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# Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults

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SUMMARY

*Objective:* The role of inflammation in osteoarthritis (OA) pathogenesis is unclear, and the associations between inflammatory cytokines and cartilage loss have not been reported. We determined the associations between serum levels of interleukin (IL)-6 and tumor necrosis factor-α (TNF-α), knee radiographic OA (ROA) and cartilage loss over 2.9 years in older adults.

*Methods:* A total of 172 randomly selected subjects (mean 63 years, range 52–78, 47% female) were studied at baseline and approximately 3 (range 2.6–3.3) years later. IL-6 and TNF-α were assessed by radioimmunoassay. T1-weighted fat-suppressed magnetic resonance imaging of the right knee was performed at baseline and follow-up to determine knee cartilage volume. Knee ROA of both knees was assessed at baseline.

*Results:* At baseline, quartiles of IL-6 and TNF-α were associated with increased prevalence of medial tibiofemoral joint space narrowing (OARSI grade ≥1) in multivariate analyses [odds ratio (OR): 1.42 and 1.47 per quartile, respectively, both $P < 0.05$]. Longitudinally, baseline IL-6 predicted loss of both medial and lateral tibial cartilage volume ($\beta$: −1.19% and −1.35% per annum per quartile, $P < 0.05$ and $P < 0.01$, respectively), independently of TNF-α. Change in IL-6 was associated with increased loss of medial and lateral tibial cartilage volume ($\beta$: −1.18% and −1.06% per annum per quartile, both $P < 0.05$) and change in TNF-α was also negatively associated with change in medial cartilage volume ($\beta$: −1.27% per annum per quartile, $P < 0.05$).

*Conclusions:* Serum levels of IL-6 and TNF-α are associated with knee cartilage loss in older people suggesting low level inflammation plays a role in the pathogenesis of knee OA.



## Introduction

Osteoarthritis (OA) is one of the most common diseases among older people and is a leading cause of disability. It affects the whole joint structure including articular cartilage, synovial membrane, subchondral bone, meniscus, and periarticular muscles[1]. The structural changes of OA are due to a combination of risk factors, ranging from common factors such as aging, obesity, being female, smoking, genetics and joint injury, to mechanical and metabolic factors[1–4]. Inflammation has been implicated in the pathogenesis of OA. Synovitis is common in early[5] and advanced[6] OA, and it has been associated with knee pain[7] and progression of cartilage degeneration[8]. The inflammatory changes in OA synovium include synovial hypertrophy and hyperplasia with an increased number of lining cells[9], and an infiltration of the sublining tissue with a mixed population of inflammatory cells including synovial macrophages[10], activated B and T lymphocytes[6]. Local levels of pro-inflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor (TNF) α, and IL-6 produced by these cells are detectable even in early OA but generally at lower levels than in rheumatoid arthritis[11–13], and *in vitro* and animal studies have documented that these cytokines can enhance cartilage degradation or induce bone resorption[9,14,15].

Multiple studies have demonstrated that circulating levels of C-reactive protein (CRP), a marker of low-grade systemic inflammation, are modestly elevated in OA and are associated with decreased cartilage volume and disease progression[16–18]; however, the associations between IL-6 and IL-1, the primary regulators of CRP, and severity and progression of OA have been rarely reported. A recent

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study in women reported that prevalent radiographic OA (ROA) was significantly associated with both IL-6 and CRP, and that incident ROA was significantly predicted by IL-6[19]. The same study found no significant associations between TNF-α and prevalent or incident OA[19], while a recent Dutch study suggested that *ex vivo* production of TNF-α from whole blood samples upon lipopolysaccharide stimulation was associated with radiological progression of knee OA over 2 years[20].

While OA disease progression is commonly measured by radiographs, such means have been proven to have limited sensitivity due to their two-dimensional nature and measurement error. Magnetic resonance imaging (MRI) can visualize whole joint structure directly and is recognised as a more sensitive, accurate and reproducible tool than radiographic assessment to monitor OA disease progression[21]. The aim of this study was to describe the associations between circulating levels of inflammatory markers (CRP, IL-1, TNF-α and IL-6), and both ROA prevalence and MRI-detected knee cartilage loss over approximately 3 years in older adults.

