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Inflammatory Markers and the Risk of Hip and Vertebral Fractures in Men: the Osteoporotic Fractures in Men (MrOS)

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Abstract

Cytokines play major roles in regulating bone remodeling, but their relationship to incident fractures in older men is uncertain. We tested the hypothesis that men with higher concentrations of pro-inflammatory markers have a higher risk of fracture. We used a case-cohort design and measured inflammatory markers in a random sample of 961 men and in men with incident fractures including 120 clinical vertebral, 117 hip, and 577 non-spine fractures; average follow-up 6.13 years (7.88 years for vertebral fractures). We measured interleukin (IL)-6, C-reactive protein (CRP), tumor necrosis factor alpha (TNF α), soluble receptors (SR) of IL-6 (IL-6SR) and TNF (TNF α .SR1 and TNF α .SR2), and IL-10. The risk of non-spine, hip, and clinical vertebral fracture was compared across quartiles (Q) of inflammatory markers using Cox proportional hazard models with tests for linear trend. In multivariable-adjusted models, men with the highest (Q4) TNF α cytokine concentrations and their receptors had a 2.0–4.2-fold higher risk of hip and clinical vertebral fracture than men with the lowest (Q1). Results were similar for all non-spine fractures, but associations were smaller. There was no association between CRP and IL-6SR and fracture.

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Disclosures

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Additional Supporting Information may be found in the online version of this article.

Men in the highest Q of IL-10 had a 49% lower risk of vertebral fracture compared with men in Q1. Among men with 3 inflammatory markers in the highest Q, the hazard ratio (HR) for hip fractures was 2.03 (95% confidence interval [CI] 1.11–3.71) and for vertebral fracture 3.06 (1.66–5.63). The HRs for hip fracture were attenuated by 27%, 27%, and 15%, respectively, after adjusting for appendicular lean mass (ALM), disability, and bone density, suggesting mediating roles. ALM also attenuated the HR for vertebral fractures by 10%. There was no association between inflammation and rate of hip BMD loss. We conclude that inflammation may play an important role in the etiology of fractures in older men.

Keywords

CYTOKINES; AGING; OSTEOPOROSIS; GENERAL POPULATION STUDIES; FRACTURE RISK ASSESSMENT

Introduction

Cytokines play a major role in bone remodeling, with several in vitro and rodent studies showing the involvement of inflammatory markers in the pathogenesis of osteoporosis.^(1–3) Most data focus on the influence of immune cells on osteoclast-mediated bone resorption. However, pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF α) can also lead to increased bone resorption by stimulating osteoblast production of pro-inflammatory cytokines and receptor activator of nuclear factor-kappa B ligand (RANKL).⁽⁴⁾ Thus, chronic inflammation favors bone resorption by disrupting the balance of activity of both the osteoclasts and osteoblasts. In humans, elevated pro-inflammatory cytokines have been linked to an increased risk of fracture.^(5–8) and bone loss.^(9–11) However, there is a paucity of data on inflammatory markers and fractures in older men. Most studies were carried out in older women. Two studies that included men did not show sex-specific models.^(5,12) To our knowledge, a single study examined the association between C-reactive protein (CRP) and non-spine fractures and reported that men in the upper CRP tertile had an 80% higher risk of fracture that was independent of bone mineral density (BMD).⁽¹³⁾ This study was limited to a single inflammatory marker and a single fracture outcome.

The aim of the current analysis was to test the hypothesis that older men with higher pro-inflammatory markers and inflammatory burden are at an increased risk of hip, clinical vertebral, and all clinical non-spine fractures in a dose-response manner and that these men will also experience faster rates of hip bone loss.

Materials and Methods

Study population

In 2000 to 2002, 5994 men enrolled in the Osteoporotic Fractures in Men Study (MrOS), a longitudinal cohort study designed to determine risk factors for osteoporosis, fractures, and falls in six US clinical centers.⁽¹⁴⁾ Men were recruited primarily through mass mailings targeted to age-eligible men.⁽¹⁵⁾ The MrOS study has been described in more detail previously.⁽¹⁴⁾ Briefly, all men were ≥ 65 years, able to walk independently, and did not

report bilateral hip replacements; 35% of men were aged 65 to 69 years, and 11% were aged 80 years or older at baseline. Written informed consent was obtained from each participant, and the protocol was approved by the institutional review boards at each institution.

Case-cohort design

Our study design was a case cohort,⁽¹⁶⁾ nested within the prospective cohort of 5994 men recruited into MrOS. Men with at least five 1-mL aliquots of archived, unfrozen serum were eligible for inclusion. We randomly selected a subcohort of 980 men; inflammation data were missing on 6 men, leaving 974 in the analysis. We chose all cases of non-spine fracture ($n = 577$) including 117 hip fractures. We also identified 120 men with a clinical vertebral fracture.

Fracture outcomes

Participants were contacted by mail every 4 months after baseline (>97% of follow-up contacts completed) and were queried about fractures. All fractures were confirmed by radiographic report. In the case of a self-reported vertebral fracture, a copy of the community spinal imaging study (X-rays, CT, and/or MRI studies) was obtained. Clinical vertebral fractures were confirmed by the study radiologist who used a semiquantitative method⁽¹⁷⁾ to establish that the community imaging study showed a new deformity of higher grade than was present in the same vertebra on the baseline study film. We performed three different sets of analyses on each fracture outcome. Individuals with multiple fractures were only counted once within each outcome. The average follow-up for non-spine fractures was 6.96 years and for vertebral fractures, 7.88 years.

Inflammatory markers

Fasting morning blood samples were obtained at the baseline visit and then processed and stored centrally at -80°C until assay. All cytokine assays were performed at the Laboratory for Clinical Biochemistry Research (LCBR), University of Vermont, under the direction of Dr Russell Tracy.

Interleukin (IL)-6 was measured using a high-sensitivity ELISA from R&D Systems (Minneapolis, MN, USA) employing a quantitative sandwich enzyme immunoassay technique. The assay range is 0.16 to 12.0 pg/mL with interassay coefficients of variation (CVs) ranging from 6.11% to 8.47%. Expected values for IL-6 in normal, healthy individuals are <10 pg/mL.

