



# Chemokine CXCL9, a marker of inflammaging, is associated with changes of muscle strength and mortality in older men

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## Abstract

**Summary** Our study examined associations of the CXC motif chemokine ligand 9 (CXCL9), a pro-inflammatory protein implicated in age-related inflammation, with musculoskeletal function in elderly men. We found in certain outcomes both cross-sectional and longitudinal significant associations of CXCL9 with poorer musculoskeletal function and increased mortality in older men. This requires further investigation.

**Purpose** We aim to determine the relationship of (CXCL9), a pro-inflammatory protein implicated in age-related inflammation, with both cross-sectional and longitudinal musculoskeletal outcomes and mortality in older men.

**Methods** A random sample from the Osteoporotic Fractures in Men (MrOS) Study cohort ( $N=300$ ) was chosen for study subjects that had attended the third and fourth clinic visits, and data was available for major musculoskeletal outcomes (6 m walking speed, chair stands), hip bone mineral density (BMD), major osteoporotic fracture, mortality, and serum inflammatory markers. Serum levels of CXCL9 were measured by ELISA, and the associations with musculoskeletal outcomes were assessed by linear regression and fractures and mortality with Cox proportional hazards models.

**Results** The mean CXCL9 level of study participants ( $79.1 \pm 5.3$  years) was  $196.9 \pm 135.2$  pg/ml. There were significant differences for 6 m walking speed, chair stands, physical activity scores, and history of falls in the past year across the quartiles of CXCL9. However, higher CXCL9 was only significantly associated with changes in chair stands ( $\beta = -1.098$ ,  $p < 0.001$ ) even after adjustment for multiple covariates. No significant associations were observed between CXCL9 and major osteoporotic fracture or hip BMD changes. The risk of mortality increased with increasing CXCL9 (hazard ratio quartile (Q)4 vs Q1 1.98, 95% confidence interval 1.25–3.14;  $p$  for trend  $< 0.001$ ).

**Conclusions** Greater serum levels of CXCL9 were significantly associated with a decline in chair stands and increased mortality. Additional studies with a larger sample size are needed to confirm our findings.

**Keyword** Cytokines · Functional performance · Mortality · Osteoporotic fractures

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## Introduction

Aging involves a diverse set of temporal biological changes that increases the risk for a myriad of chronic diseases and mortality. The “nine tenants of aging” have been proposed to explain the physiological maladaptation evident with age: including the loss of proteostasis, decreased cellular adaptation, stem cell exhaustion, deregulated nutrient sensing, telomere attrition, mitochondrial dysfunction, macromolecular damage, cellular senescence, altered intercellular communication, and unfavorable epigenetic reprogramming [1]. At the center of these pathways is the age-dependent rise in inflammation (“inflammaging”) [2], a process characterized by an elevation in proinflammatory markers that accumulate in the tissue and cause tissue damage [3]. Age-related inflammation has been associated with an increased risk of developing chronic diseases, such as cardiovascular disease [4], hip fracture [5], type 2 diabetes, sarcopenia, and cancer [6].

A major limitation in the development of interventions to slow the aging process has been the accurate prediction of an individual’s biological age [7]. This is important because, similar to disease models, wherein an endpoint is necessary to evaluate improved disease outcome, an endpoint is necessary to determine if an intervention delays aging. Prior work has utilized the age-associated changes in DNA methylation pattern to correlate chronological age with biological age, dubbed the DNA methylation clock [8]. Indeed, interventions shown to extend lifespan, such as caloric restriction, have been associated with improved epigenetic remodeling and are suggestive of decreasing biological age [9, 10]. However, epigenetic reprogramming is only one contributor to physiological remodeling with age, whereas inflammation has been implicated as having a central role.

Prior work characterizing the immunome of 1001 individuals resulted in the development of an inflammatory clock of aging, which was highly correlated with several aging parameters including multimorbidity, immunosenescence, cardiovascular aging, frailty, and conversely, longevity [11]. Among the multiple pro-inflammatory proteins identified, the CXC-motif chemokine ligand 9 (CXCL9) had the strongest correlation with inflammation. This observation was notably consistent with previous studies that have shown elevated CXCL9 was not only associated with frailty [11] but is also a major predictor of morbidity and mortality [12], along with falls and fractures in older adults [13–15].

