**Cross-sectional and longitudinal associations between serum inflammatory cytokines and knee bone marrow lesions in patients with knee osteoarthritis**

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**ABSTRACT**

***Objective.*** To describe cross-sectional and longitudinal associations between serum levels of interleukin (IL) - 6, IL-17A, IL-17F, IL-23 and knee bone marrow lesions (BMLs) in patients with knee osteoarthritis (OA).

***Design*.** Patients (n=192) with symptomatic knee OA (mean 63 years, range 50-79, female 53%) were assessed at baseline and after 24 months. At each time point, serum IL-6, IL-17A, IL-17F and IL-23 were measured using Bio-Plex® Multiplex Immunoassays with Luminex xMAP technology. Knee BMLs were scored using the modified whole organ MRI score (WORMS) from T2 weighted fat-supressed fast spin echo magnetic resonance imaging (MRI). Multivariable linear regression and log binominal regression were used to determine the associations between cytokines and BMLs.

***Results****.* Baseline IL-6 (quarters) were significantly associated with total knee BMLs (p<0.01 for the trend) as well as associated with an increase in BML score (p=0.05 for the trend), after adjustment for confounders. Baseline IL-17F and IL-23 (highest quarters vs others) was associated with an increase in BML score in females (p=0.04 for IL-17F; p=0.01 for IL-23), but not in males, in multivariable analyses. In contrast, IL-17A was not significantly associated with BMLs in either females or males.

***Conclusion.*** IL-6 is associated with increased knee BMLs in both females and males with OA. Serum IL-17F and IL-23 predicted increased knee BML scores in females only, suggesting that inflammation is involved in BML pathogenesis in knee OA, especially in women.

**Trail Registration.** ClinicalTrials.gov identifier: NCT01176344; Australian New Zealand Clinical Trials Registry: ACTRN12610000495022

**INTRODUCTION**

Osteoarthritis (OA) is the most frequent type of arthritis worldwide. It is characterized by progressive loss of cartilage, deterioration of subchondral bone and mild synovial inflammation [[1](#_ENREF_1)]. While OA has traditionally been regarded as a non-inflammatory type of arthritis, there is a growing evidence that the clinical course of OA may be driven by systemic and localized inflammation [[2-4](#_ENREF_2)], albeit at much lower levels than the recognised inflammatory arthropathies [[5](#_ENREF_5), [6](#_ENREF_6)].

IL-6 is an inflammatory cytokine with pro- and anti-inflammatory effects, both inside and outside of the joints [[7-9](#_ENREF_7)]. Although emerging evidence suggests that low-level systemic inflammation is involved in OA pathogenesis [[2](#_ENREF_2), [10](#_ENREF_10)], the role of IL-6 in OA remains controversial. In a spontaneous aging model of OA, mice deficient in IL-6 displayed increased levels of cartilage loss, suggesting a potentially protective role of IL-6 in the development of OA. In contrast, higher levels of serum IL-6 were associated with higher prevalence of osteophytes in older adults [[3](#_ENREF_3)]. Circulating levels of IL-6 have been associated with the prevalence of knee OA [[5](#_ENREF_5)].

IL-17A and IL-17F are the prototypical members of the IL-17 cytokine family, which are produced by CD4 (+) T-helper 17 cells (Th-17) [[11](#_ENREF_11)]. IL-17A and IL-17F share the most homology at the amino acid level [[12](#_ENREF_12)], have overlapping but also distinct effector functions in a range of autoimmune diseases [[13](#_ENREF_13)]. The development of CD4 (+) Th-17 cells is differentiated by IL-6, and stabilised by IL-23 [[11](#_ENREF_11)]. Produced by antigen presenting cells (APCs), IL-23 is a heterodimeric protein composed of a p19 and a p40 subunit. Several groups have demonstrated that IL-1β, IL-6 and IL-23 promote human Th-17 cells differentiation from CD4+ cells, resulting in the expression of IL-17A, IL-17F, and IL-6 [[14](#_ENREF_14), [15](#_ENREF_15)]. Thus, IL-6, IL-23 and IL-17 form a new axis through Th-17 cells, which has an important role in autoimmunity and chronic inflammation [[16-19](#_ENREF_16)].