## Materials and methods

### *Subjects*

The study was carried out in southern Tasmania from March until August 2002. The follow-up study was conducted approximately 3 years later (range 2.6–3.3 years). Subjects between ages 50 and 79 years were selected randomly from the roll of electors in southern Tasmania (population 229,000) with an equal number of men and women. Institutionalized persons were excluded. This study was conducted as part of the Tasmanian Older Adult Cohort Study, an ongoing, prospective, population-based study in 1100 subjects aimed at identifying the environmental, genetic, and biochemical factors associated with the development and progression of OA and osteoporosis (the overall response rate was 57% at baseline and 82% retention for follow-up). Subjects with rheumatoid arthritis were excluded from analyses. The first 172 subjects were selected to perform the measurements of serum inflammatory markers at baseline and follow-up. At follow-up, these measurements were not performed in nine subjects due to insufficient serum sample, leaving 163 subjects. The study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee, and written informed consent was obtained from all participants.

### *Anthropometrics and questionnaire*

Height was measured to the nearest 0.1 cm (with shoes, socks, and headgear removed) using a stadiometer. Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707, Bradford, MA) that were calibrated using a known weight at the beginning of each clinic. Body mass index [BMI; weight (kg)/height$^2$ (m$^2$)] was also calculated. Self-report of smoking status and diseases including asthma, cardiovascular disease, and diabetes were recorded by questionnaire. Steps per day were measured using a pedometer worn on their dominant side for seven consecutive days except during sleeping or water based activities.

### *Serum inflammatory markers measurement*

Serum was isolated and refrigerated overnight in plastic tubes, at which time aliquots were prepared and stored at −80°C. The IL-1 β, IL-6 and TNF-α were measured at baseline and then at follow-up with a solid-phase, two-site chemiluminescent enzyme immunometric assay method by use of IMMULITE IL-1 β, IMMULITE IL-6 and IMMULITE TNF-α (all from EURO/DPC Llanberis, Gwynedd, United Kingdom). Samples with undetectable cytokine concentrations were assigned a value corresponding to the lower limit of detection of the assay (1.5 pg/ml for IL-1 β, 2 pg/mL for IL-6 and 1.7 pg/mL for TNF-α). The coefficients of variation (CVs) in our hands were 3% for IL-1 β, 8% for IL-6 and 6% for TNF-α[22].

Testing high-sensitivity CRP (hs-CRP) was performed by using the CRP-Latex (II) immunoturbidimetric assay (Abbott Diagnostic's c8000 Architect). The lower detection limit of the assay is 0.01 mg/L. The CV in our hands was of the order of 4.8%[22].

Changes in these markers were calculated as: change per annum = (follow-up value − baseline value)/(time between two visits in years).

### *Knee X-ray and knee pain assessment*

A standing anteroposterior semiflexed view of the right and left knees with 15° of fixed knee flexion was performed in all subjects at baseline and scored individually for osteophytes and joint space narrowing (JSN) on a scale of 0–3 (0 = normal and 3 = severe) according to the Osteoarthritis Research Society International (OARSI) atlas as previously described[23]. The presence of medial or lateral tibiofemoral JSN or osteophytes was defined as any score of ≥1 in that compartment. The presence of JSN or osteophytes in the whole tibiofemoral compartment was defined as the presence of that feature in either of the medial or lateral compartments.

Knee pain (on flat surface, going up/down stairs, at night, sitting/lying and standing upright) was assessed by self-administered questionnaire using the Western Ontario McMaster Osteoarthritis Index (WOMAC) with a 10-point scale from 0 (no pain, stiffness or no function problems) to 9 (most severe pain, stiffness or severe function problems)[24]. Each component of joint pain was summed to create a total pain (0–45) score. Prevalent knee pain was defined as a total score of ≥1.

### *Knee cartilage volume and tibial bone area measurements*

MRI scans of the right knee were performed at baseline and follow-up. Knees were imaged in the sagittal plane on a 1.5-T whole body magnetic resonance unit (Picker, Cleveland, OH) and a fat-saturated T1-weighted spoiled gradient echo sequence was used. Knee cartilage volume was determined by means of image processing on an independent workstation as previously described[2,25]. The volumes of individual cartilage plates (medial tibial, and lateral tibial) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis. These data were then resampled by means of bilinear and cubic interpolation (area of 312 and 312 μm and 1.5 mm thickness, continuous sections) for the final 3-D rendering. The CVs for cartilage volume measures in our hands were 2.1–2.6%[25]. Rates of change in cartilage volume were calculated as: percentage change per annum = [100 × [(follow-up volume − baseline volume)/baseline volume]/(time between two scans in years)].

Tibial bone area at the medial and lateral compartments was determined as previously described[23].