IL-6 soluble receptors (SR), TNF α .SR1, and TNF α .SR2 were measured using an ELISA from R&D Systems. A monoclonal antibody specific for each cytokine receptor was coated on the assay plate and a polyclonal anti-cytokine receptor antibody was used as the sandwich antibody; the amount of cytokine receptor was then determined by colorimetric reaction. The assay range for IL-6SR was 3120 to 200,000 pg/mL. The manufacturer's normal range for IL-6 is approximately 15,000 to 46,000 pg/mL with interassay CVs ranging from 4.68% to 8.83%. The assay range for TNF α SR1 and SR2 is 78 to 6000 pg/mL with interassay CVs ranging from 5.42% to 8.59% for TNF α .SR1 and 2.87% to 3.54% for TNF α .SR2.

IL-10 and TNF α were measured using the Human Serum CVD3 Multiplex Kit from Millipore Corp. (Billerica, MA, USA), using flow cytometry on the Bio-Rad Bioplex 200 Luminex instrument. The assay range for IL-10 is 0.13 to 2000 pg/mL with interassay CVs ranging from 4.94% to 10.66%. The TNF α assay range is 0.13 to 2000 pg/mL with interassay CVs ranging from 4.93% to 9.13%.

CRP was measured using the BNII nephelometer from Dade Behring utilizing a particle-enhanced immunonephelometric assay. The assay range is 0.16 to 1100 μ g/mL. Expected values for CRP in normal, healthy individuals are 3 μ g/mL. Interassay CVs ranged from 1.52% to 3.68%.

The complexity and interrelatedness of human cytokines make it unlikely that one biomarker would capture an individual's entire inflammatory burden. Therefore, a composite inflammatory burden score was considered more likely to represent systemic inflammation than a high concentration of just one inflammatory marker.^(5,18) Participants were assigned a pro-inflammatory burden score—a composite variable summing the number of pro-inflammatory markers (IL-6, IL-6 SR, TNF α , TNF α -SR1, TNF α -SR2, CRP) that were in the highest quartile. In sensitivity analyses, we created a score based on TNF α , TNF α -SR1, and TNF α -SR2. This methodology is not unprecedented; previous studies have found relationships between inflammatory burden scores and fracture in older women.^(6,7,19)

Covariates

Height was measured by Harpenden stadiometer and weight was measured by digital or balance-beam scale. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

A self-administered questionnaire was used to collect information on demographics, education, self-rated health status, medical history (Parkinson's disease, congestive heart failure [CHF], chronic obstructive pulmonary disease [COPD], heart attack, hypertension, diabetes, stroke, osteoarthritis, and rheumatoid arthritis [RA]), tobacco use (current/past smoking status), and alcohol consumption (drinks per week). At baseline, calcium and vitamin D intake and supplement use were captured using a modified Block Food-Frequency Questionnaire. Participants were asked to bring all prescription medications they had been taking for at least the most recent month to the clinic visit. All medications were entered into an electronic database, verified by pill bottle examination, and each medication was matched to its ingredients based on the Iowa Drug Information Service (IDIS) Drug Vocabulary (College of Pharmacy, University of Iowa, Iowa City, IA, USA).⁽²⁰⁾ Physical activity was quantified using the Physical Activity Scale for the Elderly (PASE).⁽²¹⁾

Potential mediators

We considered several potential mediators in the causal pathway between inflammation and fractures. Physical function was assessed using gait speed. Gait speed was measured in meters per second on a standard 6-m walk course. Men also reported limitations in three instrumental activities of daily living (IADLs) (meal preparations, shopping, and housework) and two physical tasks (walking 2 to 3 blocks and climbing 10 stairs). We analyzed the number of men with >1 IADL difficult versus none.

We also measured hip BMD and appendicular lean mass (ALM) by dual-energy X-ray absorptiometry (DXA) and evaluated them as potential mediators. Participants had hip DXA scans completed on Hologic 4500 scanners (Hologic, Waltham, MA, USA) as previously described.⁽²²⁾ Briefly, centralized quality-control procedures, certification of DXA operators, and standardized procedures for scanning were used to ensure reproducibility of DXA measurements. Each clinic scanned a spine and hip phantom throughout the study to monitor longitudinal changes in measures of BMD, and correction factors were applied to participant data as appropriate. To adjust for inter-clinic differences, statistical models include indicator variables for clinical center. The precision of DXA scans of the spine and hip is 1% to 2% in clinical settings;⁽²³⁾ the CV of the MrOS DXA scanners estimated using a central phantom ranged from 0.3% to 0.7% for the total hip.

Longitudinal analysis of hip BMD was carried out in the random subcohort. Annualized rate (%) of total hip and femoral neck BMD change were calculated comparing baseline scans (2000–2002) and visit 2 (2002–2005), an average of 4.6 years apart. Whole-body DXA scans were used to measure lean mass in the extremities, ALM.

Statistical analysis

We calculated descriptive statistics for all variables and compared participants by level of pro-inflammatory burden score. For this, we used chi-square test (or Fisher's exact test for low expected cell counts) for categorical variables, one-way ANOVA test for normally distributed continuous data, and Kruskal-Wallis tests for nonparametrically distributed data. Next, we compared mean or median concentrations of inflammatory markers by incident fracture status. For this, we used a two-sample *t* test for normally distributed continuous data and Wilcoxon-Mann-Whitney test for skewed continuous data.

