Original Article

Inflammatory Markers and Risk of Hip Fracture in Older White Women: The Study of Osteoporotic Fractures†

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Abstract

Hip fractures are the most devastating consequence of osteoporosis and impact 1 in 6 white women leading to a 2-3 fold increased mortality risk in the first year. Despite evidence of inflammatory markers in the pathogenesis of osteoporosis, few studies have examined their effect on hip fracture. To determine if high levels of inflammation increase hip fracture risk and explore mediation pathways, a case-cohort design nested in a cohort of 4709 white women from the Study of Osteoporotic Fractures was used. A random sample of 1171 women was selected as the subcohort (mean age 80.1 ± 4.2 years) plus the first 300 women with incident hip fracture. Inflammatory markers interleukin-6 (IL-6) and soluble receptors (SR) for IL-6 (IL-6 SR) and tumor necrosis factor (TNF SR1 and TNF SR2) were measured and participants were followed for a median (interquartile range) of 6.3 (3.7, 6.9) years. In multivariable models, the hazard ratio (HR) of hip fracture for women in the highest inflammatory marker level (quartile 4) was 1.64 (95% confidence interval [CI], 1.09-2.48, p trend=0.03) for IL-6 and 2.05 (95% CI, 1.35-3.12, p trend <0.01) for TNF SR1 when compared with women in the lowest level (quartile 1). Among women with 2 and 3-4 inflammatory markers in the highest quartile, the HR of hip fracture was 1.51 (95% CI, 1.07-2.14) and 1.42 (95% CI, 0.87-2.31) compared with women with 0-1 marker(s) in the highest quartile (p trend = 0.03). After individually adjusting for 7 potential mediators, cystatin-C (a biomarker of renal function) and bone mineral density (BMD) attenuated HRs among women with the highest inflammatory burden by 20% and 15%, respectively, suggesting a potential mediating role. Older white women with high inflammatory burden are at increased risk of hip fracture in part due to poor renal function and low BMD.

Key Words: Inflammatory markers, cytokines and cytokine soluble receptors, hip fracture, case-cohort design, older white women

Introduction

Hip fractures contribute the greatest to morbidity and mortality among all osteoporotic fractures.(1) The burden of hip fractures is particularly high among women, and increases exponentially with age. It is estimated that 1 in 6 white women will have a hip fracture in their lifetime.(2) Additionally, women who sustain a hip fracture have a 2-3 fold increased risk of mortality in the first year.(3,4)

The inflammation hypothesis of aging suggests that inflammation plays a major role in the aging process through an increase in vascular permeability, tissue damage, and cell death.(5) Elevated levels of pro-inflammatory markers have also been linked with an increased risk of chronic conditions and death.(6-9) Moreover, pro-inflammatory cytokines interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor-alpha (TNF-α) have been shown to influence bone remodeling, with several in vitro and rodent studies showing their involvement in the pathogenesis of osteoporosis.(10,11) Several longitudinal studies among older women have found an association between high levels of inflammatory makers and increased bone loss.(12-15) Further, Cauley et al. showed that elevated inflammatory markers are a risk factor for incident non-traumatic fractures.(16) We also recently reported on inflammatory markers and risk of hip fracture using data from the Women's Health Initiative (WHI).(17) We found that women with elevated levels of inflammatory markers for all three cytokine-soluble receptors (IL-6 SR, TNF SR1, and TNF SR2) had almost a 3-fold risk of hip fractures.(17) However, BMD was measured on only a subset of WHI women, and thus, we were not able to account for BMD in our analysis. Another limitation of that study was that we used a nested case-control design, and as a result, we were unable to calculate person-time risk. Additionally, our previous studies did not include many women over the age of 80 years, a demographic that has the highest predisposition for hip fracture.

In the current analysis, we address these limitations by examining the prospective association of inflammatory markers on risk of hip fracture in older white women enrolled in the Study of Osteoporotic Fractures (SOF). We hypothesized that this association is mediated through several pathways including BMD and cystatin-C (a biomarker of renal function).