### *Data analysis*

*T*-tests or $\chi^2$-tests (where appropriate) were used to compare means or proportions. Quartiles of IL-6 or TNF-α were used in analysis, because nearly a quarter of subjects had IL-6 levels under the lower limits of detection. The associations between quartiles of IL-6/TNF-α and presence of knee ROA (JSN or osteophytes), both

before and after adjustment for potential confounding factors including sex, age, BMI, smoking, steps per day, knee JSN if osteophytes, osteophytes if JSN, asthma, cardiovascular diseases, and diabetes, were analysed by logistic regression using generalized estimating equations (GEE's) to account for the correlation of data between both knees. An exchangeable correlation matrix was used as observations are clustered (and not time dependent) and all models used robust variance estimates to ensure valid standard errors, irrespective of correlation structure or whether the residuals are not identically distributed across subjects. Univariable and multivariable linear regression analyses were used to examine the associations between quartiles of IL-6/TNF-α or change in quartiles and cartilage volume or change in cartilage volume before and after adjustment for potential confounding factors. Further adjustment for each biomarker (TNF-α for IL-6 or IL-6 for TNF-α) was made to test the independence of these two predictors from each other. Analyses were also conducted using the lowest quartile (quartile 1) as a reference, to compare the individual effect of quartile 2, 3 or 4 categorically in predicting outcome measures. Standard diagnostic checks of model fit and residuals were routinely made and points with high influence were investigated. The linearity for the associations between quartiles of IL-6 or TNF-α and outcome measures was determined.

Interactions between IL-6/TNF-α and other variables (such as sex) on ROA or change in cartilage volume were investigated. A *P*-value less than 0.05 (two-tailed) or a 95% confidence interval not including the null point was regarded as statistically significant. All statistical analyses were performed on SPSS version 12.0 for Windows (Chicago, IL), or Stata statistical software, release 10.0 [College Station (TX): Stata Corporation, 2006].

## Results

A total of 172 subjects (47% female) aged between 52 and 78 (mean 63 years) participated and 163 had complete data at follow-up. Knee pain was prevalent in 45% of subjects. There were no significant differences in demographic factors, ROA and cartilage loss between the current cohort and the subjects who did not have serum markers measured (data not shown). The serum levels of inflammatory markers were generally low with 98.4% of IL-1 β undetectable in this sample, so this was not further analysed. The median level of IL-6 was 2.9 pg/mL, TNF-α was 7.3 pg/mL and hs-CRP was 2.2 mg/L. The numbers of IL-6 and TNF-α measurements below their respective lower limits of detection were 51 (~26%) and 25 (~13%) at baseline; and 32 (~19%) and 17 (~10%) at follow-up. Characteristics of the subjects are presented in Table I. There were no significant differences between subjects with high (≥median) and low (<median) levels of IL-6 in terms of sex, BMI, smoking, disease status, tibial cartilage volume, and tibial bone area; however, subjects with high levels of IL-6 (≥median) were older, had less steps per day and higher levels of TNF-α and hs-CRP, and greater prevalence of JSN and left tibiofemoral osteophytes (Table I). The interactions between sex and IL-6 or TNF-α on JSN or loss of cartilage volume were all non significant (data not shown), so men and women were combined for analyses.

At baseline, quartiles of IL-6 were associated with prevalent JSN (grade ≥1) in the medial tibiofemoral compartments but not with JSN in lateral tibiofemoral compartment (Table II). After adjustment for sex, age, BMI, smoking, steps per day and other potential confounders, IL-6 was associated with 1.4-fold increased odds per quartile of medial tibiofemoral JSN ($P = 0.015$, Table II). The association became non-significant after further adjustment for TNF-α ($P = 0.099$, data not shown). IL-6 was also associated with 1.3-fold increased odds per quartile of whole tibiofemoral (prevalence in either medial or lateral compartment) JSN ($P = 0.016$). Quartiles of IL-6 were not significantly associated with osteophytes (Table II); however, high levels of IL-6 (≥median) were associated with greater prevalence of osteophytes in whole tibiofemoral compartment of left knee compared with low levels of IL-6, before (Table I) and after adjustment for potential confounders including TNF-α (OR = 7.4, $P = 0.021$). When each quartile was examined as an independent predictor for prevalent JSN, quartiles 3 and 4 of IL-6 were associated with greater prevalence of JSN, but were not significant, despite an overall significant positive trend for medial JSN (Table III).