The association between inflammatory markers and fracture was analyzed using proportional hazards regression models modified for the case-cohort sampling design.⁽¹⁶⁾ Our analytic approach was defined a priori in an analysis plan. We used quartiles of inflammatory markers because there are not well-established clinical cutpoints for cytokines. Quartile cut-offs for each inflammatory marker were determined from the distribution in the random subcohort because this group is likely to have concentrations of inflammatory markers representative of the general population. We calculated hazard ratios (HR) and 95% confidence intervals (CI) across quartiles of individual inflammatory markers with quartile 1 (lowest) serving as the referent group. We performed a test for trend to evaluate potential dose-response relationships. Base models adjusted for age, clinic site, and race. Multivariable models included the base models plus height, weight, medication use (corticosteroids, nonsteroidal anti-inflammatory drugs [NSAIDs], and/or statins), self-reported comorbidities (CHF, COPD, hypertension, diabetes, stroke, osteoarthritis), health status, smoking status, and total calcium and vitamin D intake. We also compared risk of fracture in men with 0 (referent), 1, 2, and 3 inflammatory markers in the highest quartile with test for trends. As a sensitivity analysis and for comparison with the quartile comparisons, we also calculated continuous inflammatory markers HRs and 95% CI for one standard deviation (SD) increase in the inflammatory marker. Because of skewed distributions, we transformed TNF α , IL-10, and CRP using a logarithm scale base 2 and

calculated the HR per doubling of the inflammatory marker. These results are reported in Supplemental Table S2.

To adjust for multiple comparisons, we calculated *p* values corresponding to a 5% false discovery rate (FDR) for each outcome using the quartile predictor models.⁽²⁴⁾ We considered several potential mediators in the causal pathway between inflammatory burden and fracture including ALM, difficulty with 1 IADL, total hip BMD, and physical function as assessed by gait speed. An attenuation of 10% in the HR of the group with the highest inflammatory burden (three to six inflammatory markers in the highest quartile) was used to support the hypothesis of mediation based on prior published criterion.⁽²⁵⁾ The percentage reduction in HR was calculated as $[(\text{HR Multivariable model} - \text{HR mediator model}) / (\text{HR multivariable model} - 1)] * 100$.

Linear regression was used to study longitudinal changes in BMD. Least square means of annualized changes in hip BMD were determined across the inflammatory burden index adjusting for the same set of covariates as those used in the adjusted multivariable fracture model.

Results

Generally, the characteristics of men with three or more pro-inflammatory markers in the top quartile (23% of the random cohort) suggested a higher risk of fracture. These men were older, more likely to be white, heavier, drank less alcohol, were less physically active, were less likely to report good/excellent health, were more likely to be current smokers, and had lower ALM (Table 1). They also were more likely to report at least one IADL as difficult, as well as COPD, hypertension, myocardial infarction, CHF, diabetes, and stroke. Their mean total dietary calcium and vitamin D intake was, however, higher than men with no pro-inflammatory markers in the highest quartile (36% of the random subcohort). There was no difference in falls, BMD, fracture history, self-reported rheumatoid arthritis, and use of NSAIDs, statins, or corticosteroids across the number of “high” cytokines. Descriptive characteristics of men by fracture status are presented in Supplemental Table S1.

Fracture

Men who experienced a hip fracture compared with those without a hip fracture had statistically significant higher mean concentrations of IL-6, TNF α , and TNF α SRs but had similar concentrations of IL-6SR, CRP, and IL-10 (Table 2). Men with an incident clinical vertebral fracture had higher IL-6, IL-6SR, TNF α , TNF α SR1, and TNF α SR2, but there was no difference between groups in CRP. Men who experienced an incident vertebral fracture had a 19% lower median IL-10 than men without a vertebral fracture. Compared with men who did not experience an incident non-spine fracture, those who had any incident non-spine fracture had higher mean concentrations of TNF soluble receptors but there was no difference in other pro-inflammatory cytokines, CRP or IL-10.

The models showing the HR of fracture for increases in each inflammatory marker treated as a continuous measure are summarized in Supplemental Table S2. In multivariable-adjusted models, a doubling of TNF α was associated with about a 60% increase in hip fracture, but

there was no association between other inflammatory markers and hip fractures. All of the proinflammatory markers, except CRP, were significantly associated with an increased risk of clinical vertebral fractures with HR ranging from 1.31 to 1.75. A doubling of IL-10 was associated with a 20% decreased risk of clinical vertebral fractures in continuous models. For all non-spine fractures, one SD increase in TNF α SR2 was associated with a 12% increase in fracture risk, but there was no association with other markers.

Examination of a dose-response relationship between increasing quartiles of individual inflammatory markers and hip fracture showed a significant dose response for TNF α , TNF α SR1, and TNF α SR2 and hip fracture risk (Table 3). Men in the highest quartile of TNF α or TNF α SRs had a 2.1- to 2.9-fold increased risk of hip fracture compared with men in the lowest quartile. These associations were independent of many important covariates including medications, other chronic diseases, and general health status. Additional adjustment for total hip BMD yielded similar trends (Supplemental Table S3). There was no dose-response association across quartiles of IL-6, IL-6SR, CRP, and IL-10 and hip fracture.

The dose-response association between quartiles of inflammatory markers and clinical vertebral fracture was consistent across individual markers with positive associations with TNF α , TNF α SR1, and TNF α SR2 with HR in the highest versus lowest quartile ranging from 2.87 to 4.16. Additional adjustment for total hip BMD yielded similar findings (Supplemental Table S3). The association with IL-6 was attenuated and no longer statistically significant in the multivariable model. In contrast, men in the highest quartile of IL-10 had a 49% lower risk of experiencing a vertebral fracture compared with men in the lowest quartile. Additional adjustment for BMD had no effect on this result. There was no association between IL6SR or CRP and vertebral fractures.

Similar findings were observed for all non-spine fractures. Men with the highest TNF α SRs 1 and 2 had a 46% to 68% greater risk of non-spine fracture compared with men with the lowest. Adjustment for total hip BMD attenuated the association between TNF α and non-spine fracture Supplemental Table S3). There was no association of non-spine fractures across quartiles of other inflammation markers.

In models adjusting for multiple comparisons using FDR, to be significant, the p values for hip fracture had to be $p < 0.021$; clinical vertebral fracture, $p < 0.029$; and non-spine fractures, $p < 0.014$.

Inflammatory burden

Men with three or more (of six) pro-inflammatory markers had a 1.4 to 3.15 increased risk of fracture (Fig. 1). Limiting our analysis to TNF α , TNF α -SR1, and TNF α -SR2 yielded similar results.