Taken together, these reports suggest increased levels of CXCL9 were related to both worse “inflammaging” and musculoskeletal function. The aim of this study was to assess CXCL9 levels in serum from a random sample of

subjects in a cohort of older men to determine its relationship with musculoskeletal function at the concurrent time point of serum collection and subsequent follow-up for fractures and mortality.

## Methods

### Study population

The Osteoporotic Fractures in Men (MrOS) Study is a longitudinal cohort study designed to study risk factors for osteoporosis and fractures in older men. Between March 2000 and April 2002, men ages 65 or older were recruited from six centers across the US (Birmingham, AL; Minneapolis, MN; Palo Alto, CA; Monongahela Valley near Pittsburgh, PA; Portland, OR; and San Diego, CA). To be eligible for the study, participants had to be able to walk without aid, and must not have had bilateral hip replacements. A total of 5994 men were enrolled, and baseline examinations were completed.

The current study used data from Visit 3 (V3) and Visit 4 (V4) of MrOS, collected from March 2007 through March 2009 and May 2014 through May 2016. Of the 4681 men who completed V3, the number of subjects from the original cohort at V3 that met our inclusion criteria had both inflammatory marker data (interleukin-6, interleukin-1 beta, and tumor necrosis factor alpha) and six or more vials of serum available was 900 subjects. We then randomly selected 300 subjects with a random number assigned to each subject and started at the lowest random number until  $n = 300$  was reached for this analysis. Since this was a pilot study, without external funding, we were only able to perform this study on 300 subjects.

### Assessment of muscle strength, physical performance, and physical activity

Participants completed clinical examinations and a self-administered questionnaire [16] during V3 to collect information about age (years), history of falls in the previous 12 months (0, 1, 2–3,  $\geq 4$  falls), and history of low trauma fractures from adulthood (yes/no) and after the age of 50 years (0, 1, 2,  $\geq 3$  fractures). Multimorbidity index (MMI) scores were assessed using self-reported chronic conditions such as myocardial infarction, stroke, congestive heart failure, diabetes, cancer, COPD, rheumatoid arthritis, osteoarthritis, depression, visual impairment, Parkinson’s disease, and Alzheimer’s disease [17]. Measurements of bone mineral density (BMD) were taken at the femoral neck [16] using dual-energy x-ray absorptiometry (DXA) machines (Hologic, Inc., Bedford, MA) [18].

Walking speed (m/s) was assessed by a 6-m walking test at the usual pace. Chair stands were assessed by measuring the time in seconds required to do five repeated extended stands from a full sitting position on an armless chair, with arms crossed over the chest. Chair stand speed was calculated and used to create a variable equal to the estimated number of chair stands per 10 s. The coefficients of variation for walking speed and chair stands were reported as 2.4% and 4.9%, respectively [19].

The Physical Activity Scale for the Elderly (PASE) was self-administered at V3 as described previously [20].

### Detection of CXCL9 and cytokine measurements

Serum from V3 was stored in cryovials at  $-80^{\circ}\text{F}$  until they were thawed and experiments performed. CXCL9 was measured in 300 participants at V3 using Invitrogen ELISA Human CXCL9/MIG (catalog number EHCXCL9X10) per the manufacturer's instructions. All volumes totaled 100  $\mu\text{l}$ /well. Standards were included in each assay, and standard curves were used for the estimation of CXCL9 concentrations (pg/ml). The minimal detection limit was 20 pg/ml, and the range of the standard curve was 6000–8.23 pg/ml. Intra-assay coefficient of variation (CV) was  $<10\%$ .

Inflammatory cytokines including C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-1 beta (IL-1 $\beta$ ), and tumor necrosis factor alpha (TNF $\alpha$ ) were all assayed as described previously [5].

### Fracture and mortality outcomes

Participants in MrOS were contacted every 4 months after the baseline examination to ask about clinical fracture events and ascertain vital status. Self-reported fractures were adjudicated by physician review of radiology reports. Clinic staff was notified of a participant's death when following up on missed contacts. Deaths were centrally adjudicated by physician review of death certificates and hospital discharge summaries (when available) to broadly assign the cause of death using the International Classification of Diseases, Ninth Revision codes.

