed (time in seconds to walk 6 m at usual pace expressed as m/s). BMD at the total hip was measured using dual energy x-ray absorptiometry; details of the BMD measurement methods and precision are published elsewhere. (15)

Frailty status was defined using criteria similar to those proposed by Fried and colleagues (16;17) using data collected in the Cardiovascular Health Study. Frailty was identified by the presence of 3 or more of the following 5 components: [1] shrinking as defined by weight loss of 5% or more between the Year 8 and Year 10 examinations ; [2] weakness as defined by grip strength in the lowest quintile stratified by BMI quartile; [3] exhaustion as defined by an answer of no" to the question, "Do you feel full of energy?" on the Geriatric Depression Scale; [4] slowness as defined by walk speed in the lowest quintile stratified by median height; and [5] low physical activity as defined by kilocalories expended per week from walking in the lowest quintile.

Measures of $25(OH)D_2$ and $25(OH)D_3$ were performed at the Mayo Clinic using liquid chromatography-mass spectrometry (18) 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 levels of pro-inflammatory cytokines (TNFα, IL-6) and their soluble receptors (TNFα sR1, TNFα sR2, and IL-6 sR) using ELISA kits (R&D systems, Minneapolis, MN) at the University of Maryland Cytokine Core Laboratory.

Statistical Analysis

Characteristics of participants in the random subcohort at the Year 10 examination (baseline for this analysis) were compared using across quartiles of cystatin C using chi-square tests for categorical variables and ANOVA for continuous variables. Characteristics of women with and without hip fracture were also compared and these comparisons were between the 1093 women in the random subcohort who did not experience an incident hip fracture and the 300 incident hip fracture cases. The association between cystatin C and incident hip fracture was analyzed using proportional hazards regression models modified for the case-cohort sampling design. (7) Hazard ratios (HR) and 95% confidence intervals (CI) stratified according to a history of hip fracture prior to the Year 10 examination were first calculated across quartiles of cystatin C (cutpoints were determined from distribution of cystatin C among women in the random subcohort) with quartile 1 serving as the referent group. Since there was not evidence of an interaction between cystatin C and a history of hip fracture prior to Year 10 examination for the prediction of risk of hip fracture after Year 10 examination, a weighted HR on both strata was reported for subsequent analyses.

Models were initially adjusted for age and then further adjusted for clinical site, BMI and total hip BMD (base model). To obtain final multivariable risk estimates, we subsequently added to the base model characteristics associated with cystatin C or hip fracture at $p < 0.10$. Tests for trend were performed by including cystatin C (ordinal variable with 4 levels) as an independent variable in the models.

To determine whether the association between cystatin C and risk of hip fracture varied by fracture location, we analyzed the association between cystatin C and risk of trochanteric fracture and that between cystatin C and risk of femoral neck fracture. We also examined whether the association between cystatin C and hip fracture persisted when the analysis was limited to women without evidence of clinical chronic kidney disease defined by an $eGFR_{Cr}$ ≥60 mL/min/1.73 m^2. To explore biological mechanisms that might underlie the independent association observed between cystatin C and hip fracture, levels of calcitropic hormones (25(OH)D, PTH) and markers of chronic inflammation (TNFα, IL-6, TNFα sR1, TNFα sR2, and IL-6SR) were added one at a time to the base model.

To compare cystatin C with standard measures of renal function in predicting hip fracture, analyses were performed substituting traditional measures of renal function (creatinine, $eGFR_{Cr}$) for cystatin C. Finally, we examined the association between chronic kidney disease (CKD) as defined by an eGFR <60 ml/min/1.73 m^2 calculated using both cystatin C-based and creatinine-based equations and risk of hip fracture.