Bone marrow lesions (BMLs), observed as ill-defined hyperintense signals in subchondral bone of the knees on magnetic resonance imaging (MRI) scans, are an important feature of knee OA. They are strongly associated with knee pain [[20](#_ENREF_20), [21](#_ENREF_21)], and OA incidence [[22](#_ENREF_22)] and progression [[23](#_ENREF_23), [24](#_ENREF_24)]. Additionally, BMLs predict knee joint space loss on x-ray [[23](#_ENREF_23)], cartilage defect progression [[25](#_ENREF_25)] and cartilage loss on MRI [[26](#_ENREF_26)], as well as knee replacement surgery [[27](#_ENREF_27), [28](#_ENREF_28)]. However, thus far, the etiology of BML formation is largely unknown. An experimental study reported BMLs might originally correspond to an acute inflammatory response, oedema, contusion and/or necrosis, which were eventually replaced by permanent bone marrow remodelling such as fibrosis and myxomatous connective tissue [[29](#_ENREF_29)]. BMLs could be caused by an inflammatory reaction to cartilage breakdown products, or other factors in intruded synovial fluid [[30](#_ENREF_30)]. Our previous study reported that high serum hs-CRP was associated with increased knee BMLs in patients with knee OA, suggesting that systemic inflammation may play a role in the pathogenesis of BMLs in knee OA patients [[31](#_ENREF_31)].

Although IL-6/IL-23/IL-17 axis has been implicated in the pathogenesis of many inflammatory conditions [[12](#_ENREF_12)], its’ roles in human OA are unclear. Therefore, the aim of this study was to describe cross-sectional and longitudinal associations between serum IL-6, IL-17A, IL-17F, IL-23 and knee BMLs in patients with knee OA.

**SUBJECTS AND METHODS**

This study was a sub-analysis from the Vitamin D Effects on Osteoarthritis (VIDEO) Study, which was a multi-centre parallel-group, randomized, placebo-controlled and double-blind clinical trial to evaluate the effects of vitamin D supplementation in patients with symptomatic knee OA and a low 25-hydroxyvitamin D (25OHD) levels. Measures of IL-6, IL-17A, IL-17F and IL-23 were made from the 192 patients (mean 63 years, range 49-79 years, female 53%) recruited in Hobart. Inclusion and exclusion criteria were the same as for the VIDEO study [[32](#_ENREF_32)]. Briefly, eligible subjects met the American College of Rheumatology (ACR) criteria for clinical knee OA [[33](#_ENREF_33)], and had a pain score more than 20 mm on a 100 mm visual analogue scale (VAS). They also had an ACR function class rating of I, II and III [[34](#_ENREF_34)], and relatively good health, with a score of 0 to 2 out of a maximum score of 4 on a global investigator assessment of disease status [[35](#_ENREF_35)], where 0 indicates very good health and 4 indicating very poor health.

Subjects were included if their serum 25OHD levels were > 12.5 nmol/L and < 60 nmol/L and were randomly assigned to receive either a monthly capsule of 50,000 IU (1.25 mg) vitamin D3 (cholecalciferol) or identical placebo for two years. Exclusion criteria included grade 3 radiographic changes according to Altman’s atlas [[36](#_ENREF_36)], severe knee pain on standing (more than 80 mm on a l00 mm VAS), contraindication to MRI, rheumatoid or psoriatic arthritis, lupus, cancer, and severe cardiac or renal impairment.

The VIDEO study was approved by the Tasmania Health and Human Medical Research Ethics Committee (reference number H1040). Written informed consent was obtained from all participants.