Participants in this analysis were followed up for an average of 9.7 years after the V3 examination to ascertain incident fractures and mortality information. Major osteoporotic fracture (MOF) outcomes included hip, proximal humerus, wrist, and clinical vertebral fractures.

### Statistical analysis

The baseline characteristics of the participants were evaluated across quartiles of the CXCL9 using  $\chi^2$  tests for homogeneity for categorical data, ANOVA (normally distributed), or Kruskal Wallis tests (skewed data) for continuous

variables. Normality was assessed using skewness and kurtosis metrics. Spearman correlation ( $r$ ) coefficients and scatterplots were used to describe the unadjusted and age-adjusted correlations of the independent (CXCL9) with outcomes (musculoskeletal outcomes and their changes over time, absolute changes in total hip BMD, and cytokines variables). Linear regression was used to ascertain if serum levels of CXCL9 predict a cross-sectional and longitudinal decline in physical function. We adjusted for potential confounding variables; regression Model 1 was unadjusted; Model 2 was adjusted for age, race, and site; Model 3 was further adjusted for BMI and PASE; and finally, Model 4 was further adjusted for IL-1 $\beta$ , IL-6, TNF $\alpha$ , and CRP. As the distribution of CXCL9 values was skewed, we log-transformed the variable prior to inclusion in the model. Finally, we used the Cox proportional hazards model to estimate hazard ratios (HRs) for the association between quartiles of CXCL9 (with the lowest quartile as the reference group) and incident risk of MOF and mortality. Analyses were completed in SAS version 9.4 (SAS Institute, Cary, NC).

## Results

### Participant characteristics

A total of 300 older men were included in this analysis whose V3 characteristics are reported by quartiles of CXCL9 (Table 1). The mean age was  $79.1 \pm 5.3$  years, and the mean BMI was  $26.90 \pm 3.8$  kg/m $^2$ . During 7.3 years of follow-up (9.7 years for MOF and mortality), 15 (5.0%) incident MOF and 205 (75.7%) deaths occurred. Men in the higher quartiles of CXCL9 were older ( $p < 0.001$ ) than those with lower CXCL9. In addition, men in the higher quartiles of CXCL9 generally had lower levels of physical activity, fewer chair stands, and slower walking speeds (all  $p < 0.05$ ). At V3, 78 (26%) men reported falls within 12 months, and those men in the higher quartiles of CXCL9 had a higher prevalence of falls within the last 12 months than those in the lowest quartile. There were no significant differences in total hip and femoral neck BMD (at V3) across quartiles of CXCL9. Serum levels of CRP and TNF $\alpha$  were greater with increasing quartiles of CXCL9 (all  $p < 0.05$ ) while no differences were found with either IL-6 or IL-1 $\beta$ .

### Correlations of physical function, BMD, cytokines, and CXCL9

CXCL9 correlated with 6 m walk speed ( $r = -0.27$ ,  $p < 0.001$ ), chair stands ( $r = -0.22$ ,  $p < 0.001$ ), decline in walking speed ( $r = -0.18$ ,  $p = 0.035$ ), and decline in chair stands ( $r = -0.29$ ,  $p < 0.001$ ) over time; however, correlations with chair stands and decline in walking speed were