Similarly to IL-6, quartiles of TNF-α were associated with JSN in the medial tibiofemoral compartments but not with JSN in lateral tibiofemoral compartment (Table II). After adjustment for potential confounders, TNF-α was associated with 1.5-fold increased odds per quartile of medial tibiofemoral JSN ($P = 0.007$) (Table II). This

**Table I**
Characteristics of participants

| | IL-6 < median ($n = 85$) | IL-6 ≥ median ($n = 87$) | *P*-values |
|---|---|---|---|
| Age (year) | 61.4 (6.3) | 63.8 (7.8) | 0.029* |
| Female sex (%) | 48 | 45 | 0.656** |
| BMI (kg/m$^2$) | 27.1 (3.5) | 27.8 (4.8) | 0.269* |
| Knee pain (%) | 45 | 46 | 0.868** |
| Knee pain severity (0–45) | 3.1 (5.3) | 3.0 (5.2) | 0.921* |
| Ever smoking (%) | 53 | 48 | 0.494** |
| Steps per day (×1000) | 11.1 (2.7) | 8.8 (3.7) | <0.001* |
| Asthma (%) | 17 | 13 | 0.434** |
| Cardiovascular diseases (%) | 4 | 5 | 0.678** |
| Diabetes (%) | 5 | 6 | 0.706** |
| Right knee JSN (%) | 47 | 65 | 0.017** |
| Left knee JSN (%) | 51 | 73 | 0.003** |
| Right tibiofemoral osteophytes (%) | 5 | 12 | 0.233** |
| Left tibiofemoral osteophytes (%) | 4 | 14 | 0.025** |
| Medial tibial cartilage volume (ml) | 2.4 (0.6) | 2.3 (0.6) | 0.438* |
| Lateral tibial cartilage volume (ml) | 2.9 (0.7) | 2.8 (0.7) | 0.631* |
| Medial tibial bone area (mm$^2$) | 21.2 (3.2) | 21.0 (3.0) | 0.661* |
| Lateral tibial bone area (mm$^2$) | 12.1 (1.9) | 12.4 (2.3) | 0.808* |
| Hs-CRP (mg/l) | 1.6 (1.0, 3.7) | 2.6 (1.4, 5.4) | 0.009** |
| TNF-α (pg/ml) | 6.1 (4.4, 8.6) | 9.3 (6.5, 13.2) | <0.001** |

Mean standard definition (SD) or median (interquartile range) except for percentages. IL-6 median: 2.9 pg/ml. * Unpaired *t*-test except ** $\chi^2$-tests.

**Table II**
Associations between knee ROA at the right and left knees and cytokines: cross-sectional data

| | Univariable OR (95% CI) | Multivariable* OR (95% CI) |
|---|---|---|
| *IL-6 (per quartile)* | | |
| Medial JSN | **1.37 (1.04, 1.80)** | **1.42 (1.07, 1.87)** |
| Lateral JSN | 1.25 (0.90, 1.73) | 1.23 (0.88, 1.71) |
| Medial OP | 1.08 (0.63, 1.87) | 0.81 (0.49, 1.32) |
| Lateral OP | 1.62 (0.71, 3.67) | 1.47 (0.66, 3.25) |
| *TNF-α (per quartile)* | | |
| Medial JSN | **1.50 (1.13, 1.98)** | **1.47 (1.11, 1.95)** |
| Lateral JSN | 1.07 (0.76, 1.51) | 1.07 (0.77, 1.49) |
| Medial OP | 1.03 (0.60, 1.76) | 0.78 (0.47, 1.30) |
| Lateral OP | 1.12 (0.52, 2.42) | 1.16 (0.55, 2.48) |

OP: osteophytes. Dependent variable: presence of JSN or osteophytes; Independent variable: quartiles of IL-6 (≤2.0, >2.0–2.9, >2.9–4.0, ≥4.0 pg/ml) or TNF-α (≤5.1, >5.1–7.3, >7.3–11.6, ≥11.6 pg/ml). Bold denotes statistically significant result.
* Adjusted for sex, age, BMI, smoking, steps per day, knee pain, knee JSN if osteophytes, osteophytes if JSN, asthma, cardiovascular diseases, and diabetes.

**Table III**
Associations between knee radiographic JSN at the right and left knees and cytokines: comparisons between quartiles of cytokines in cross-sectional data