RESULTS

Among the women in the random subcohort (n=1170), the mean (SD) cystatin C concentration was 1.14 (0.30) mg/L and mean (SD) creatinine concentration was 0.82 (0.26) mg/dL; mean $eGFR_{cysC}$ was 68.3 (19.0) mL/min/1.73 $m^2$and mean $eGFR_{Cr}$ was 70.4 (16.1) mL/min/1.73 m^2. The correlation between serum cystatin C and creatinine was moderate in

magnitude (Spearman Correlation Coefficient=0.71). CKD was evident in 384 (32.8%) women in the random subcohort as defined by an $eGFR_{cysC}$ <60 mL/min/1.73 $m^2$and 294 (25.4%) women in the random subcohort as defined by an $eGFR_{Cr}$ <60 mL/min/1.73 m^2.

Higher cystatin C among women in the random subcohort was associated ($p \leq 0.05$) with older age, poorer health status, history of cardiovascular disease, self-reported diabetes mellitus, higher BMI, greater frailty status, and lower $eGFR_{Cr}$ (Table 1). There were no differences across quartiles of cystatin C in smoking status, fracture history, fall history, or hip BMD.

Compared to women without hip fracture, women who experienced incident hip fracture were older, more likely to report prior fracture, and more likely to be classified as frail (Table 2). In addition, women with hip fractures were thinner, had lower hip BMD, higher cystatin C, and lower $eGFR_{Cr}$. There was some evidence that poorer health status (p=0.06) and falling (p=0.07) were more common among women who experienced subsequent hip fracture. There were no differences in smoking status, self-reported diabetes or cardiovascular disease between hip fracture cases and women without hip fracture.

Association between Serum Cystatin C and Hip Fracture

In models adjusted for age, clinical site, BMI, and total hip BMD, higher cystatin C was associated with an increased risk of hip fracture after the Year 10 examination among women with and those without a history of prior hip fracture. Among the 74 women with a prior history, the risk of hip fracture was 4.5-fold higher in women in quartile 4 compared with those in quartile 1 (HR 4.54, 95% CI 1.13–18.20, p-trend 0.03). Among the 1319 women without a prior history, the risk of hip fracture was 1.7-fold higher in women in quartile 4 compared with those in quartile 1 (HR 1.69, 95% CI 1.07–2.66, p-trend 0.04). While the association appeared more pronounced among women with a history of prior hip fracture, the test for interaction between cystatin C and prior history of hip fracture for the prediction of incident hip fracture after Year 10 examination did not reach the level of significance (p=0.18). The weighted HR on both strata for quartile 4 (vs. quartile 1) was 1.91 (95% CI 1.24–2.95, p-trend 0.008) (Figure 1). This association was modestly attenuated in the final multivariable model adjusted for age, clinical site, BMI, total hip BMD, health status, medical conditions including diabetes mellitus and cardiovascular disease, prior fracture, fall history, and frailty status. The risk of hip fracture was 1.7-fold higher in women in quartile 4 compared with those in quartile 1 (multivariable HR 1.74, 95% CI 1.11–2.72, p-trend 0.03).

Women with higher cystatin C were at increased risk of trochanteric (HR base model quartile 4 vs. quartile 1: 1.94, 95% CI 1.04–3.63) and femoral neck fractures (HR base model quartile 4 vs. quartile 1: 2.05, 95% CI: 1.21–3.53)), though evidence of a graded association between cystatin C and fracture was present only for femoral neck fracture (p-trend 0.10 for trochanteric fracture and 0.007 for femoral neck fracture).

The association between higher cystatin C and an increased risk of hip fracture persisted when the analysis was limited to 1021 women without clinical CKD (e.g. those with $eGFR_{Cr}$ ≥60 mL/min/1.73 m^2); compared with women in quartile 1 (referent group), those in quartile 4 had a 1.9-fold increased risk of hip fracture (HR base model: 1.89, 95% CI 1.07–3.35, p-trend 0.06).

The association between cystatin C and hip fracture was not explained by alterations in calcitropic hormones (PTH, 25(OH)D) or inflammatory markers (TNFα, IL-6, TNFα sR1, TNFα sR2, IL-6, IL-6 sR) (Table 3). Further adjustment of the base model for each of the potential biological mediators had little impact on the association.