Methods

Study population

From 1986 to 1988, a total of 9704 Caucasian women who were at least 65 years old were recruited for participation in the initial examination of the prospective SOF. Women were recruited from population-based listings in four areas of the United States irrespective of BMD. SOF initially excluded 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.(18)

Of the original cohort, 7008 surviving women provided at least questionnaire data for the Year 10 examination conducted between 1997 and 1998; 1648 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

Our study design was a case-cohort(19) within the cohort of 4709 white women providing serum specimens at the Year 10 (1997-1998) examination. We randomly selected 1171 women out of the 4709 women to serve as the random subcohort (mean age 80.1 ± 4.2 years). We also selected the first 300 women with incident hip fracture during follow-up (146 femoral neck fractures and 150 intertrochanteric fractures), which also included 77 cases from the random subcohort. The final analytic sample consisted of 1339 participants (1124 from the random subcohort) after excluding women without inflammatory marker values. Follow-up for hip fractures occurred every four months from year 10 to year 16 (2002-2004), with more than 95% of follow-up contacts completed; median ((interquartile range (IQR)) follow-up was 6.3 (3.7- 6.9) years. Hip fractures (including the location) were confirmed by review of radiographic reports.

Inflammatory Markers

Fasting morning blood was collected at year 10 (1997-1998) examination and processed for serum which was stored at -70 Celsius until thawed. Cytokines IL-6, and soluble receptors (SR) of IL-6 (IL-6 SR) and TNF-α (TNF SR1 and TNF SR2) were measured with DuoSet ELISA kits (R&D Systems, Minneapolis, MN, USA) at the University of Maryland Cytokine Core Laboratory. The coefficients of variation (CVs) of IL-6, IL-6 SR, TNF SR1, and TNF SR2 were 8%, 4%, 5%, and 6%, respectively, indicating robust reliability.

The complexity and interrelatedness of cytokines involved makes it unlikely that one biomarker would capture the entire inflammatory burden, so high levels of two or more inflammatory markers were considered more likely represent systemic inflammation than a high level of just one inflammatory marker.(20,21) Therefore, the primary predictor was a composite measure of inflammation that combines the number of four cytokines and soluble cytokine receptors in the highest quartile. This composite measure was predefined based on our prior work.(16) Quartile cutoffs for each inflammatory marker were determined from their distribution in the random subcohort, because this group is likely to provide the expected concentration of inflammatory markers in the population that gave rise to the hip fractures cases.

Potential Confounders

All covariates are from the year 10 with the exception of history of rheumatoid arthritis (RA) (year 8). Participants were asked about age, smoking status, health status, prevalent medical conditions including diabetes mellitus, cardiovascular disease (history of myocardial infarction, congestive heart failure, or stroke), RA, and previous fractures since age 50. Use of prescription and non-prescription (e.g., vitamins) medications was obtained by clinic interviewers through a medication inventory review. Participants provided information on supplementation and medication. Information on vitamin D supplements, non-steroidal anti-inflammatory drugs (NSAIDs), steroid use, and selective serotonin reuptake inhibitors (SSRIs) in the past 30 days was ascertained. Information on the use of calcium at least once a week in the last 30 days was obtained. Physical activity has been assessed using a modified version of the Harvard Alumni Questionnaire(22,23) and expressed as a weighted score of kilocalories expended

per week from walking. Weight has been 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).

Potential mediators

We considered several potential mediators in the causal pathway between inflammation and hip fractures. The self-report of prevalence of falls in the year prior to the year 10 visit was ascertained. Physical function was measured using walk speed (time in seconds to walk 6 meters at usual pace expressed as m/s). BMD of the total hip was measured using dual energy x-ray absorptiometry; details of the BMD measurement methods and precision are published elsewhere.(24)

Frailty status was defined using criteria similar to those proposed by Fried and colleagues(25,26) 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. Women with no components of frailty were considered robust, whereas those with one or two components were considered to be pre-frail.

Total 25(OH)D was the sum of measures serum 25(OH)D2 [Intra-assay CVs were 4.4%, 3.3%, and 4.2% at 14, 41, and 124 ng/mL, respectively] and serum 25(OH)D3 [Intra-assay CVs were 3.8%, 2.4%, and 4.7% at 25, 54, and 140 ng/mL, respectively], which were performed at the Mayo Clinic using liquid chromatography-mass spectrometry. Total intact parathyroid hormone (PTH) was measured using an immunoradiometric assay (Scantibodies Laboratory, Inc., Santee, CA) at the Columbia University Laboratory with inter-assay and intra-assay CVs of 8.4% and 5.6%, respectively. Renal function was measured by serum cystatin-C, assayed at the University of Minnesota Medical Center in 2010 using a BN100 nephelometer (Dade Behring Inc., Deerfield, IL) and a particle-enhanced immunonepholometric assay(27) (assay range 0.23-8.00 mg/L with inter-assay CVs 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%]).