**Table 1** Baseline characteristics of participants across quartiles of CXCL9 at Visit 3

	10.00 ≤ Q1 < 107.31 (N = 75)	107.31 ≤ Q2 < 160.64 (N = 75)	160.64 ≤ Q3 < 246.59 (N = 75)	246.59 ≤ Q4 < 857.00 (N = 75)	p for trend
Age (years)	76.92 ± 4.47	78.08 ± 4.65	79.20 ± 4.54	82.33 ± 5.87	< .001
BMI (kg/m <sup>2</sup> )	26.65 ± 3.18	27.53 ± 3.49	26.94 ± 4.32	26.47 ± 4.17	0.565
Any prevalent fractures before V3, n (%)	48 (64.00)	50 (66.67)	43 (57.33)	43 (57.33)	0.244
Chair stands (n)	4.76 ± 1.63	4.18 ± 1.74	4.26 ± 1.68	3.79 ± 1.95	0.002
6 m walking speed (m/sec)	1.21 ± 0.20	1.12 ± 0.22	1.13 ± 0.21	1.05 ± 0.25	< .001
Multimorbidity Index score	0.93 ± 0.95	1.05 ± 1.09	1.05 ± 1.21	1.01 ± 0.99	0.662
PASE score	142.23 ± 69.30	142.07 ± 66.88	142.80 ± 53.11	117.78 ± 52.35	0.023
Total hip, BMD (g/cm <sup>2</sup> )	0.94 ± 0.13	0.95 ± 0.17	0.93 ± 0.14	0.92 ± 0.17	0.296
Femoral neck, BMD (g/cm <sup>2</sup> )	0.76 ± 0.13	0.78 ± 0.14	0.75 ± 0.12	0.76 ± 0.15	0.485
History of falls in the last 12 months, n (%)	12 (16.00)	23 (30.67)	17 (22.67)	26 (34.67)	0.034
% fat from DXA	26.63 ± 4.85	27.92 ± 4.40	25.19 ± 5.69	26.60 ± 5.95	0.320
CXCL9 (pg/ml)	80.06 ± 18.50	132.68 ± 15.28	197.37 ± 23.12	377.40 ± 147.17	< .001
CRP (ug/ml)	1.37 ± 1.75	2.99 ± 3.98	3.17 ± 4.72	3.15 ± 3.42	0.004
IL-1β (pg/ml)	0.04 ± 0.08	0.05 ± 0.12	0.08 ± 0.19	0.06 ± 0.09	0.187
IL-6 (pg/ml)	1.01 ± 0.58	2.36 ± 6.00	2.18 ± 3.97	1.72 ± 1.11	0.308
TNFα (pg/ml)	4.89 ± 1.35	5.41 ± 1.49	5.96 ± 1.57	6.15 ± 1.98	< .001

Values are presented as mean ± standard deviation, or number (%)

BMD, bone mineral density; BMI, body mass index; CRP, C-reactive protein; CXCL9, CXC motif chemokine ligand 9; DXA, dual-energy X-ray absorptiometry; IL-1β, interleukin-1beta; IL-6, interleukin-6; PASE, physical activity scale for the elderly; TNFα, tumor necrosis factor alpha; V3, visit 3

**Table 2** Correlation of CXCL9 serum levels, physical performance, and inflammatory markers at Visit 3 and changes in physical performance and bone mineral density (BMD) from Visit 3 to Visit 4

	Unadjusted	Age-adjusted
Walking speed	−0.27 <sup>c</sup>	−0.17 <sup>b</sup>
Chair stand	−0.22 <sup>c</sup>	−0.10
Δ walking speed	−0.18 <sup>a</sup>	−0.14
Δ chair stand	−0.29 <sup>c</sup>	−0.27 <sup>b</sup>
Δ BMD in total hip	−0.07	−0.04
Δ BMD in femur neck	−0.04	−0.02
CRP	0.22 <sup>c</sup>	0.21 <sup>c</sup>
IL-1β	0.12 <sup>a</sup>	0.07
IL-6	0.27 <sup>c</sup>	0.23 <sup>c</sup>
TNFα	0.30 <sup>c</sup>	0.26 <sup>c</sup>

CRP, C-reactive protein; CXCL9, CXC motif chemokine ligand 9; IL-1β, interleukin-1beta; IL-6, interleukin-6; TNFα, tumor necrosis factor alpha

<sup>a</sup>p < 0.05

<sup>b</sup>p < 0.01

<sup>c</sup>p < 0.001

attenuated after adjustment for age (Table 2). Higher CXCL9 was also correlated with higher levels of CRP ( $r = 0.22$ ,  $p < 0.001$ ), IL-1β ( $r = 0.12$ ,  $p = 0.031$ ), IL-6 ( $r = 0.27$ ,  $p < 0.001$ ), and TNFα ( $r = 0.30$ ,  $p < 0.001$ ), but associations

with IL-1β were attenuated after adjustment for age. In addition, there were no statistically significant correlations between CXCL9 and changes in total hip and femoral neck BMD over time.