The associations between the number of “high” pro-inflammatory cytokines, fracture, and tests of potential mediating factors are shown in Table 4. Men with three or more high inflammatory cytokines had a twofold increased risk (HR = 2.03 [1.11–3.71] of hip fracture, p trend = 0.06). However, additional adjustment of the multivariable model with ALM (p trend = 0.16), 1 IADL difficult (p trend = 0.20), and hip BMD (p trend = 0.17) attenuated

these trends. Adjustment for ALM, IADL difficulty, and BMD attenuated the HR (in men with three or more inflammatory markers in the highest quartile) by 27%, 27%, and 15%, respectively. There was no attenuation in the risk of hip fracture in models adjusting for gait speed.

Men with three or more “high” inflammatory cytokines had greater than a threefold increased risk of clinical vertebral fractures compared with men with no “high” cytokines (p trend = 0.0006). Further adjustment for ALM attenuated this HR by 10% meeting our criteria for mediation. Adjustment for IADL difficulty, BMD, or gait speed had no effect on the association between increasing number of “high” cytokines and clinical vertebral fracture.

There was a trend between increasing number of “high” cytokines and non-spine fractures in the base model (p trend = 0.02), but this association was attenuated in the multivariable model (p trend = 0.11). Further adjustment for ALM, IADL difficulty, BMD, and gait speed had little effect on these results between non-spine fracture and inflammatory burden.

Rates of bone loss

Although the average annualized rate of bone loss at the total hip and femoral neck was numerically higher in men with the greatest inflammatory burden, the tests for trend did not reach statistical significance (Supplemental Table S4).

Discussion

In this case-cohort study, men with TNF cytokine concentrations in the highest quartile including both TNF soluble receptors had a >twofold higher risk of hip and clinical vertebral fractures, two of the most common and clinically important osteoporotic fractures, compared with men in the lowest quartile for these cytokines. In addition, when compared with men in the lowest quartile, men with the highest TNF cytokines had about a 40% to 60% increased risk of all non-spine fractures. These associations were independent of many important covariates including height, weight, smoking, general health status, and several comorbid conditions including both COPD and cardiovascular disease. Additional adjustment for BMD yielded similar findings for hip and clinical vertebral fractures. These results extend our previous findings in women to men.^(5–8)

High concentrations of multiple cytokines likely represent a more specific indicator of systemic inflammation than a high concentration of just one inflammatory marker. Thus, we examined a composite inflammation index. Men with three or more pro-inflammatory markers had a two- to threefold increased risk of hip and clinical vertebral fracture, respectively, and a 30% increased risk of any non-spine fracture. A sensitivity analysis limiting our composite index to TNF α and TNF α SR1 and 2 showed similar results. The magnitude of the association between inflammatory burden and hip and non-spine fracture was similar to that previously reported in women.^(5–8)

To our knowledge, our study is the first to examine the association between individual cytokines, our composite inflammation index, and clinical vertebral fractures. In continuous

models, all pro-inflammatory markers except CRP were associated with an increased risk of clinical vertebral fracture. This association was particularly strong, showing a greater than threefold increased risk in men with the highest inflammatory burden and highest TNF cytokines. Perhaps this reflects the greater proportion of trabecular bone in the spine and the greater bone turnover in trabecular bone. TNF α has been shown to have direct effects on bone turnover. TNF α stimulates osteoclast differentiation in vitro and in vivo.^(26,27) This can be accomplished indirectly through suppression of osteoprotegerin expression and stimulation of RANKL in mesenchymal cells.⁽²⁸⁾ TNF α also has been shown to activate osteoclast precursors directly by acting synergistically with RANKL. This mechanism may lead to increased risks of fracture at trabecular bone-rich sites.

Although mean IL-6 concentrations were higher in men with an incident hip and clinical vertebral fracture and risk of these fractures were significantly higher among men in the highest versus the lowest quartiles in minimally adjusted models, these associations were attenuated after further covariate adjustment. We found no association between IL-6SR and fractures. It is not clear why we found weaker or no relationship between IL-6 or IL-6SR and fracture because IL-6 is also a multifunctional cytokine that has a number of effects on bone.⁽²⁹⁾ IL-6 but not IL-6SR was associated with an increased risk of hip fracture in the Study of Osteoporotic Fractures (SOF).⁽⁷⁾ Nevertheless, in the Women's Health Initiative (WHI), the risk of hip fracture was 43% higher among participants in the highest IL-6SR quartile, but it was not statistically significant.⁽⁶⁾ The association between IL-6 and fractures also was not significant in the Health ABC study.⁽⁵⁾

We tested for several mediators of the association between inflammation and fracture. Elevated cytokines have been associated with loss of muscle mass,⁽³⁰⁾ poor mobility,⁽³¹⁾ and disability.⁽³²⁾ In our study, men with three or more cytokines in the top quartile had slower gait speed. Nevertheless, addition of gait speed to the multivariable model had no effect on results. On the other hand, a self-reported indicator of disability (≥ 1 IADL difficult) attenuated the association between high inflammatory burdened and hip fractures, suggesting that effects of inflammation on disability may contribute to their association with hip fracture. We previously showed in women enrolled in SOF that BMD was an important mediator between inflammatory burden and hip fracture.⁽⁷⁾ In SOF, addition of total hip BMD to the multivariable model attenuated the association by 50% such that it was no longer significant. We found about 10% of mediation by BMD for hip fracture models; the association was no longer significant. Lack of mediation by BMD for vertebral fractures may suggest alternate pathways for different fracture sites whereby inflammation may influence fractures.

Appendicular lean mass attenuated the effect of inflammation on hip and vertebral fracture, suggesting that the effects of inflammatory markers on muscle mass contribute to their association with fracture. The age-related dysregulation of immune function may have direct adverse consequences on physical function and promote disability by causing fatigue and loss of muscle strength.^(33–35) In aging, IL-6 is thought to have a direct impact on fatigue and loss of muscle function. Injection of IL-6 produced acute and chronic atrophy of skeletal muscle in rats.⁽³⁶⁾ Exposure of skeletal muscle to TNF α led to a loss of total muscle protein

that is in part mediated by reactive oxygen species.^(37,38) Thus, the association between inflammation and fracture could be because of direct effects of inflammation on ALM.