Statistical Analysis

Participant characteristics in the random subcohort at baseline were compared by number of inflammatory markers in the highest quartile using tests of trend. Characteristics of women with and without hip fracture were compared using chi-square, t-tests, and non-parametric tests.

The association between inflammatory markers and incident hip fracture was analyzed using proportional hazards regression models modified for the case-cohort sampling design.(19) All multivariable models included seven potential confounders: age, BMI, health, diabetes, prevalent history of fracture, vitamin D supplementation, steroid use, and estrogen use [which were associated with hip fractures at $p<0.10$]. We calculated hazard ratios (HRs) and 95% confidence intervals (CI) across quartiles of individual inflammatory markers with quartile 1 (lowest) serving as the referent group, and performed a test of trend to evaluate potential dose-response relationships.

Women with 2 and 3-4 inflammatory markers in the highest quartile were compared to women with 0 or 1 (referent) inflammatory markers in the highest quartile. To investigate potential mediators by which inflammatory markers might be associated with hip fractures, we added the following 7 potential mediators individually to the MV model: frailty score, physical function (measured using walking speed), fall in past year, cystatin-C, PTH, total 25(OH)D, and BMD. An attenuation of ≥10% in the HR of the group with the highest inflammatory burden (3-4 inflammatory markers in the highest quartile) was used as support for the hypothesis of mediation based on prior published criterion.(28) The percentage reduction in HR was calculated as [(HR MV model –HR mediator model)/(HR MV model)] *100. A test of trend was performed across inflammatory marker groups to determine if there was dose-response relationship with hip fracture. No significant interactions by age (<80 vs ≥80 years) and prior hip fracture were observed. Significance level for all analyses was set at $p<0.05$.

Because only 2 of the 4 inflammatory markers were individually associated with hip fracture a sensitivity analysis was performed using the 2 significant markers (IL-6 and TNF SR1) to create a composite measure of inflammation variable (0, 1, and 2 inflammatory markers in the highest quartile) and examine if the findings differ when limiting analysis to significant markers.

Prior research suggests that femoral neck and intertrochanteric fractures have different risk factors.(29) To examine if this is true for inflammatory markers; a post-hoc analysis was performed to understand their impact on femoral neck and intertrochanteric fractures individually.

An additional post-hoc analysis was performed to determine mortality rates among women following a hip fracture in relation to number of inflammatory markers in the highest quartile. We limited the analytic sample to women in the random cohort, because the selection of the case component of our study was non-random.

Results

A higher number of inflammatory markers in the top quartile was significantly associated with potential confounders older age, greater BMI, lower physical activity, poorer health, being more likely to report diabetes, steroid use, and potential mediators poorer physical function (defined as walking speed), renal function (defined using cystatin-C), greater PTH , and being classified as frail (Table 1).

Women with an incident hip fracture were significantly older, had lower BMI, BMD, and physical function, had greater cystatin-C, and were more likely to report a previous fracture since age 50, steroid use, and be classified as frail compared with pre-frail and robust women (Table 2).

Association of individual inflammatory markers with hip fractures

In multivariable models, women in the highest quartile of IL-6 (HR=1.64; 95% CI=1.09, 2.48) and TNF SR1 (HR=2.05; 95% CI=1.35, 3.12) had a significantly higher risk of hip fractures compared to women in the lowest quartiles of these inflammatory markers (Table 3). Furthermore, there was positive dose-response between IL-6 (p-trend=0.03), and TNF SR1 and hip fractures (p-trend <0.01). There was no association of IL-6 SR and TNF SR2 with risk of incident hip fractures.

High levels of IL-6, IL-6 SR, and TNF SR1 concentrations were significantly associated with increased femoral neck fractures in multivariable models (data not shown). Conversely, no inflammatory markers were individually associated with intertrochanteric fractures.