### Associations of CXCL9 and physical function, BMD changes, and major osteoporotic fractures

In cross-sectional linear regression models, CXCL9 was inversely associated with walking speed (Table 3; unadjusted  $\beta = -0.097$ ,  $p < 0.001$ ) and chair stands in Model 1 (Table 3; unadjusted  $\beta = -0.615$ ,  $p < 0.001$ ). However, these associations were attenuated and became not significant in fully adjusted Model 4 (Table 3; fully adjusted  $\beta = -0.038$ ,  $p = 0.072$  for walking speed and  $\beta = -0.014$ ,  $p = 0.933$  for chair stand). Interestingly, there was no significant association between CXCL9 and changes in walking speed over time in both unadjusted and adjusted models (Table 3; adjusted  $\beta = -0.052$ ,  $p = 0.139$ ) while CXCL9 was inversely associated with changes in chair stands over time (Table 3; unadjusted  $\beta = -1.089$ ,  $p < 0.001$ ). Adjustment for covariates including four inflammatory markers slightly attenuated the association of changes in chair stands, but it remained significant in the fully adjusted model (Table 3; fully adjusted  $\beta = -1.098$ ,  $p < 0.001$ ). In contrast, CXCL9

**Table 3** Association of CXCL9 serum levels and physical performance at Visit 3 and changes in physical performance and bone mineral density (BMD) from Visit 3 to Visit 4

	<i>B</i>	95% CI	<i>p</i>
Walking speed			
M1	−0.097	−0.137, −0.057	<0.001
M2	−0.064	−0.105, −0.024	0.002
M3	−0.058	−0.097, −0.018	0.005
M4	−0.038	−0.078, 0.003	0.072
Chair stand			
M1	−0.615	−0.935, −0.295	<0.001
M2	−0.255	−0.583, 0.073	0.129
M3	−0.203	−0.526, 0.119	0.218
M4	−0.014	−0.343, 0.314	0.933
Δ walking speed			
M1	−0.061	−0.125, 0.002	0.060
M2	−0.046	−0.110, 0.017	0.157
M3	−0.047	−0.113, 0.018	0.158
M4	−0.052	−0.120, 0.016	0.139
Δ chair stand			
M1	−1.089	−1.560, −0.617	<0.001
M2	−1.005	−1.494, −0.516	<0.001
M3	−1.051	−1.552, −0.550	<0.001
M4	−1.098	−1.624, −0.571	<0.001
Δ BMD in total hip			
M1	−0.015	−0.031, 0.001	0.067
M2	−0.014	−0.030, 0.003	0.110
M3	−0.012	−0.029, 0.005	0.152
M4	−0.012	−0.029, 0.006	0.190
Δ BMD in femur neck			
M1	−0.009	−0.024, 0.007	0.266
M2	−0.007	−0.023, 0.009	0.412
M3	−0.005	−0.022, 0.012	0.559
M4	−0.004	−0.021, 0.013	0.640

*M1*, unadjusted; *M2*, age-, race-, and site-adjusted; *M3*, *M2* + BMI and PASE; *M4*, *M3* + CRP, IL-1 $\beta$ , IL-6, and TNF $\alpha$

was not associated with BMD changes in total hip and femoral neck (Table 3).

### Associations of CXCL9 and major osteoporotic fractures and survival

During the 9.7-year follow-up, there was attrition of the study subjects across the quartiles of CXCL9, with only 18.7% and 5.3% remaining in quartiles 3 and 4. There were 15 (5.0%) MOF and 205 (75.7%) deaths reported during the 9.7 years; the study subjects were followed. There was no association between CXCL9 and the risk of developing MOFs in both unadjusted and fully adjusted models (Table 4). However, men in the highest quartile of CXCL9 had a 3.3-fold higher risk of mortality compared to men in

the lowest quartile. Further adjustment for covariates including age and the four inflammatory markers (IL-1 $\beta$ , IL-6, TNF $\alpha$ , and CRP) only slightly attenuated these associations (hazard ratio quartile (Q) Q4 vs Q1 1.98, 95% confidence interval 1.25–3.14; *p* for trend 0.001) (Table 4).