| Model | Predictor | Compartment | OR (95% CI) | | | | *P*-value for trend |
|---|---|---|---|---|---|---|---|
| | | | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | |
| Univariable | IL-6 | Medial | 1.00 (Reference) | 0.46 (0.20, 1.10) | 1.81 (0.73, 4.47) | 1.84 (0.77, 4.38) | **0.027** |
| | | Lateral | 1.00 (Reference) | 1.06 (0.36, 3.08) | 2.20 (0.80, 6.07) | 1.66 (0.56, 4.90) | 0.178 |
| | TNF-α | Medial | 1.00 (Reference) | 1.31 (0.56, 3.07) | 1.44 (0.61, 3.39) | **3.90 (1.56, 9.77)** | **0.004** |
| | | Lateral | 1.00 (Reference) | 0.64 (0.22, 1.84) | 0.80 (0.29, 2.26) | 1.16 (0.43, 3.08) | 0.687 |
| Multivariable* | IL-6 | Medial | 1.00 (Reference) | 0.43 (0.16, 1.12) | 1.75 (0.69, 4.39) | 1.87 (0.70, 5.02) | **0.030** |
| | | Lateral | 1.00 (Reference) | 1.13 (0.34, 3.74) | 2.28 (0.75, 6.88) | 1.55 (0.43, 5.54) | 0.320 |
| | TNF-α | Medial | 1.00 (Reference) | 0.87 (0.34, 2.27) | 1.21 (0.46, 3.22) | **3.21 (1.19, 8.65)** | **0.016** |
| | | Lateral | 1.00 (Reference) | 0.42 (0.13, 1.36) | 0.58 (0.17, 1.96) | 0.75 (0.24, 2.31) | 0.874 |

Dependent variable: presence of JSN; Independent variable: quartiles 2, 3 or 4 of IL-6 (>2.0–2.9, >2.9–4.0, ≥4.0 pg/ml) or TNF-α (>5.1–7.3, >7.3–11.6, ≥11.6 pg/ml) vs quartile 1. Bold denotes statistically significant result.
* Adjusted for sex, age, BMI, smoking, steps per day, knee pain, osteophytes, asthma, cardiovascular diseases, and diabetes.

association decreased in magnitude after further adjustment for IL-6 ($P = 0.035$, data not shown). TNF-α was also associated with 1.4-fold increased odds per quartile of whole tibiofemoral (prevalence in either medial or lateral compartment) JSN ($P = 0.007$). In addition, in adjusted analysis, subjects in the highest quartile of TNF-α were 3.2 times more likely to have prevalent medial JSN than those in the lowest quartile (Table III). TNF-α analysed by either quartiles (Table II) or median cutpoint was not associated with osteophytes.

At baseline, IL-6 was not associated with tibial cartilage volume (Table I). However, over approximately 3 years, subjects in the highest quartile of IL-6 had greater loss of both medial and lateral tibial cartilage volume than those with lowest quartile of IL-6, in unadjusted analyses (both $P < 0.05$, data not shown). Quartiles of IL-6 were associated with loss of both medial and lateral tibial cartilage volume after adjustment for covariates (Table IV, $P < 0.05$ for medial and $P < 0.01$ for lateral) and these remained largely unchanged further adjustment for TNF-α ($P < 0.05$ for medial and $P < 0.01$ for lateral, data not shown). Change in quartiles of IL-6 was also significantly associated with loss of both medial and lateral tibial cartilage volume after adjustment for potential confounders (Table IV; both $P < 0.05$). These associations decreased in magnitude and only remained significant for lateral cartilage volume loss ($P < 0.05$, data not shown) after adjustment for change in TNF-α. When quartiles were examined separately in adjusted analyses, we found that the highest quartile of baseline IL-6 had 3.5% and 4.2% per year more loss of medial and lateral tibial cartilage volume, respectively, when compared to the lowest quartile (Table V). Also, quartile 3 of baseline IL-6 had 2.7% per year more loss of lateral tibial cartilage volume than quartile 1 (Table V).

Baseline TNF-α was not associated with baseline tibial cartilage volume (data not shown) and change in tibial cartilage volume (Table IV); however, change in quartiles of TNF-α was associated with loss of medial tibial cartilage volume both before and after adjustment for potential confounders (Table IV, both $P < 0.05$). This association decreased in magnitude but remained significant after further adjustment for change in IL-6 (data not shown). No quartile of TNF-α contributed significantly more to cartilage volume loss than the lowest quartile (Table V).

We did not find any significant association between hs-CRP and JSN, cartilage volume and change in cartilage volume (data not shown), or significant results using IL-6 or TNF-α as a continuous predictor.