Inflammatory Burden and hip fracture

In the multivariable model, among women with 2 and 3-4 inflammatory markers in the highest quartile, the HR of hip fracture was 1.51 (95% CI=1.07, 2.14) and 1.42 (95% CI=0.87, 2.31), in comparison with women with 0 or 1 marker in the highest quartile (s) (p trend=0.03) (Table 4). After individually adding 7 potential mediators to the multivariable model, cystatin-C (p-trend=0.24) and total hip BMD (p-trend=0.16) attenuated the HR (in women with 3-4 inflammatory markers in the highest quartile) by 19% and 15%, respectively, suggesting a potential mediating role between inflammation and hip fracture. Frailty, physical function, falls, total 25 (OH)D, and PTH did not meet our mediation criterion, nor did they greatly attenuate the dose-response between inflammation and hip fractures.

In the sensitivity analysis, among women with 1 and 2 inflammatory markers in the highest quartile of IL-6 and TNF SR1, the HR of hip fracture was 1.49 (95% CI=1.10, 2.01) and 1.72 (95% CI=1.05, 2.81), in comparison with women with 0 markers in the highest quartile (p trend=<0.01) (data not shown).

In post-hoc analyses, among women with 2 and 3-4 inflammatory markers in the highest quartile, the HR of femoral neck fracture was 1.73 (95% CI=1.10, 2.73) and 1.87 (95% CI=1.03, 3.41), respectively, in comparison with women with 0 or 1 marker in the highest quartile (s) (p trend=<0.01) in the multivariable model (data not shown). Cystatin-C (15%), physical function (10.2%), and frailty (10.2%) met mediation criterion for femoral neck fractures, in contrast to falls, total 25(OH)D, PTH, and BMD. Conversely, there was no association between number of inflammatory markers in the top quartile and incident intertrochanteric fractures.

Lastly, among women with hip fracture, the mortality rate (per 1000 women year) was 75.3, 147.5, and 177.3 among those with 0-1, 2, and 3-4 inflammatory markers in the highest quartile, respectively.

Discussion

In this prospective case-cohort study, we found that high levels of inflammatory markers were associated with increased risk of hip fracture in older women independent of age, BMI, self-reported health, physical activity, diabetes, history of fracture, vitamin D supplementation, steroid use, and estrogen use. In the mediator analysis, we

showed that this association was due in part to poor renal function and low BMD, as shown by the attenuation in HR among women with the highest number of inflammatory markers in the top quartile.

The 40-50% increased risk of hip fracture among women with 2 or 3-4 inflammatory markers in the top quartile was lower than the over 2.5-fold risk of hip fracture we observed in our prior study.(17) Our sensitivity analysis showed that limiting the analysis to inflammatory markers that were individually associated with hip fracture resulted in a more robust association (73% increased risk of hip fracture) among women with the highest inflammatory burden. Despite the increased risk, the hip fracture risk in women with the greatest inflammatory burden remained substantially larger in the WHI study(17). Perhaps this disparity may be explained by the dissimilar levels of inflammation in these cohorts. More studies in older women are required to determine the true hip fracture predictive capability of inflammatory markers.

In the mediating pathway between inflammation and hip fracture, renal function appears to play a large role. In this study and our prior study(17), controlling for cystatin-C resulted in the largest attenuation of hip fracture risk among women with the highest inflammatory burden; a decrease of 19% and 15%, respectively. High levels of cystatin-C have been shown to increase risk of hip (30-32) and non-vertebral fractures (33) in post-menopausal women. Because factors other than kidney function may affect Cystatin-C levels(34), additional mechanisms may link cystatin-C to hip fractures. For instance, high cystatin-C concentrations might indicate a pathological response to injury.(35) The biological pathway between inflammation, kidney function and hip fractures is complex and may be bi-directional. Longitudinal studies show that greater inflammation can lead to kidney function decline(36-38), and that glomerular injury can be induced directly by TNF-a,(39,40) or mediated by immune cells (i.e, macrophages).(38) Conversely, reduced renal function may result in an increase of inflammatory markers in the blood,(41) through the mechanism of increasing oxidative stress resulting in advanced oxidation protein products, which increase inflammatory marker concentrations.(42) Multiple measurements of inflammatory markers and cystatin-C over time may improve our understanding of this relationship.