Association of Creatinine and $eGFR_{Cr}$ with Hip Fracture

In unadjusted models, lower $eGFR_{Cr}$, but not higher serum creatinine, was significantly associated with an increased risk of hip fracture (Table 4). However, the association between lower $eGFR_{Cr}$ and hip fracture appeared to be explained by older age among those with lower $eGFR_{Cr}$; after adjustment for age, there was no evidence of an association. Further adjustment for other potential confounders did not alter these findings.

Association between CKD and Hip Fracture

After adjustment for age, women with CKD as defined by an $eGFR_{cysC}$ <60 mL/min/1.73 m^2 (HR 1.37, 95% CI 1.04–1.80), but not those with clinical CKD as defined by an $eGFR_{Cr}$ <60 mL/min/1.73 m^2 (HR 1.08, 95% CI 0.80–1.46), were at increased risk of hip fracture. The association between CKD as defined by an $eGFR_{cysC}$ <60 mL/min/1.73 m^2 and risk of hip fracture persisted after further adjustment for multiple potential confounders (HR 1.48, 95% CI 1.08–2.04).

DISCUSSION

In this prospective case-cohort study, older women with higher serum cystatin C had an increased risk of hip fracture independent of traditional risk factors including age, prior fracture, frailty status, body weight and hip BMD. Our findings indicate that conventional measures of reduced renal function including higher serum creatinine or lower estimated GFR calculated using a creatinine-based formula were not related to hip fracture risk.

These findings are in agreement with those of a prospective study of older adults enrolled in the Cardiovascular Health Study (CHS) (4) and a prior case-control study nested within the Women's Health Initiative Observational Study (WHI-OS) cohort (5) that reported an increased risk of hip fracture among women with higher cystatin C levels. However, neither of these previous studies examined whether the association remained after adjustment for BMD. The results from the present study extend the results of previous investigations and suggest that this association is not substantially altered despite consideration of several strong clinical risk factors and hip BMD. While higher cystatin C concentration was associated with an increased risk of incident hip fracture among women with and those without a prior hip fracture in this study, our findings suggest that this association is most pronounced among women with a prior history who are at very high risk for a second event.

The association between higher cystatin C and increased risk of hip fracture in this study persisted when women with clinical CKD as defined an $eGFR_{Cr}$ <60 mL/min/1.73 m^2 were excluded from the analysis. These results indicate that older women with higher cystatin C and preserved $eGFR_{Cr}$ are at increased risk of hip fracture. A prior study (19) reported an increased risk of progression to adverse kidney events, cardiovascular events, and death among older adults with elevated cystatin C levels and preserved $eGFR_{Cr}$. Together these studies support the contention that higher cystatin C in the presence of normal creatinine-based eGFR may identify "preclinical" state of renal dysfunction in older adults, predating the development of clinical chronic kidney disease, but associated with a higher risk of adverse health consequences.

Previous studies comparing differences in risk factor patterns between femoral neck and trochanteric fractures have reported that women with trochanteric fractures are more likely to be older and have poorer health status. Since these characteristics are also more prevalent among older adults with higher cystatin C concentrations, it was reasonable to postulate that the association between cystatin C and hip fracture might be most evident for trochanteric fractures. However, in this study, cystatin C was related to increased risks of both trochanteric fractures and femoral neck fractures. In a prior study in this cohort that utilized

serum specimens collected 10 years earlier at the baseline examination (20), reduced renal function as manifested by a lower serum creatinine clearance standardized for body surface area was most strongly associated with an increased risk of trochanteric fractures. In contrast, in the prior case-control study nested within the WHI-OS cohort (5), reduced renal function as manifested by a lower eGFR calculated using a cystatin C-based formula was most strongly associated with an increased risk of femoral neck fractures. Differences in the effect of renal function on type of hip fractures in these studies may be due to several factors, including differences in age of study participants, sample size, choice of renal function measure, or chance alone.