Adipose tissue is an active endocrine tissue.⁽³⁹⁾ Obese individuals have been shown to have higher inflammatory markers.⁽⁴⁰⁾ In particular, visceral adipose tissue appears to release IL-6 in amounts two to three times greater than the subcutaneous compartment. We adjusted for BMI, but future research should explore whether visceral adipose tissue mediates the association between cytokines and fracture.^(41,42)

Previous studies have shown associations between IL-6 and bone loss in women within 10 years of menopause, a time when bone loss is quite rapid,⁽¹¹⁾ but there was no association in older women. Similarly, IL-6 and TNF α were associated with 1-year change in BMD, but the average age of these women was 54 years.⁽¹⁰⁾ In a combined sample of men and women, IL-6 was associated with faster rates of bone loss, but the authors did not show models separately for men.⁽⁹⁾ We found no association between inflammation and rates of bone loss in our men whose average age was about 74 years. Perhaps this relates to an overall slower rate of bone loss in these older men compared with newly menopausal women.

IL-10 is a potent immunosuppressant.⁽⁴³⁾ Experimental studies have confirmed the anti-inflammatory effect of IL-10.⁽⁴⁴⁾ IL-10-deficient mice had significantly lower total bone mass, cancellous bone mass, cortical bone mass, less trabecular bone area, fewer trabecular number, and lower trabecular surface and width than age-matched wild-type mice. Trabecular separation was also higher in the IL-10-deficient mice.⁽⁴⁵⁾ These data suggest that circulating IL-10 could prevent fractures. We found that men with higher IL-10 concentrations had a significantly lower risk (20%) of clinical vertebral fractures for every doubling of IL-10 in continuous models, but there was no association with all non-spine fractures or hip fractures. Men with the highest IL-10 (Q4) compared with Q1 had a 50% lower risk of clinical vertebral risk. It has been shown that many cell types can express IL-10, highlighting the complexity of IL-10 regulations.⁽⁴⁶⁾ Perhaps IL-10 is differentially expressed in trabecular bone and, thus, has greater effects on vertebral fractures than other largely cortical bone sites.

Previous studies have found positive associations between CRP and fracture.^(12,13,47–49) In Health ABC, the association was borderline significant.⁽⁵⁾ Nevertheless, the association between CRP and BMD has been inconsistent.^(50–52) We found no association between CRP and fractures, although CRP was included as a component of our inflammatory burden index.

Our study has a number of strengths. To our knowledge, we are the first to examine a broad range of inflammatory markers and their association with fracture risk in older men. We tested for associations with hip, vertebral, all non-spine fractures, and rates of bone loss. We also tested for possible mediation by a number of potential factors including ALM and BMD. Finally, we adjusted for many important confounders.

Our study was also subject to a number of limitations. Most MrOS men are white, and our results are not generalizable to non-white men. Our inflammatory markers were measured in serum and may not reflect concentrations of cytokines in the bone microenvironment. We

created a composite index of inflammation where each inflammatory marker is given equal weight. However, individual markers may have stronger association than others. For example, a secondary composite score consisting of TNF α and the TNF α -SR1 and 2 was created because these cytokines had the strongest association with fractures. However, results were similar. We analyzed seven cytokines in relationship to three skeletal outcomes. For each outcome, the probability of a type I error at $\alpha = 0.05$ is 30%. Therefore, we also adjusted for multiple comparisons using the FDR. We relied on self-report of medical conditions including RA. Finally, residual confounding owing to unmeasured covariates is an inherent limitation to all observational studies.

In conclusion, inflammation may play an important role in the etiology of fractures in older men. These associations may reflect effects of inflammation on ALM, disability, and BMD, although we found no association with rates of bone loss. Exploration of anti-inflammatory pathways as potential therapeutic agents may be warranted.

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Authors' roles: Study concept and design: JAC. Data collection: JAC, KEE, and ESO. Data analysis and interpretation: JAC, SLH. Drafting manuscript: JAC. Critical review and final approval of manuscript content: JAC, KEB, SLH, YKC, MED, KEE, HF, ESO, and RB. Statistical analysis: Ms Stephanie Harrison performed the statistical analyses and is independent of any commercial funder. She had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analyses.

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Fig. 1. Hazard ratio of fractures by number of high (quartile 4) inflammatory markers. (A) Composite score of TNF α , TNF α SR1, TNF α SR2, IL-6, IL-6SR, and CRP. (B) Composite score of TNF α , TNF α SR1, and TNF α SR2 age-, clinic-, and race-adjusted models.