To our knowledge this is the first study to examine if the association between inflammatory markers and hip fracture is mediated by BMD. Further adjusting for BMD in our multivariable model reduced the HR among women with the highest inflammatory burden by 15% meeting our mediation criterion of ≥10%. Cytokines TNF-α

and IL-6 have been shown to stimulate osteoclast differentiation in vitro and in vivo. This can be accomplished indirectly through suppression of OPG expression and stimulation of receptor activator of NF-kB (RANK) in mesenchymal cells,(43) and directly by acting synergistically with RANKL.(44) In addition several epidemiological studies show that high concentrations of inflammatory markers are associated with increased bone loss.(12-15) Thus, lowering inflammation may lead to reduced BMD loss and subsequently lower hip fracture rates.

Commensurate with our prior study, (17) frailty, physical function, falls, and serum 25(OH)D did not mediate the association between inflammation and hip fractures. Furthermore, PTH was not a mediator in this study. PTH was strongly correlated with high levels of inflammation at baseline; whereas it was not associated with incident hip fractures, which is consistent with findings from prior longitudinal studies.(45,46)

Similar to our previous findings (17), inflammatory markers individually were significantly associated with hip fractures. For instance, women in the highest quartile of TNF SR1 had an over 2-fold risk of hip fractures compared to women in the lowest quartile. Similarly, women in the top IL-6 quartile were 65% more likely to have a hip fracture compared to women in the lowest quartile. Although, a composite measure of the burden of inflammation is ideal, our findings suggest that having one elevated inflammatory marker concentration may also increase an older woman's risk of hip fracture.

To our knowledge this is the first study to examine the role of inflammatory markers on different types of hip fracture (femoral neck vs. intertrochanteric). The effect of inflammation on hip fractures differed by hip fracture type. Women with 3-4 markers in the top quartile had an almost 2 fold increased risk of femoral neck fracture, whereas the effect on intertrochanteric fractures was null. High levels of inflammation increased the risk of femoral neck fractures independent of BMD, which surprisingly did not mediate this association. Cystatin-C, physical function, and frailty mediated the association between inflammatory markers and femoral neck fracture. This finding is consistent with prior research that shows that hip fracture etiology may vary by hip fracture type. For instance, femoral neck fractures were largely predicted by low BMD, poor function, and steroid use, whereas aging and poor health status were factors that were more linked with intertrochanteric fractures using women from the same cohort.(29) More studies are needed to determine if these findings can be replicated.

Among women with a hip fracture, the mortality rate was highest among those with 3-4 markers in the top quartile. The combination of both high levels of inflammation and hip fracture leading to a high mortality risk is not surprising given the link between elevated inflammatory marker levels and mortality.(8) Targeting this high risk population for intervention may reduce their mortality rate.

Our study had several strengths. We examined multiple markers of inflammation in relation to incident hip fractures (the most serious consequence of osteoporosis) in a high risk population (white women with a mean age of 80 years at baseline). All hip fractures were confirmed objectively by review of radiographic reports and reliability of inflammatory marker measurements was robust. We accounted for many potential confounders and explored several important mechanisms (i.e., kidney function and BMD) of mediation. Our study was subject to some limitations. First, our findings are not generalizable to nonwhite women or men. Second, we measured inflammatory marker concentrations in the serum; and these levels may differ in the bone microenvironment and over time. Third, not all potential confounders or mediators were measured objectively, with some variables measured through self-report (i.e., physical activity, falls in the past year, and health status) which may lead to recall and social desirability bias. Fourth, accounting for rates of bone loss in the analysis prior to hip fracture or right censoring may have improved the understanding of our hypothesis of mediation; however this type of analysis was significantly underpowered in this cohort. Finally, residual confounding due to unmeasured factors is a component of all observational studies. Oei L et al. recently showed that Mendelian randomization analyses did not yield evidence for a causal relationship between serum high-sensitivity C-reactive protein (CRP) and increased hip fracture risk, and that is association may be explained by untested confounding factors. (47)

In summary, inflammatory markers may be important biologic factors in fracture etiology, particularly for hip fractures, with studies showing consistent findings across different cohorts. Further, the association between inflammatory burden and hip fractures was due in part to poor renal function and low BMD. Lowering inflammation may be an effective approach to reducing hip fracture risk in older women.

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Disclosure

The authors state that they have no conflicts of interest.