Several biological mechanisms may underlie an association between higher cystatin C levels and increased hip fracture risk among older women. Abnormalities in calcium, phosphorus, and vitamin D metabolism that occur in even mild renal insufficiency among older adults may lead to decreased formation of 1,25-dihydroxyvitamin D by the kidney, resulting in reduced fractional calcium absorption, secondary hyperparathyroidism, greater bone resorption, and higher hip fracture rates. (21;22) However, the association between higher cystatin C and hip fracture women in this study persisted despite adjustment for 25-hydroxyvitamin D and PTH. Some, but not all prospective studies, have reported an association between lower vitamin D status and increased fracture risk (23;24) and the association between PTH levels and hip fracture risk among older adults is controversial. (25;26) Moderate impairment in renal function has also been associated with higher levels of inflammatory factors and homocysteine, as well as anemia. (27–29) Adjustment for pro-inflammatory cytokines TNF-α, IL-6 or their soluble receptors had little impact on the association between cystatin C and hip fracture observed in this study suggesting that the increased risk of hip fracture was not explained by the effect of renal dysfunction on chronic inflammatory processes. In contrast, a previous prospective study reported that levels of TNF-α cytokine soluble receptors in part mediated the association between cystatin C and risk of nonvertebral fracture that was observed among postmenopausal white women. (30) Higher cystatin C may be a marker for poorer health status and frailty (31) that may increase risk of falls and related hip fractures, though the association between cystatin C and hip fracture in this study remained after adjustment for these characteristics. While adjustment for initial hip BMD also did not alter the association, a prior investigation (32) reported that older men with higher cystatin C concentrations experienced increased rates of hip bone loss. Thus, higher rates of bone loss among women with elevated cystatin C concentrations may in part mediate the association between cystatin C and hip fracture. The independence of the association between cystatin C and hip fracture in this study is in agreement with results of a previous investigation in WHI-OS that reported persistence of the association in postmenopausal women despite adjustment for markers of bone resorption and formation, frailty and co-morbid conditions, 25(OH) D, hemoglobin, or homocysteine. (5)

In this study, cystatin C concentration (but not serum creatinine concentration or level of $eGFR_{Cr}$) was independently associated with hip fracture risk. Use of a cystatin C-based equation vs. use of a creatinine-based equation yielded a higher prevalence of CKD in this cohort of older women and CKD defined using a cystatin-C based equation, but not a creatinine-based equation, was associated with a higher hip fracture risk. Similarly, a recent study from Alberta, Canada utilizing a province-wide laboratory database and administrative outcome dataset found no evidence of an association of CKD defined using a creatinine-based equation with risk of hip fracture, including among adults 75 years and older. (33) These results might reflect the lower dependence of cystatin C on muscle mass and its greater accuracy in estimating GFR in older adults with modest to moderate reductions in renal function. Creatinine levels may be misleading indicator of renal function in aged populations because of age-related declines in muscle mass result in lower creatinine production. Hence, even with a reduction in GFR to <60 mL/min/1.73 m^2, serum creatinine

may not rise appreciably in elderly persons. Thus, low creatinine in many older adults may be the result of reduced muscle mass or frailty, rather than normal renal function, and this misclassification might lead to a U or J-shaped association between creatinine level and hip fracture. GFR-estimating equations based on serum creatinine, such as the CKD-EPI equation used in this study, include variables for age, race and sex as surrogates for creatinine generation by muscle. However, these variables do not account for lower creatinine production due to malnutrition, inflammation, or loss of muscle bulk in older adults with chronic illnesses in whom creatinine based equations may overestimate GFR. (34)

This study has a number of strengths including use of cystatin C and creatinine-based measures of renal function, the large well-characterized cohort with comprehensive assessment of hip fracture risk factors, and consideration of multiple potential confounders and potential biologic mediators of the association. However, this study has several limitations. The cohort was comprised of older white women and findings do not necessarily apply to other population groups. Importantly, we had no direct measure of GFR and factors other than GFR may affect cystatin C levels. (35) Thus, the possibility of a mechanism linking cystatin C with hip fracture risk, unrelated to renal function, cannot be excluded and residual confounding may exist. On the other hand, the discrepancy between findings for cystatin C versus those for conventional creatinine-based measures observed in this study may reflect the greater accuracy of cystatin C in detecting mild to moderate renal dysfunction in older adults. Severe chronic kidney disease (e.g. eGFR <30 mL/min/1.73 m^2) was uncommon in this cohort and future studies should include older adults with a wider range of renal function. Finally, this study examined the association between markers of renal function and risk of hip fracture. While results suggest that cystatin C may be a promising biomarker for identification of older adults at increased risk of hip fracture, findings from this study do not demonstrate that its measurement provides additional prognostic information in the clinical practice setting beyond that which is readily available by assessing traditional risk factors.