**Table IV**
Associations between cytokines at baseline, change in cytokines and change in knee cartilage volume: longitudinal data

| | Multivariable* β (95% CI) | Multivariable** β (95% CI) |
|---|---|---|
| *Baseline IL-6 (quartiles)* | | |
| Medial tibial (%/year per quartile) | **−1.07 (−1.98, −0.17)** | **−1.19 (−2.21, −0.18)** |
| Lateral tibial (%/year per quartile) | **−0.80 (−1.58, −0.03)** | **−1.35 (−2.23, −0.46)** |
| *IL-6 change in quartile* | | |
| Medial tibial (%/year per quartile) | **−1.08 (−1.90, −0.25)** | **−1.18 (−2.10, −0.25)** |
| Lateral tibial (%/year per quartile) | −0.55 (−1.26, 0.16) | **−1.06 (−1.86, −0.25)** |
| *Baseline TNF-α (quartiles)* | | |
| Medial tibial (%/year per quartile) | −0.59 (−1.47, 0.30) | −0.60 (−1.61, 0.41) |
| Lateral tibial (%/year per quartile) | −0.19 (−0.97, 0.58) | −0.29 (−1.20, 0.63) |
| *TNF-α change in quartile* | | |
| Medial tibial (%/year per quartile) | **−1.24 (−2.00, −0.45)** | **−1.27 (−2.20, −0.33)** |
| Lateral tibial (%/year per quartile) | −0.37 (−1.03, 0.30) | −0.51 (−1.36, 0.33) |

β: regression coefficient. Dependent variable: change in cartilage volume, %/year. Independent variable: quartiles of IL-6 (≤2.0, >2.0–2.9, >2.9–4.0, ≥4.0 pg/ml) or TNF-α (≤5.1, >5.1–7.3, >7.3–11.6, ≥11.6 pg/ml). Bold denotes statistically significant result.
* Adjusted for baseline IL-6 or TNF-α (for change in IL-6 or TNF-α, respectively) or change in IL-6 or TNF-α (for baseline IL-6 or TNF-α, respectively).
** Further adjusted for sex, age, BMI, smoking, steps per day, tibial bone area, knee pain, asthma, cardiovascular diseases, and diabetes.

## Discussion

This study is the first, to our knowledge, to determine the associations between circulating inflammatory markers and cartilage loss in a population-based study of older adults. Cross-sectionally, serum levels of IL-6 and TNF-α were both associated with JSN in the medial tibiofemoral compartment (both $P < 0.05$) but not with tibial cartilage volume. Longitudinally, serum levels of IL-6 predicted loss of medial and lateral tibial cartilage volume, and change in IL-6 and TNF-α were associated with loss of medial and lateral tibial cartilage volume. The associations of IL-6 and TNF-α were, in part, dependent of each other, except for the associations between baseline IL-6 and cartilage loss. In contrast, serum levels of hs-CRP were not associated with JSN, tibial cartilage volume and change in tibial cartilage volume.

IL-6 is produced by T cells, B cells, monocytes, fibroblasts[26], as well as osteoblasts from subchondral bone, osteophytes of OA under mechanical loading and adipocytes of the infrapatellar fat pad[27–29]. IL-6 can stimulate synoviocyte proliferation and osteoclast activation, leading to synovial pannus formation and matrix metalloproteinases production, which induces joint and cartilage destruction[26]. IL-6 has been reported to correlate with clinical

**Table V**
Associations between cytokines at baseline and change in knee cartilage volume: comparisons between quartiles of cytokines in longitudinal data

| Model | Predictor | Compartment | β (95% CI) | | | | *P*-value for trend |
|---|---|---|---|---|---|---|---|
| | | | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | |
| Multivariable* | IL-6 | Medial | 0.00 (Reference) | −1.64 (−4.33, 1.04) | −2.52 (−5.82, 0.78) | −2.58 (−5.62, 0.45) | **0.020** |
| | | Lateral | 0.00 (Reference) | −2.27 (−4.60, 0.07) | −2.04 (−4.93, 0.84) | **−2.82 (−5.31, −0.32)** | **0.043** |
| | TNF-α | Medial | 0.00 (Reference) | 0.67 (−1.53, 2.86) | −0.05 (−3.09, 2.99) | −2.17 (−5.21, 0.86) | 0.192 |
| | | Lateral | 0.00 (Reference) | 0.32 (−1.82, 2.47) | −1.20 (−3.82, 1.42) | −0.37 (−2.98, 2.25) | 0.622 |
| Multivariable** | IL-6 | Medial | 0.00 (Reference) | −1.07 (−3.49, 1.34) | −2.56 (−5.38, 0.26) | **−3.50 (−6.62, −0.37)** | **0.022** |
| | | Lateral | 0.00 (Reference) | −2.10 (−4.20, 0.00) | **−2.65 (−5.10, −0.19)** | **−4.21 (−6.93, −1.50)** | **0.003** |
| | TNF-α | Medial | 0.00 (Reference) | 0.46 (−2.05, 2.97) | −0.14 (−3.00, 2.72) | −1.73 (−4.85, 1.40) | 0.241 |
| | | Lateral | 0.00 (Reference) | −0.07 (−2.34, 2.20) | −0.62 (−3.22, 1.98) | −0.78 (−3.62, 2.07) | 0.539 |