**Inflammatory markers measurements.** Serum levels of IL-6, IL-17A, IL-17F and IL-23 were measured at baseline and after 24 months using enzyme-linked immunosorbent assay [[6](#_ENREF_6)]. The limits of detection are 0.49 pg/ml, 1.84 pg/ml, 14.6 pg/ml and 34.4 pg/ml respectively. The proportion of participants with IL-6 below limit of detection was 17%. The majority of study participants had IL-17A, IL-17F and IL-23 levels below limits of detection, with the proportions of measurements below their limits of detection for IL-17A, IL-17F and IL-23 were 78%, 60% and 77%, respectively. The coefficients of variation (CVs) were 5.8%-6.3%.

**Assessment of bone marrow lesions.** BMLs in the diseased knee, or of the less painful knee if both knees (aiming to avoid “ceiling effects” of the treatment on disease outcomes when designed in original randomized controlled trail (RCT) study) were affected were assessed on MR images. These were acquired with a 1.5T whole-body magnetic resonance unit (Picker, Cleveland, Ohio, USA) using a commercial transmit-receive extremity coil at baseline and 24 months later. Image sequence included the following: Fat-saturated T2-weighted fast spin echo (FSE), flip angle 90º, repetition time 3067 ms, echo time 112 ms, field of view 16 cm, 45 partitions, 228×256-pixel matrix; sagittal images were obtained at a partition thickness of 2 mm.

BMLs were measured by one trained observer (ZZ) using the modified whole organ MRI score (WORMS) [[37](#_ENREF_37)]. In the modified WORMS, the medial and lateral tibial and femoral compartments are divided into three sub-regions (anterior, central, posterior), and the tibia has an additional subspinous sub-region which represents the portion of the tibia beneath the tibial spines. Thus, a total of 15 sub-regions were scored for each knee [[37](#_ENREF_37)].

BMLs were scored according to the maximal percentage of bone area that the lesion occupied within the total sub-region. We scored grade 0 if no BMLs were present; grade 1 if lesion size=<25% of the subregion; grade 2, 25% to 50% of the subregion; grade 3, > 50% of the subregion [[37](#_ENREF_37)]. Total knee BML scores were obtained by summing the BML scores of all the sites, giving a potential total knee BMLs score range of 0 to 45. Baseline and follow-up MRIs were scored in pairs in chronological order to minimise measurement error [[38](#_ENREF_38)]. The intra-rater reliability (ICC=0.93-0.98) and inter-rater reliability (ICC=0.91-0.97) were excellent. Presence of BMLs in the whole knee was defined as a BML score of ≥1 at any subregion. For longitudinal analyses we defined an increase in BML score of greater than one between baseline and follow-up to be the outcome of interest.

**Anthropometrics.** 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 (Model 707; Seca Delta, Hamburg, Germany) that were calibrated using a known weight at the beginning of each clinic. Body mass index (BMI) [weight (kg)/height2 (m2)] was calculated.

**25OHD assays.** Serum 25OHD was assayed by the Liaison method at baseline, utilizing a direct competitive chemiluminescent immunoassays (DiaSorin Inc, Stillwater, Minnesota, USA). The intra-assay and inter-assay coefficients of variation were 3.2% and 6.0%, respectively [[39](#_ENREF_39)].

**DATA ANALYSIS**

Student’s t-tests, χ2 tests or Wilcoxon rank-sum test (when appropriate) were used to compare means, proportions or median between those with or without baseline knee BMLs.

Baseline IL-6 was highly right-skewed, hence quarters were used for subsequent analyses, the other cytokines were dichotomised at the highest quarters. Baseline BML score had a right-skewed and zero-inflated distribution, thus multivariable negative binomial regression models were used to determine associations between cytokines and total knee BML scores, adjusted for age, sex, BMI and vitamin D level. Negative binomial regressions were used to address the problems of the unusual distribution of the BML score variable at baseline, which was bounded (at zero), highly skewed, and with a large proportion of zero scores. Alternative models were investigated but ultimately rejected. Serum levels of 25-(OH)D were associated with inflammatory markers including IL-6, IL-17, IL-23 [[40-42](#_ENREF_40)], as well as BMLs [[43](#_ENREF_43), [44](#_ENREF_44)]; therefore, 25-(OH)D levels were used for adjustment as a potentially confounding factor.