In conclusion, older women with higher cystatin C, but not higher serum creatinine or lower $eGFR_{Cr}$, have an increased risk of hip fracture independent of traditional risk factors. Future studies evaluate whether measurement of cystatin C can improve medical decision making in identifying older adults at high hip fracture risk.

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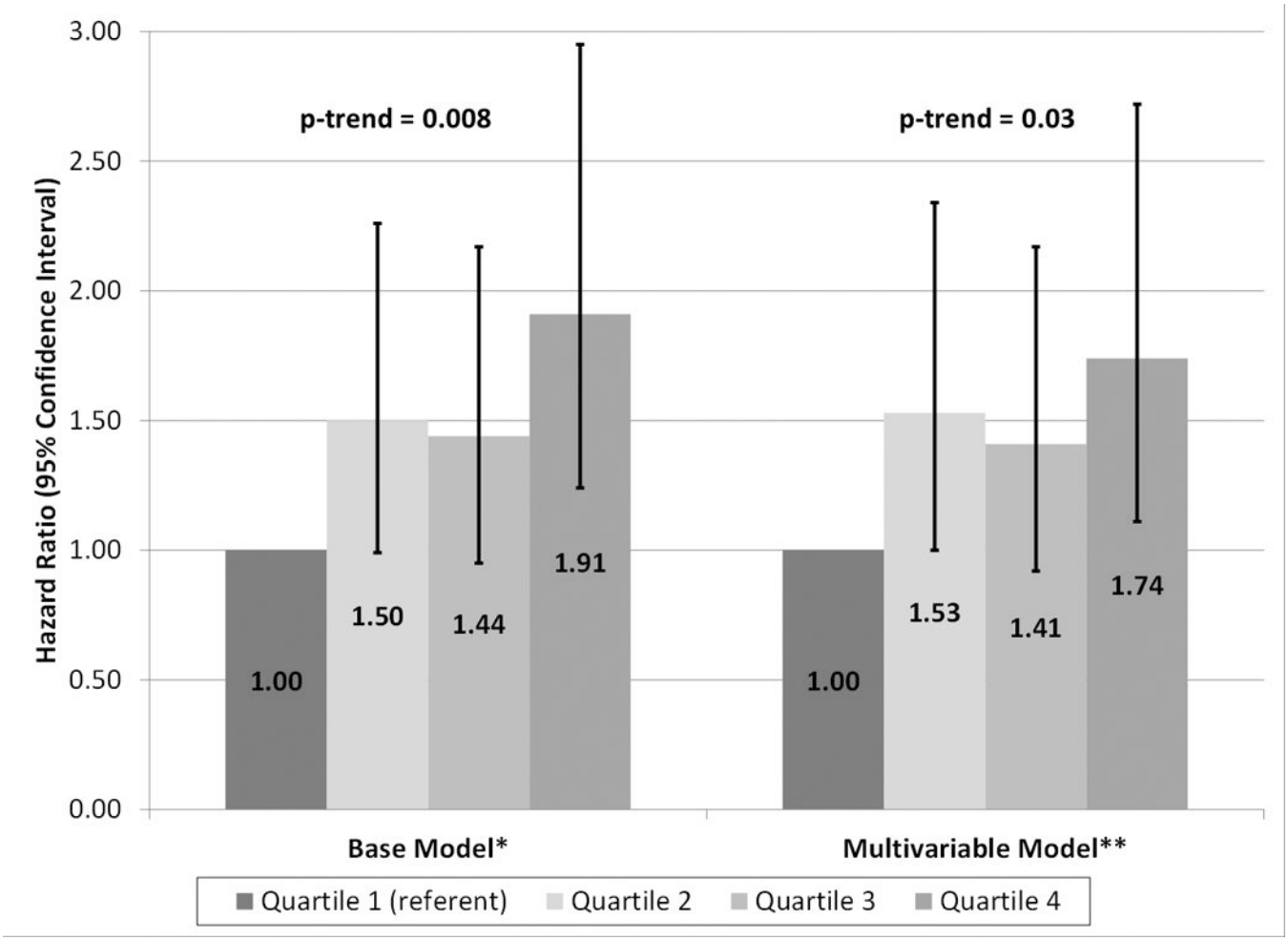