Dependent variable: change in cartilage volume, %/year; Independent variable: quartiles 2, 3 or 4 of IL-6 (>2.0–2.9, >2.9–4.0, ≥4.0 pg/ml) or TNF-α (>5.1–7.3, >7.3–11.6, ≥11.6 pg/ml) vs quartile 1. Bold denotes statistically significant result.
* Adjusted for baseline IL-6 or TNF-α (for change in IL-6 or TNF-α, respectively) or change in IL-6 or TNF-α (for baseline IL-6 or TNF-α, respectively).
** Further adjusted for sex, age, BMI, smoking, steps per day, tibial bone area, knee pain, asthma, cardiovascular diseases, and diabetes.

manifestations and laboratory markers of rheumatoid arthritis, and therapies involving blockade of IL-6 functions have constituted a new therapeutic strategy for rheumatoid arthritis and other inflammatory diseases[30]. In contrast, the role of IL-6 in OA remains much less studied.

In the Hartley guinea pig model of OA, serum levels of IL-6 were found to correlate positively with total histological score of OA (assessing cartilage structure abnormalities and proteoglycan loss), independently of age and weight[14]. The findings from clinical studies are far less convincing. In 41 patients with knee OA, IL-6 expression in synovial membrane was not associated with Kellgren–Lawrence radiological score and WOMAC pain[31], and in 29 patients with knee or hip OA, IL-6 in serum and urine was not associated with clinical measures such as soft tissue swelling[32]. These two studies are both limited by small sample size. Penninx *et al.*[33] reported that the serum concentration of IL-6 was not associated with WOMAC pain, stiffness and function and ROA, but tended to be associated with slower walking speed in 272 patients with knee OA. Toncheva *et al.*[34] reported that serum levels of IL-6 were significantly elevated in patients with active OA (expressing swelling, local hyperthermia and high erythrocyte sedimentation rate) but not in those with inactive OA (lacking above symptoms), compared with healthy controls; however, the association with JSN or cartilage loss was not reported. Livshits *et al.* reported that circulating IL-6 predicted the incidence of whole-joint knee ROA assessed by the Kellgren–Lawrence scoring system in a population of women[19]. Furthermore, Sakao *et al.*[35] reported that IL-6 expression was significantly enhanced in subchondral bone osteoblasts with knee OA compared to subchondral bone osteoblasts without knee OA, and IL-6 expression was greater in patient with severe cartilage damage than those with mild cartilage damage.

In this study, we found that serum levels of IL-6 were associated with JSN in the medial tibiofemoral compartment and whole compartment. Importantly, baseline IL-6 levels were associated with loss of tibial cartilage volume, independently of TNF-α, and change in IL-6 was also associated with change in tibial cartilage volume over approximately 3 years. These associations were independent of potential confounders including age, sex, BMI, tibial bone area, smoking, physical activity and other disease status. These results suggest that IL-6 may play a role in cartilage loss at an early stage of knee OA. This may have clinical significance; for example, if rates of loss are constant over time it can be estimated that patients with low level IL-6 (<2 pg/ml, 0.6% per annum loss in this sample) will never lose the amount of medial tibial cartilage required to reach end-stage OA[36] (when 60% of cartilage is lost) as it will take 100 years, but this deterioration will take only 15 years for patients with high IL-6 levels (>4.0 pg/ml, 4.1% per annum loss). This effect would be of major clinical importance. Furthermore, we found that prevalence of osteophytes was greater in subjects with high levels of serum IL-6 (≥median) than those with low levels of IL-6. This is in line with a recent report which demonstrated that IL-6 production in osteoblasts isolated from the OA osteophytes was significantly higher than that of osteoblasts from subchondral bone without OA[27], and suggest that IL-6 may play a role in osteophyte formation in OA, though this needs to be confirmed by longitudinal studies.

TNF-α appears to play a pivotal role in cartilage matrix degradation and bone resorption in OA. It can induce the production of other cytokines (such as IL-6), matrix metalloproteinases and prostaglandins[9], and inhibit the synthesis of proteoglycans and type II collagen[6]. TNF-α mRNA levels were up-regulated in OA-affected cartilage compared to normal cartilage[37]. Penninx *et al.*[33] reported that in patients with knee OA, serum levels of TNF-α were not associated with WOMAC knee pain, stiffness and radiographic scores, but higher serum levels of the soluble receptors TNF-sR1 and TNF-sR2 were significantly associated with greater knee pain and stiffness, and tended to be associated with worse radiographic scores. A Dutch group reported that high innate *ex vivo* production of TNF-α in whole-blood assay upon lipopolysaccharide stimulation was not associated with an increased risk of OA[38]; however, longitudinally, patients in the highest quartile of TNF-α production had a 6-fold increased risk of JSN progression compared to those in the lowest quartile over 2 years[20].