Log binominal regression was used to assess associations between baseline cytokines and presence of an increase in total knee BMLs, adjusted for age, sex, BMI, vitamin D levels and baseline total knee BMLs. Log-binomial regression models were used to estimated relative risks where the outcome was dichotomous. Logistic regression was also considered for this type of analysis but rejected since the resultant odds rations would overestimate the more appropriate relative risks due to the common nature of the outcome (an increase in BMLs from baseline to follow-up). Interactions between sex and cytokines were tested in all multivariable models. When significant interactions were identified, estimates are presented separately for each sex.

A *p* value less than 0.05 (two tailed) was regarded as statistically significant. All statistical analyses were performed on Stata version 12.0 for Windows (StataCorp, College Station, TX, USA).

**RESULTS**

**Characteristics of the study population at baseline.** A total of 192 subjects (53% female) aged between 49 and 79 years old (mean 63 years) were included. 80% had BMLs visible on MR imaging at baseline. Characteristics of the participants at baseline are presented in Table 1. No statistically significant differences were found between participants with and without baseline BMLs in terms of age, sex, BMI and baseline serum cytokine levels (Table 1).

**Associations between IL-6 and BMLs.** Patients in the higher quarters of baseline IL-6 had higher baseline total knee BML scores (Figure 1a), and were more likely to have an increase in total knee BMLs over 2 years (Figure 1b).

Table 2 describes cross-sectional and longitudinal associations between serum IL-6 and total knee BMLs over 2 years. Baseline IL-6 (quarters) were statistically significantly associated with baseline total knee BML score, after adjustment for age, sex, BMI and vitamin D levels (Table 2). Higher baseline IL-6 (quarters) were statistically significantly associated with greater risks of increased BML scores over 2 years, after adjustment for age, sex, BMI, vitamin D levels and baseline total knee BMLs, in log binomial regression analyses (Table 2). There were no statistically significant interactions between sex and IL-6 (data not shown), therefore men and women were combined for analyses.

**Associations between IL-17A, IL-17F, IL-23 and BMLs.** Table 3 describes cross-sectional and longitudinal associations between serum IL-17A, IL-17F and IL-23 and total knee BMLs over 2 years in males and females. Interactions between sex and IL-17F, IL-23 on BMLs were statistically significant (both p<0.1), so men and women were analysed separately. Additionally, no statistically significant sex interaction was found for IL-17A, however since this interleukin is part of the same family as the others and they are located adjacent to each other and exhibit a similar cysteine knot configuration, we present results separately for males and females also.

Dichotomized baseline IL-17A, IL-17F and IL-23 (highest quartile vs others or detectable vs undetectable) were not associated with baseline total knee BMLs in females or males, before or after adjustment for age, BMI and vitamin D levels, in multivariable negative binominal regression analyses (Table 3). In females, those with higher baseline IL-17F and IL-23 had 1.9-fold and 2.1-fold higher risks of increased BML scores, after adjustment for age, BMI, vitamin D levels and baseline total knee BMLs, in log binomial regression analyses (Table 3, Figure 2). However, no statistically significant associations were found in male group (Table 3). IL-17A was not statistically significantly associated with an increase in BMLs in either females or males (Table 3).

**DISCUSSION**

This study is the first, to our knowledge, to assess longitudinal associations between IL-6, IL-23 and IL-17, and knee BMLs in patients with knee OA. Our data showed that higher serum IL-6 was associated with greater likelihood of an increase in knee BMLs over 2 years, and that high levels of IL-17F and IL-23 predicted an increase in knee BMLs in females (but not males) with knee OA. These results add to the growing evidence that systematic inflammation is involved in the pathogenesis of knee OA, particularly among females [[2](#_ENREF_2), [3](#_ENREF_3)].