Table 1

Descriptive Characteristics of Men by Inflammatory Burden

Variable	No. of high pro-inflammatory cytokines (cytokines in top quartile) ^a				p Trend
	0 (n = 346) (36%)	1 (n = 257) (26%)	2 (n = 148) (15%)	3 or more (n = 223) (23%)	
Age (years), mean ± SD	72.90 ± 5.60	72.52 ± 5.13	74.57 ± 6.10	76.17 ± 6.45	<0.0001
Race (white versus other)	314 (90.75)	235 (91.44)	141 (95.27)	212 (95.07)	0.0328
Body mass index (kg/m ²), mean ± SD	27.06 ± 3.27	27.27 ± 3.65	27.40 ± 3.72	27.85 ± 0.9	0.0125
Weight (kg), mean ± SD	82.84 ± 11.57	83.49 ± 12.37	82.69 ± 13.17	84.70 ± 14.71	0.15
Height (cm), mean ± SD	174.88 ± 6.64	174.93 ± 6.60	173.57 ± 7.04	174.16 ± 7.36	0.08
Self-reported health (excellent/good), n (%)	315 (91.04)	225 (87.55)	125 (85.03)	170 (76.23)	<0.0001
Smoking, n (%)					
None	143 (41.33)	99 (38.52)	57 (38.51)	73 (32.74)	0.0084
Past	196 (56.65)	150 (58.37)	83 (56.08)	137 (61.43)	
Current	7 (2.02)	8 (3.11)	8 (5.41)	13 (5.83)	
Drinks/week, mean ± SD	5.18 ± 1.3	4.76 ± 7.37	4.08 ± 5.96	3.57 ± 6.35	0.004
PASE score, mean ± SD	156.65 ± 68.19	155.37 ± 63.59	150.42 ± 68.75	131.51 ± 74.71	<0.0001
1 IADL, n (%)	46 (13.33)	30 (11.67)	32 (21.62)	82 (36.94)	<0.0001
Parkinson's disease, n (%)	2 (0.58)	2 (0.78)	0 (0.00)	3 (1.35)	0.64
COPD, n (%)	22 (6.36)	30 (11.67)	19 (12.84)	37 (16.59)	0.0016
Hypertension, n (%)	132 (38.15)	99 (38.52)	65 (43.92)	123 (55.16)	<0.0001
Heart attack, n (%)	48 (13.87)	32 (12.45)	23 (15.54)	49 (21.97)	0.0094
Congestive heart failure, n (%)	8 (2.31)	7 (2.72)	8 (5.41)	19 (8.52)	0.0002
Diabetes, n (%)	35 (10.12)	20 (7.78)	23 (15.54)	40 (17.94)	0.0016
Stroke, n (%)	10 (2.89)	13 (5.06)	11 (7.43)	22 (9.87)	0.0003
Osteoporosis, n (%)	10 (2.89)	12 (4.67)	4 (2.70)	11 (4.93)	0.34
Osteoarthritis, n (%)	61 (17.63)	49 (19.07)	24 (16.22)	48 (21.52)	0.37
Rheumatoid arthritis, n (%)	16 (10.3)	13 (11.11)	5 (7.25)	17 (14.41)	0.49
Fallen in past 12 months, n (%)	66 (19.08)	43 (16.73)	25 (16.89)	56 (25.11)	0.12
Appendicular lean mass	24.48 ± 3.16	24.59 ± 3.41	23.70 ± 3.41	23.91 ± 3.71	0.0106
Total hip BMD (g/cm ²), mean ± SD	0.96 ± 0.14	0.96 ± 0.13	0.95 ± 0.13	0.94 ± 0.15	0.23
Fracture after age 50 years, n (%)	82 (23.84)	53 (20.70)	35 (23.65)	57 (25.79)	0.54

Variable	No. of high pro-inflammatory cytokines (cytokines in top quartile) ^a				<i>p</i> Trend
	0 (<i>n</i> = 346) (36%)	1 (<i>n</i> = 257) (26%)	2 (<i>n</i> = 148) (15%)	3 or more (<i>n</i> = 223) (23%)	
Use of NSAIDs, <i>n</i> (%)	53 (16.01)	31 (12.60)	20 (14.39)	43 (20.09)	0.23
Use of statins, <i>n</i> (%)	98 (29.61)	73 (29.67)	30 (21.58)	60 (28.04)	0.37
Use of corticosteroids, <i>n</i> (%)	18 (5.44)	32 (13.01)	14 (10.07)	22 (10.28)	0.08
Total calcium intake, mean ± SD	740.40 ± 347.78	806.18 ± 362.97	807.89 ± 380.01	811.50 ± 405.63	0.02
Total vitamin D intake, mean ± SD	144.13 ± 103.23	158.25 ± 108.18	166.55 ± 116.99	166.05 ± 121.38	0.0129

PASE = Physical Activity Scale for the Elderly; COPD = chronic obstructive pulmonary disease; BMD = bone mineral density; NSAIDS = nonsteroidal anti-inflammatory drugs.
Note: Six are missing IL-6 values so they are not included.

^aIL-6, IL-6SR, TNFα, TNFαSR1, TNFαSR2, C-reactive protein.

Table 2

Mean (Standard Deviation) by Fractures or Median (IQR) Status: All Non-Spine Fractures, Hip Fractures, and Clinical Vertebral Fractures

Inflammatory marker	No	Yes	<i>p</i>
Hip fracture	(<i>n</i> = 888)	(<i>n</i> = 117)	—
TNFα (pg/mL) ^a	4.27 (3.40)	5.13 (3.14)	0.01
TNFα.SR1 (pg/mL)	2049 (596)	2300 (571)	<0.0001
TNFα.SR2 (pg/mL)	3619 (787)	4019 (788)	<0.0001
IL-6 (pg/mL)	2.96 (1.87)	3.58 (2.17)	0.0051
IL6SR (pg/mL)	48,457 (12,180)	50,250 (12,481)	0.14
IL-10 (pg/mL) ^a	8.88 (6.81)	8.94 (7.43)	0.74
C-reactive protein (μg/mL) ^a	1.39 (2.12)	1.65 (2.67)	0.26
Clinical vertebral fracture	(<i>n</i> = 961)	(<i>n</i> = 120)	—
TNFα (pg/mL)	4.29 (3.4)	4.94 (2.76)	0.0201
TNFα.SR1 (pg/mL)	2051 (597)	2221 (541)	0.0031
TNFα.SR2 (pg/mL)	3625 (785)	4088 (845)	<0.0001
IL-6 (pg/mL)	2.95 (1.83)	3.52 (2.35)	0.0135
IL6SR (pg/mL)	48,424 (12,128)	51,557 (13,779)	0.0088
IL-10 (pg/mL) ^a	8.95 (6.74)	7.29 (7.14)	0.0145
C-reactive protein (μg/mL) ^a	1.41 (2.12)	1.57 (3.09)	0.52
Non-spine fracture	(<i>n</i> = 888)	(<i>n</i> = 577)	—
TNFα (pg/mL)	4.27 (3.40)	4.39 (2.38)	0.42
TNFα.SR1 (pg/mL)	2048 (596)	2131 (566)	0.008
TNFα.SR2 (pg/mL)	3619 (787)	3768 (771)	0.0004
IL-6 (pg/mL)	2.96 (1.87)	3.13 (1.96)	0.10
IL6SR (pg/mL)	48,457 (12,180)	49,072 (12,425)	0.35
IL-10 (pg/mL) ^a	8.88 (6.81)	9.16 (6.85)	0.27
C-reactive protein (μg/mL) ^a	1.39 (2.12)	1.42 (2.29)	0.22

^aMedian (IQR).