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Tables

Table 1: Characteristics of 1124 women in the random subcohort by number of 4 cytokines and soluble cytokine receptors in the highest quartile¶

Characteristic	Number of cytokines and soluble cytokine receptors in the highest quartile			
	0-1 (N=809)	2 (N=212)	3-4 (N=103)	p-trend
Potential confounders				
Age (years), mean (SD)	79.7 (4.0)	80.7 (4.5)	81.7 (4.8)	<0.01
BMI (kg/m^2), mean (SD)	26.0 (4.5)	27.2 (4.9)	28.1 (5.5)	<0.01
Physical activity (kcal/week), mean (SD)	608 (643)	516 (631)	369 (400)	<0.01
Current smoker, %	4.2%	1.9%	2.9%	0.20
Health status, excellent/good, %	83.6%	77.4%	65.7%	<0.01
Diabetes, %	3.2%	7.6%	6.9%	<0.01
Cardiovascular disease, %	10.1%	11.3%	17.7%	0.04
RA, %	4.3%	6.1%	5.9%	0.28
Previous fracture since age 50, %	51.1%	52.6%	65.1%	0.02
Calcium use at least once a week in past 30 days, %	55.4%	53.8%	46.6%	0.12
Vitamin D supplementation in past 30 days, %	59.3%	55.7%	46.6%	0.01
NSAID use in past 30 days, %	23.4%	18.4%	23.3%	0.46
SSRI use in past 30 days, %	4.3%	4.3%	6.8%	0.37
Steroid use in the past 30 days, %	4.3%	5.2%	11.7%	<0.01
Estrogen use in the past 30 days, %	20.9%	20.3%	13.6%	0.13
Bisphosphonate use in the past 30 days, %	5.9%	6.1%	2.9%	0.34
Potential mediators				
Frailty status				
Robust	31.8%	22.2%	19.6%	<0.01
Pre-frail	56.5%	56.6%	53.9%	
Frail	11.7%	21.2%	26.5%	
Fall in past year, %	30.9%	35.4%	35.3%	0.19
Total hip BMD (g/cm^2), mean (SD)	0.72 (0.13)	0.74 (0.14)	0.70 (0.15)	0.13
Physical function[§], m/s, mean (SD)	0.93 (0.21)	0.84 (0.21)	0.80 (0.24)	<0.01
Renal function (cystatin-C), mg/L, mean (SD))	0.94 (0.18)	1.11 (0.24)	1.43 (0.48)	<0.01
Total 25(OH)D, ng/ml, mean (SD)	25.0 (9.4)	25.3 (12.5)	26.0 (10.6)	0.62
PTH, pg/ml, mean (SD)	34.0 (17.2)	37.8 (20.9)	46.6 (31.8)	<0.01

Abbreviations: BMI, body mass index; RA, rheumatoid arthritis; NSAID use, non-steroidal anti-inflammatory medications; BMD, bone mineral density; PTH, parathyroid hormone

¶Quartile cutpoints were determined from distribution of inflammatory markers among women in the random subcohort

§Defined as walking speed

Table 2: Characteristics of 1339 women with and without incident hip fracture

Characteristic	Hip Fracture Cases (N=288)	Non-cases (N=1051)	P-value
Potential confounders			
Age (years), mean (SD)	81.9 (4.5)	80.0 (4.2)	<0.01
Physical activity (kcal/week), mean (SD)	511 (545)	578 (631)	0.08
Current smoker, %	3.8%	3.6%	0.86
Health status, excellent/good, %	76.0%	81.1%	0.05
Diabetes, %	5.6%	4.4%	0.39
Cardiovascular disease, %	11.2%	11.2%	0.97
RA, %	5.2%	4.8%	0.75
Previous fracture since age 50, %	69.4%	51.3%	<0.01
Calcium use at least once a week in past 30 days, %	55.2%	54.2%	0.77
Vitamin D supplementation in past 30 days, %	62.9%	56.9%	0.07
NSAID use in past 30 days, %	17.4%	22.7%	0.05
SSRI use in past 30 days, %	6.9%	4.3%	0.06
Steroid use in the past 30 days, %	8.7%	5.1%	0.02
Estrogen use in the past 30 days, %	20.6%	16.0%	0.08
Bisphosphonate use in the past 30 days, %	5.7%	5.2%	0.74
Potential mediators			
Frailty status			
Robust	21.2%	29.4%	<0.01
Pre-frail	57.6%	55.9%	
Frail	21.2%	14.7%	
Fall in past year, %	37.8%	32.1%	0.07
Total hip BMD (g/cm^2), mean (SD)	0.63 (0.11)	0.73 (0.13)	<0.01
Physical function[§], m/s, mean (SD)	0.82 (0.21)	0.91 (0.21)	<0.01
Renal function (cystatin-C), mg/L, mean (SD)	1.08 (0.35)	1.01 (0.26)	<0.01
Total 25(OH)D, ng/ml, mean (SD)	26.4 (12.6)	25.1 (10.2)	0.11
PTH, pg/ml, mean (SD)	36.8 (32.1)	35.6 (19.6)	0.53