In this study, we found that serum levels of TNF-α were significantly associated with JSN in tibiofemoral compartments. Our results associating TNF-α with prevalent JSN do not match the negative findings of Livshits *et al.*[19], perhaps due to their different measure of ROA. Although serum levels of TNF-α at baseline were not associated with loss of cartilage volume, change in TNF-α levels was associated with change in cartilage volume over approximately 3 years. These results suggest that TNF-α plays a role in cartilage loss of early OA but the causal relationship needs to be explored in future studies.

It was expected that the significant associations detected in this study might decrease in magnitude or even because non-significant after adjustment for TNF-α for IL-6 or IL-6 for TNF-α, because serum levels of IL-6 and TNF-α were related to each other. This was true for most analyses. The associations between baseline IL-6 and longitudinal cartilage volume loss remained largely unchanged after adjusting for TNF-α, and the association between baseline TNF-α and medial JSN remained significant after adjusting for IL-6. In contrast, there are no significant associations between TNF-α and longitudinal cartilage volume loss, and the association between baseline IL-6 and medial JSN became non-significant after adjusting for TNF-α. The reasons for these inconsistencies are unclear, but it may suggest that IL-6 plays a more important role in cartilage loss

at an earlier stage and TNF-α plays a more important role at a later stage when JSN is seen on X-ray. These preliminary findings need to be replicated by further studies with larger sample size and longer study period.

We did not find any cross-sectional associations between tibial cartilage volume and either IL-6 or TNF-α in this study, despite finding associations between change in those variables and change in cartilage volume. The reasons for this are unclear, but may be due to the fact that knee cartilage volume measured at a given point in time cannot distinguish normal from swollen cartilage[39]. Although IL-1 has been demonstrated to play a major role in cartilage destruction of OA by *in vitro* and animal studies[9], serum levels of IL-1 were mostly undetectable in this study, suggesting IL-1 may be not as important as IL-6 and TNF-α in initiating cartilage loss of early OA in the general population. We also did not find any association between serum levels of hs-CRP, JSN and cartilage loss in older adults, even though serum hs-CRP levels significantly correlated with serum IL-6 levels. This differs from previous reports in established OA[16,17], though studies of CRP in incident knee OA are equivocal[19,40], suggesting CRP may not be the best marker of inflammation in early OA.

The strengths of the present study lie in the measurements of inflammatory markers on two occasions which allowed us to assess the variation in these markers over time. Variation in inflammatory markers may be less affected by natural antagonists in the serum than cross-sectional levels of these markers. Our study has several potential limitations. First, the response rate at baseline was 57%, possibly due to the demands on study participants in that each visit took 3 h. This did leave the possibility open for selection bias. However, there were no significant differences in age and gender between those responded and those did not. We also had high rates of retention (82%) to offset this. Second, our modest sample size may not allow us to examine weak associations between some inflammatory markers (such as hs-CRP) and osteoarthritic changes. A third limitation is our measurement of inflammatory marker levels in serum rather than levels in synovial fluid, which may represent a more appropriate and sensitive measure for inflammatory markers in knee OA (such as IL-6 and TNF-α). Also, as we did not find significant results using predictors as continuous variables, we cannot rule out the possibility that the relationships are model-specific, and replications using an assay with higher sensitivity which may provide significant results with continuous IL-6 or TNF-α data are required in future studies. Lastly, measurement error may result in some non-differential misclassification; however, as all measures (e.g., cartilage volume, JSN, and inflammatory markers) were highly reproducible, this is likely to be low.

In conclusion, circulating levels of IL-6 in older people are associated with knee JSN and knee cartilage loss, and level of TNF-α is associated with JSN, suggesting low level inflammation may play a role in the pathogenesis of knee OA.

## Contributions

Study design: Jones, Cicuttini, Ding; Acquisition of data: Jones, Parameswaran, Burgess, Ding; Analysis and interpretation of data: Stannus, Jones, Cicuttini, Ding; Manuscript preparation: Stannus, Jones, Parameswaran, Quinn, Burgess, Cicuttini, Ding; Statistical analysis: Stannus, Quinn, Ding.

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## Conflict of interest

GJ serves on an advisory board, has performed clinical trials and given talks for Roche, who make an IL-6 receptor blocker. However, Roche did not fund this study nor did they have any input into the writing of this manuscript.

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