Figure 1. Association between Cystatin C Level and Risk of Hip Fracture

Quartile cutpoints: 0.94, 1.08, 1.24 mg/L

*adjusted for age, clinical site, BMI, and total hip BMD

**Base model further adjusted for health status, fall history, prior fracture, prevalent CVD, diabetes, and frailty status

Table 1

Characteristics of Women in the Random SubCohort According to Quartile of Cystatin C

Variable	Quartile of Cystatin C, mg/L				
	Q1 (n=281)	Q2 (n=284)	Q3 (n=303)	Q4 (n=302)	p-value
Age, years, mean (SD)	78.8 (3.5)	79.3 (3.7)	80.2 (4.0)	81.8 (4.8)	<0.001
Current smoker, n (%)	14 (5.0)	11 (3.9)	8 (2.6)	12 (4.0)	0.53
Health status, fair/poor/very poor, n (%)	244 (86.8)	234 (82.4)	249 (82.2)	217 (72.1)	<0.001
Diabetes, n (%)	13 (4.6)	6 (2.1)	14 (4.6)	21 (7.0)	0.05
Cardiovascular disease, n (%)	28 (10.0)	24 (8.5)	24 (7.9)	53 (17.6)	<0.001
Previous fracture since age 50, n (%)	136 (48.4)	136 (48.6)	171 (56.6)	173 (57.3)	0.04
Fall in past year, n (%)	87 (31.0)	93 (32.8)	87 (28.9)	106 (35.1)	0.41
BMI, kg/m^2, mean (SD)	25.1 (4.1)	26.2 (4.5)	27.0 (4.8)	27.3 (5.2)	<0.001
Frail status, n (%)					<0.001
Robust	114 (40.6)	96 (33.8)	72 (23.8)	49 (16.3)	
Pre-frail	140 (49.8)	159 (56.0)	193 (63.7)	167 (55.5)	
Frail	27 (9.6)	29 (10.2)	38 (12.5)	85 (28.2)	
Total hip BMD, g/cm^2, mean (SD)	0.71 (0.13)	0.73 (0.13)	0.72 (0.13)	0.72 (0.14)	0.14
$eGFR_{Cr}$, mL/min/1.73m^2, mean (SD)	83.4 (8.0)	76.6 (10.0)	70.0 (11.2)	52.9 (14.7)	<0.001

Quartile cutpoints: 0.94, 1.08, 1.24 mg/L

Quartile mean (SD) and range: Q1 0.86 (0.07), 0.43–0.94; Q2 1.00 (0.04), 0.94–1.07; Q3 1.15 (0.05), 1.08–1.23; Q4 1.52 (0.34), 1.24–4.02

Abbreviations: BMI, body mass index; BMD, bone mineral density; $eGFR_{Cr}$, creatinine-based estimated glomerular filtration rate