Abbreviations: BMI, body mass index; RA, rheumatoid arthritis; NSAID use, non-steroidal anti-inflammatory medications; BMD, bone mineral density; PTH, parathyroid hormone

[§]Defined as walking speed

Table 3: Hazard ratios (95% CI's) of hip fracture according to quartiles of individual cytokine and soluble cytokine receptors¶

	Q1	Q2	Q3	Q4	P-trend
IL-6					
Unadjusted	Ref	1.47 (1.00, 2.15)	1.44 (0.98, 2.10)	1.80 (1.24, 2.61)	<0.01
MV model*	Ref	1.39 (0.92, 2.09)	1.32 (0.87, 2.01)	1.64 (1.09, 2.48)	0.03
IL-6 SR					
Unadjusted	Ref	1.16 (0.80, 1.70)	1.31 (0.90, 1.90)	1.47 (1.02, 2.13)	0.03
MV model*	Ref	1.39 (0.92, 2.10)	1.42 (0.95, 2.13)	1.43 (0.95, 2.14)	0.10
TNF SR1					
Unadjusted	Ref	1.34 (0.91, 1.97)	1.34 (0.91, 1.97)	2.06 (1.42, 2.97)	<0.01
MV model*	Ref	1.56 (1.03, 2.38)	1.50 (0.98, 2.30)	2.05 (1.35, 3.12)	<0.01
TNF SR2					
Unadjusted	Ref	1.16 (0.80, 1.69)	1.31 (0.91, 1.89)	1.32 (0.91, 1.90)	0.11
MV model*	Ref	1.14 (0.77, 1.70)	1.21 (0.81, 1.80)	1.18 (0.78, 1.79)	0.42

¶ Quartile cutpoints were determined from distribution of inflammatory markers among women in the random subcohort
*Multivariate models controlled for age, BMI, self-reported health, diabetes, prevalent history of fracture, vitamin D, steroid use, and estrogen use.
IL-6 quartile cutoffs (pg/ml) are 1.2, 2.5, and 4.0; IL-6 SR quartile cutoffs (pg/ml) are 33773.7, 42705.7 and 53625.0. TNF SR1 quartile cutoffs (pg/ml) are 2572.5, 3160.7, and 4037.5; TNF SR2 quartile cutoffs (pg/ml) are 8486.5, 11198.3, and 14530.6.

Table 4. Hazard ratios (95% CIs) of hip fracture, by number of high[¶] inflammatory markers

	0-1	2	3-4	P trend
Crude analysis	ref	1.49 (1.08, 2.04)	1.85 (1.24, 2.76)	<0.01
MV model[†]	ref	1.51 (1.07, 2.14)	1.42 (0.87, 2.31)	0.03
MV model[†] + frailty	ref	1.49 (1.05, 2.11)	1.36 (0.83, 2.22)	0.05
MV model[†] + physical function	ref	1.41 (0.98, 2.02)	1.31 (0.80, 2.17)	0.11
MV model[†] + falls	ref	1.51 (1.07, 2.14)	1.38 (0.84, 2.26)	0.04
MV model[†] + renal function (cystatin-C)	ref	1.43 (1.00, 2.03)	1.15 (0.64, 2.08)	0.23
MV model[†] + total 25(OH)D	ref	1.49 (1.05, 2.11)	1.32 (0.80, 2.18)	0.07
MV model[†] + PTH	ref	1.52 (1.07, 2.15)	1.42 (0.87, 2.32)	0.03
MV model[†] + BMD	ref	1.43 (0.99, 2.07)	1.21 (0.73, 2.01)	0.17

[¶] Number of inflammatory markers in the highest quartile according to the distribution of inflammatory markers among women in the random subcohort

[†] MV model controlled for age, BMI, self-reported health, diabetes, prevalent history of fracture, vitamin D, steroid use, and estrogen use.