## Discussion

Our study investigated the association between the serum chemokine CXCL9 and physical function, bone mineral density changes cross-sectionally and over time, and the risk of MOF and mortality osteoporotic fracture in older men. We observed that men with higher serum levels of CXCL9 had slower walking speeds and were more likely to experience a significant decline in timed chair stands. In contrast, there was no significant association between CXCL9 serum levels and BMD changes or risk of MOF. Lastly, mortality risk was significantly higher in participants with higher CXCL9 compared with those with lower CXCL9 levels.

Inflammation plays a critical role in the accumulation of diseases of aging, including cancer, cardiovascular disease, neurodegenerative disorders, and others [6, 21, 22]. Increased pro-inflammatory cytokines, such as CRP and IL-6, have been associated with immunosenescence [23, 24], but the relationship with chronic inflammation has resulted in conflicting outcomes [25]. CXCL9 is a T-cell chemoattractant induced by interferon-gamma (IFN- $\gamma$ ) and is a selective ligand for the CXC motif chemokine receptor 3. CXCL9 is mainly secreted by monocytes, endothelial cells, fibroblasts, and cancer cells in response to IFN- $\gamma$ , which are synergistically enhanced by TNF $\alpha$  [26]. A recent study shows that serum levels of CXCL9 may be the most robust immunome contributor to age-related chronic inflammation. CXCL9 was associated with cardiovascular aging in nominally healthy individuals [11] and with falls and fractures in an elderly population [13–15], suggesting that it may also be associated with incident frailty. In the same study [11], canonical markers of acute infection, except for IL-1 $\beta$ , were not major contributors to age-related chronic inflammation, suggesting that acute inflammatory markers may not be associated with age-related chronic inflammation. In this study, the association of CXCL9 with physical performance especially longitudinal changes of chair stand was robust, even after adjustment for covariates including pro-inflammatory markers, suggesting that the chronic inflammation may be the major contributor to physical decline. Chair stand has been used as a surrogate measure of strength and physical performance in sarcopenia diagnosis [27, 28]. However, other unexplored, indirect mechanisms also seem to contribute to the occurrence of this event and deserve further investigation.

**Table 4** Hazard ratios (HR) and 95% confidence intervals (CI) for developing major osteoporotic fractures and mortality

	Quartile 1 (n=75)	Quartile 2 (n=75)	Quartile 3 (n=75)	Quartile 4 (n=75)	p for trend
Major osteoporotic fractures					
N (%)	2 (2.67)	5 (6.67)	5 (6.67)	3 (4.00)	
M1	Reference	3.00 (0.58–15.48)	3.24 (0.62–16.88)	2.42 (0.40–14.72)	0.326
M2	Reference	3.28 (0.57–18.82)	3.54 (0.64–19.50)	0.85 (0.11–6.44)	0.941
M3	Reference	3.53 (0.60–20.68)	3.92 (0.71–21.65)	0.81 (0.10–6.90)	0.864
M4	Reference	2.56 (0.42–15.62)	8.24 (1.01–67.31)	0.85 (0.06–12.00)	0.680
Mortality					
N (%)	43 (63.24)	43 (65.15)	54 (79.41)	65 (94.20)	
M1	Reference	1.16 (0.76–1.78)	1.86 (1.25–2.79)	3.33 (2.25–4.93)	<0.001
M2	Reference	1.05 (0.68–1.61)	1.76 (1.17–2.65)	1.98 (1.28–3.06)	<0.001
M3	Reference	1.08 (0.70–1.67)	1.80 (1.19–2.72)	2.04 (1.31–3.19)	<0.001
M4	Reference	1.08 (0.69–1.68)	1.76 (1.13–2.72)	1.98 (1.25–3.14)	0.001

M1, unadjusted; M2, age-, race-, and site-adjusted; M3, M2 + BMI and PASE; M4, M3 + CRP, IL-1 $\beta$ , IL-6, and TNF $\alpha$