Table 2

Characteristics of Women With and Without Incident Hip Fracture

Characteristic	Hip Fracture		
	Cases (n=300)	Women without Hip Fx (n=1093)	P value
Age, years, mean (SD)	81.9 (4.4)	80.0 (4.2)	<0.001
Current smoker, n (%)	11 (3.7)	42 (3.9)	0.90
Health status fair/poor/very poor, n (%)	227 (76.2)	886 (81.1)	0.06
Diabetes, n (%)	17 (5.7)	50 (4.6)	0.42
Cardiovascular disease, n (%)	34 (11.4)	123 (11.3)	0.94
Previous fracture since age 50, n (%)	208 (69.3)	561 (51.6)	<0.001
Fall in past year, n (%)	111 (37.4)	348 (31.9)	0.07
BMI, kg/m^2, mean (SD)	24.6 (4.3)	26.6 (4.8)	<0.001
Frail status, n (%)			<0.001
Robust	63 (21.0)	316 (28.9)	
Pre-frail	169 (56.3)	613 (56.1)	
Frail	68 (22.7)	163 (14.9)	
Total hip BMD, g/cm^2, mean (SD)	0.63 (0.11)	0.73 (0.13)	<0.001
Cystatin C, mg/L, mean (SD)	1.23 (0.39)	1.12 (0.29)	0.003
$eGFR_{Cr}$, $mL/min/1.73m^2$, mean (SD)	67.6 (17.7)	70.5 (15.9)	0.01

Abbreviations: BMI, body mass index; BMD, bone mineral density; $eGFR_{Cr}$, creatinine-based estimated glomerular filtration rate

Table 3

Association between Cystatin C and Hip Fracture Adjusted for Potential Biologic Mediators

	Relative Hazard (95% CI) by Quartile of Cystatin C				
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	**p-trend**
Base model*	1.00 (referent)	1.50 (0.99–2.26)	1.44 (0.95–2.17)	1.91 (1.24–2.95)	0.008
Base model + 25(OH)D	1.00 (referent)	1.45 (0.96–2.19)	1.36 (0.90–2.06)	1.76 (1.14–2.72)	0.02
Base model + PTH	1.00 (referent)	1.60 (1.04–2.44)	1.45 (0.94–2.23)	1.98 (1.26–3.11)	0.009
Base model + TNFα	1.00 (referent)	1.39 (0.67–2.91)	1.56 (0.80–3.03)	1.92 (0.92–4.03)	0.09
Base model + TNFα-sR1	1.00 (referent)	1.56 (1.01–2.39)	1.42 (0.92–2.18)	1.91 (1.22–3.00)	0.01
Base model + TNFα-sR2	1.00 (referent)	1.54 (1.00–2.35)	1.37 (0.89–2.10)	1.74 (1.07–2.81)	0.05
Base model + IL-6	1.00 (referent)	1.49 (0.97–2.30)	1.46 (0.95–2.23)	1.92 (1.22–3.02)	0.01
Base model + IL-6R	1.00 (referent)	1.55 (1.01–2.37)	1.42 (0.93–2.17)	1.95 (1.25–3.05)	0.009

Quartile cutpoints: 0.94, 1.08, 1.24 mg/L

*model adjusted for age, clinical site, BMI, and total hip BMD

Table 4

Standard Measures of Renal Function and Risk of Hip Fracture

	Relative Hazard (95% CI) by Quartile				
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	**p-trend**
Creatinine					
Unadjusted model	1.00 (referent)	0.80 (0.55–1.17)	1.15 (0.81–1.63)	1.26 (0.89–1.78)	0.07
Age-adjusted model	1.00 (referent)	0.76 (0.52–1.12)	1.02 (0.71–1.46)	1.04 (0.73–1.50)	0.48
Base model *	1.00 (referent)	0.86 (0.57–1.30)	1.15 (0.78–1.71)	1.27 (0.85–1.90)	0.12
$eGFR_{Cr}$					
Unadjusted model	1.55 (1.08–2.23)	1.35 (0.94–1.95)	1.14 (0.79–1.66)	1.00 (referent)	0.01
Age-adjusted model	1.01 (0.67–1.51)	1.01 (0.69–1.49)	0.84 (0.57–1.25)	1.00 (referent)	0.65
Base model *	1.23 (0.80–1.92)	1.11 (0.74–1.67)	0.84 (0.55–1.27)	1.00 (referent)	0.14

Quartiles cutpoints: (creatinine) 0.67, 0.77, 0.90 mg/dL; ($eGFR_{Cr}$) 59.7, 73.0, 83.7 mL/min/1.73m^2

* adjusted for age, clinical site, BMI, and total hip BMD