Association Between Circulating Cytokines and C-reactive Protein, Incident Hip Fractures, Clinical Vertebral Fractures, and Non-Spine Fractures^c

Inflammatory marker	Hip fractures		Clinical vertebral fractures		Non-spine fractures	
	Base models ^a HR (95% CI)	Multivariable models ^b HR (95% CI)	Base models ^a HR (95% CI)	Multivariable models ^b HR (95% CI)	Base models ^a HR (95% CI)	Multivariable models ^b HR (95% CI)
TNFα quartiles (pg/mL)						
Q1: <2.85	Referent	Referent	Referent	Referent	Referent	Referent
Q2: 2.85–<3.98	1.51 (0.77, 2.96)	1.36 (0.61, 3.00)	1.36 (0.71, 2.58)	1.40 (0.70, 2.80)	1.05 (0.78, 1.41)	1.06 (0.76, 1.46)
Q3: 3.98–<5.33	1.67 (0.86, 3.23)	1.74 (0.83, 3.63)	2.17 (1.17, 4.04)	2.16 (1.08, 4.32)	1.08 (0.80, 1.45)	1.08 (0.78, 1.50)
Q4: 5.33–<81.74	2.64 (1.43, 4.90)	2.85 (1.44, 5.65)	2.66 (1.45, 4.88)	2.87 (1.46, 5.64)	1.35 (1.00, 1.81)	1.36 (0.98, 1.88)
<i>p</i> trend	0.0020	0.0020	0.0003	0.0007	0.05	0.07
TNFαSR1 quartiles (pg/mL)						
Q1: <1710.1	Referent	Referent	Referent	Referent	Referent	Referent
Q2: 1710.1–<1970.8	1.03 (0.49, 2.18)	1.00 (0.46, 2.18)	0.95 (0.46, 1.95)	0.91 (0.41, 1.99)	0.97 (0.71, 1.31)	0.98 (0.71, 1.36)
Q3: 1970.8–<2325.9	2.17 (1.09, 4.34)	1.80 (0.87, 3.71)	2.90 (1.56, 5.40)	3.39 (1.68, 6.80)	1.49 (1.10, 2.02)	1.45 (1.04, 2.02)
Q4: 2325.9–<6066.5	2.33 (1.15, 4.73)	2.17 (1.05, 4.48)	2.78 (1.44, 5.35)	3.17 (1.45, 6.90)	1.48 (1.09, 2.03)	1.46 (1.04, 2.07)
<i>p</i> trend	0.0024	0.0099	<0.0001	0.0001	0.0012	0.006
TNFαSR2 quartiles (pg/mL)						
Q1: <3131.5	Referent	Referent	Referent	Referent	Referent	Referent
Q2: 3131.5–<3569.7	1.05 (0.52, 2.10)	1.09 (0.50, 2.36)	0.89 (0.42, 1.88)	0.98 (0.44, 2.18)	1.09 (0.81, 1.48)	1.17 (0.84, 1.62)
Q3: 3569.7–<4133.4	1.39 (0.71, 2.72)	1.53 (0.74, 3.15)	2.57 (1.34, 4.93)	3.15 (1.55, 6.38)	1.53 (1.13, 2.07)	1.67 (1.20, 2.31)
Q4: 4133.4–<6153.8	2.05 (1.07, 3.93)	2.08 (1.04, 4.17)	3.98 (2.10, 7.55)	4.16 (2.02, 8.59)	1.66 (1.21, 2.26)	1.68 (1.20, 2.35)
<i>p</i> trend	0.0129	0.019	<0.0001	<0.0001	0.0002	0.0005
IL-6 quartiles (pg/mL)						
Q1: <1.76	Referent	Referent	Referent	Referent	Referent	Referent
Q2: 1.76–<2.44	1.22 (0.61, 2.45)	0.92 (0.43, 1.99)	0.99 (0.53, 1.82)	1.07 (0.56, 2.05)	0.99 (0.73, 1.34)	0.93 (0.67, 1.29)
Q3: 2.44–<3.65	1.71 (0.87, 3.35)	1.43 (0.66, 3.13)	1.44 (0.81, 2.55)	1.28 (0.66, 2.47)	1.15 (0.85, 1.55)	1.06 (0.76, 1.47)
Q4: 3.65–<12.27	1.74 (0.89, 3.41)	1.20 (0.55, 2.62)	1.90 (1.08, 3.37)	1.61 (0.83, 3.11)	1.25 (0.92, 1.70)	1.03 (0.73, 1.44)
<i>p</i> trend	0.06	0.39	0.01	0.14	0.09	0.70
IL-6SR quartiles (pg/mL)						
Q1: <38635	Referent	Referent	Referent	Referent	Referent	Referent
Q2: 38635–<48633	1.22 (0.66, 1.23)	1.08 (0.52, 2.22)	1.09 (0.62, 1.93)	1.14 (0.60, 2.14)	1.15 (0.86, 1.55)	1.15 (0.83, 1.58)