The exact mechanism that explains the relationship between increased levels of CXCL9 and the faster physical decline and higher mortality risk among older men has not been clearly defined. In the paper by Sayed et al., CXCL9 was validated as an indicator of cardiovascular pathology independently of age [11]. In both human and mouse models, an increase in CXCL9 in older endothelial cells was related to endothelial dysfunction [11]. Moreover, the knockdown of CXCL9 in endothelial cells not only rescued the endothelial cell dysfunction but also reversed the aging phenotype, suggesting a critical role of CXCL9 in endothelial cell senescence [11]. In line with these findings, recent meta-analyses suggested an association between frailty and endothelial dysfunction in old adults, implying that chronic inflammation in the vascular endothelium and age-associated elevated inflammation are involved in physical frailty through direct and indirect means [29, 30]. Given the role of CXCL9 as a regulator of vascular function and cellular senescence, levels should correlate well with physical decline and mortality as demonstrated in our study.

Regarding the longitudinal changes of BMD and incident major osteoporotic fractures in elderly males, we found there were no associations with CXCL9 in both unadjusted and adjusted analyses. However, in another recently reported study, investigators examined the association between serum levels of CXCL9 and incident hip fracture risk. Serum samples were collected prior to 6.2 years to the fractures as the assays for CXCL9 were not available until recently [15]. Increasing CXCL9 levels were associated with increasing hip fracture risk in men but not in women [15]. Moreover, it was a matched case–control study where the number of fractures was pre-determined to detect significant risk estimates. In a case-cohort study with a random sample of 961 men from MrOS, there was a significant association between TNF cytokine and risk of hip and vertebral fractures during

an average follow-up of 6.1 years [5]. Men in the highest quartile of TNF $\alpha$  had a greater than twofold higher risk of hip and clinical vertebral fractures, but there was no association between inflammatory markers and hip BMD loss [5]. Whereas, in our study, we studied a random sample of 300 subjects who had a serum sample available for assessment of CXCL9, and there were only 15 MOFs limiting our statistical power.

Few studies have examined the association between inflammatory markers and mortality [31–34]. In a study from the Bambui-Epigen (Brazil) cohort of aging, the investigators examined the associations between baseline serum levels of cytokines and chemokines including CXCL9 with mortality risk in 1191 Brazilians aged 60 years and over during 12.8 years of follow-up [35]. Higher quartiles of IL-6 level were associated with increased risk of deaths, but the associations with CXCL9 showed weak associations with mortality (hazard ratio 1.03, 95% confidence interval 1.00–1.07) [35]. However, in our study with 300 older men, the risk of mortality increased with quartiles of CXCL9 (odds ratio Q4 vs Q1 1.98, 95% confidence interval 1.25–3.14; *p* for trend <0.001). There is mounting evidence indicating that CXCL9 is the most potent contributor to age-related inflammation, and higher levels of CXCL9 are tied to an elevated risk for cardiovascular events including myocardial infarcts and strokes [11], which are leading causes of death in elderly men in the US (<https://www.cdc.gov/minorityhealth/lcod/men/2016/all-races-origins/indexhtml>).

Strengths of the present study include the collection of major musculoskeletal outcomes, information on major covariates at two study visits, approximately 7.3 years apart, and serum samples available for measurements of cytokines using a longitudinal cohort design, thus allowing analyses of temporal relationships between the inflammation and outcomes and avoiding both temporal and information biases observed in typical retrospective case–control studies.

Information was collected with standardized instruments and trained professional interviewers, which ensured data quality. There are however several limitations. Our study only included 300 randomly selected participants and was underpowered for fractures, as this was a pilot study to obtain preliminary risk estimates. The participants were primarily white men, and the results were based on a single measure of CXCL9. We also acknowledge that unappreciated confounding may be an issue. Future studies with a larger sample size are needed to confirm these findings.

## Conclusion

In conclusion, the present study demonstrated, that in older men, elevated levels of CXCL9 were associated with a faster decline in physical performance and a higher risk of mortality. However, there was no association with longitudinal changes of BMD or incident MOF. Our findings support earlier reports that this chemokine may be an integral biomarker related to both inflammation and aging. Additional studies with more diverse samples of both men and women are needed to confirm the validity of these findings.

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## Declarations

**Conflict of interest** None.

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