Inflammatory marker	Hip fractures			Clinical vertebral fractures			Non-spine fractures		
	Base models ^a HR (95% CI)	Multivariable models ^b HR (95% CI)		Base models ^a HR (95% CI)	Multivariable models ^b HR (95% CI)		Base models ^a HR (95% CI)	Multivariable models ^b HR (95% CI)	
		Referent	Referent		Referent	Referent		Referent	Referent
Q3: 48633–<56355.0	1.18 (0.64, 2.16)		1.34 (0.66, 2.71)	0.93 (0.51, 1.69)		0.98 (0.51, 1.89)	1.05 (0.78, 1.42)		1.05 (0.76, 1.45)
Q4: 56355–<99389	1.20 (0.66, 2.17)		1.37 (0.68, 2.77)	1.34 (0.77, 2.32)		1.46 (0.79, 2.71)	1.07 (0.80, 1.45)		1.12 (0.81, 1.56)
<i>p</i> trend	0.61		0.30	0.40		0.33	0.81		0.62
IL-10 quartiles (pg/mL)									
Q1: <6.35	Referent		Referent	Referent		Referent	Referent		Referent
Q2: 6.35–<9.01	1.27 (0.70, 2.31)		1.40 (0.68, 2.88)	0.60 (0.35, 1.02)		0.65 (0.35, 1.20)	1.20 (0.89, 1.62)		1.27 (0.92, 1.77)
Q3: 9.01–<13.1	0.92 (0.50, 1.69)		1.10 (0.53, 2.27)	0.42 (0.23, 0.74)		0.43 (0.23, 0.83)	1.14 (0.85, 1.54)		1.20 (0.87, 1.67)
Q4: 13.1–<17.53	1.04 (0.57, 1.87)		1.15 (0.56, 2.34)	0.50 (0.29, 0.86)		0.51 (0.27, 0.95)	1.10 (0.82, 1.49)		1.15 (0.82, 1.60)
<i>p</i> trend	0.82		0.89	0.0074		0.0173	0.62		0.52
C-reactive protein quartiles (pg/mL)									
Q1: <0.73	Referent		Referent	Referent		Referent	Referent		Referent
Q2: 0.73–<1.41	1.10 (0.59, 2.07)		0.98 (0.49, 1.95)	0.61 (0.34, 1.12)		0.65 (0.35, 1.24)	1.18 (0.87, 1.60)		1.12 (0.81, 1.56)
Q3: 1.41–<2.86	1.16 (0.62, 2.19)		0.87 (0.43, 1.75)	0.89 (0.51, 1.55)		0.86 (0.45, 1.64)	1.15 (0.85, 1.55)		1.01 (0.72, 1.41)
Q4: 2.86–<111.0	1.35 (0.73, 2.50)		0.99 (0.47, 2.09)	1.30 (0.77, 2.12)		1.05 (0.55, 1.98)	1.25 (0.92, 1.68)		1.03 (0.73, 1.45)
<i>p</i> trend	0.32		0.92	0.22		0.73	0.19		0.97

^aBase models: age, clinic site, race.

^bMultivariable models: base + height, weight, corticosteroids, NSAIDs, statins, congestive heart failure, chronic obstructive pulmonary disease, hypertension, diabetes, stroke, osteoarthritis, health status, smoking status, and total calcium and vitamin D intake.

^cThe False Discovery Rate (FDR) was used to adjust for multiple comparisons. The FDR-adjusted *p* value for hip fracture was *p* < 0.021; clinical vertebral fracture, *p* < 0.029; and non-spine fractures, *p* < 0.014.

Table 4
Hazard Ratio (95% Confidence Intervals) by Number of High Inflammatory Markers

Fracture	Quartiles				p trend
	0	1	2	3	
Hip fracture					
Base model ^a	Referent	1.50 (0.83, 2.71)	0.79 (0.37, 1.69)	2.07 (1.20, 3.56)	0.024
MV model ^b	Referent	1.47 (0.77, 2.81)	0.63 (0.26, 1.53)	2.03 (1.11, 3.71)	0.056
MV + ALM	Referent	1.42 (0.74, 2.74)	0.60 (0.24, 1.48)	1.75 (0.95, 3.23)	0.16
MV + IADL difficulty	Referent	1.39 (0.73, 2.67)	0.64 (0.26, 1.54)	1.75 (0.93, 3.29)	0.20
MV + total hip BMD	Referent	1.48 (0.72, 3.08)	0.48 (0.19, 1.24)	1.88 (0.97, 3.63)	0.17
MV + gait speed	Referent	1.56 (0.78, 3.11)	0.69 (0.28, 1.70)	2.26 (1.22, 4.19)	0.028
Clinical vertebral fracture					
Base model ^a	Referent	1.83 (1.03, 3.24)	1.88 (0.99, 3.58)	3.15 (1.84, 5.40)	<0.0001
MV model ^b	Referent	1.78 (0.96, 3.30)	1.77 (0.86, 3.67)	3.06 (1.66, 5.63)	0.0006
MV + ALM	Referent	1.72 (0.92, 3.19)	1.73 (0.83, 3.58)	2.86 (1.55, 5.29)	0.0013
MV + IADL difficulty	Referent	1.77 (0.95, 3.27)	1.75 (0.84, 3.63)	3.00 (1.62, 5.58)	0.0009
MV + total hip BMD	Referent	1.85 (0.99, 3.46)	1.70 (0.82, 3.52)	3.10 (0.62, 5.95)	0.0015
MV + gait speed	Referent	1.83 (0.99, 3.41)	1.77 (0.85, 3.67)	3.04 (1.65, 5.60)	0.0006
Non-spine fracture					
Base model ^a	Referent	1.14 (0.87, 1.50)	1.13 (0.82, 1.56)	1.40 (1.06, 1.85)	0.023
MV model ^b	Referent	1.11 (0.82, 1.49)	1.07 (0.75, 1.54)	1.31 (0.96, 1.79)	0.11
MV + ALM	Referent	1.10 (0.81, 1.48)	1.03 (0.72, 1.49)	1.27 (0.92, 1.74)	0.19
MV + IADL difficulty	Referent	1.10 (0.82, 1.49)	1.06 (0.74, 1.52)	1.20 (0.87, 1.66)	0.31
MV + total hip BMD	Referent	1.11 (0.82, 1.51)	1.03 (0.71, 1.49)	1.26 (0.91, 1.74)	0.21
MV + gait speed	Referent	1.13 (0.84, 1.52)	1.08 (0.75, 1.55)	1.31 (0.96, 1.79)	0.54

ALM = appendicular lean mass; IADL = instrumental activities of daily living; BMD = bone mineral density.

^aBase model controlled for age, clinic, and race.

^bMultivariable models: base + height, weight, corticosteroids, NSAIDs, statins, congestive heart failure, chronic obstructive pulmonary disease, hypertension, diabetes, stroke, osteoarthritis, health status, smoking status, and total calcium and vitamin D intake.