IL-6 levels are elevated locally at sites of inflammation, and can activate B cells, T cells and other inflammatory cells [[45](#_ENREF_45)]. Increased IL-6 expression has also been observed in subchondral bone and osteophytes of subjects with knee OA [[46](#_ENREF_46)]. In our present study, associations between serum IL-6 and total knee BMLs as well as increases in knee BMLs over 2 years were independent of potential confounders including age, sex, BMI and vitamin D levels, suggesting that serum IL-6 is involved in the pathogenesis of BMLs in knee OA patients and predicts BML development/progression. This is in line with our previous report in which a higher circulating levels of IL-6 was an independent predictor of worsening knee pain [[47](#_ENREF_47)].

A number of investigations have demonstrated roles for Th17 cytokines in the aetiology and progression of rheumatoid arthritis (RA) [[48](#_ENREF_48)]; the cytokines are expressed by rheumatoid synovium and can induce inflammation and osteoclastic bone resorption [[49](#_ENREF_49)]. IL-17A and IL-17F are pro-inflammatory cytokines, and have regulatory roles in host defence and chronic inflammation, resulting in tissue damage and autoimmunity [[50](#_ENREF_50), [51](#_ENREF_51)]. However, little is known about the role of IL-17 in OA. Chen et al [[52](#_ENREF_52)] reported a positive association between knee OA severity and IL-17 concentration in synovial fluid, but not in serum. Liu et al [[53](#_ENREF_53)] showed that synovial IL-17 level was correlated with severity of knee pain, but not with radiographic severity. These studies were limited by their cross-sectional design, small sample size and lack of adjustment for potential confounders. In our present study, we found that baseline IL-17F predicted increased BML scores in females but not males. Associations between IL-17A and BMLs were consistently positive in both females and males, but none reached statistical significance. IL-17A and IL-17F are expected to have similar physiological effects because they are located adjacent and exhibit a similar cysteine knot configuration [[54](#_ENREF_54)]. However, our findings suggest that IL-17F may play a more important role in human knee OA aetiology than IL-17A, but the underlying reasons for this difference are unknown. In this study, we also found that baseline serum IL-23 predicted increased BMLs in females but not males; this association was independent of potential confounders such as age, BMI and vitamin D levels. Combined with the associations that we found between IL-6, IL-17F and knee BMLs in this study, these results suggest that IL-6/IL-23/IL-17 axis may play a role in the aetiology and progression of knee OA, at least in females. Further studies investigating other OA- related features (meniscal tears, ligamentous lesions, cartilage defects, etc.) and IL-6/IL-23/IL-17 axis are required to provide more comprehensive understanding of the relationship between inflammation and knee OA progression.

The reasons for why we observed a sex difference for the association between IL-17F/IL-23 and BMLs, but not between IL-6 and BMLs are unclear. It may reflect the influence of sex hormones on the relationships of IL-17/IL-23 and knee BMLs. We previously reported that serum levels of IL-6 were associated with hip radiographic changes in females but not in males [[55](#_ENREF_55)], which was inconsistent with the present study. This may imply that there are different inflammatory pathways between hip OA and knee OA in the relationship with IL-6.

This study has several limitations. First, the original study was a randomized controlled trail (RCT); therefore, results could be affected by the intervention (vitamin D supplementation). However, all associations from longitudinal analyses remained significant after adjustment for vitamin D levels. Secondly, giving that the majority of subjects had cytokine levels under the limits of detection for most cytokines, we dichotomized predictors rather than using them as continuous variables. Therefore, we cannot completely exclude the possibility that the relationships are model-specific, and replications using predictors with higher sensitivity are required in future studies. Third, we did not measure ligamentous and meniscal status that may be risk factors of BMLs [[56](#_ENREF_56)]; however, there is no evidence showing that serum levels of inflammatory cytokines are associated with meniscal and/or ligamentous status, so meniscal and/or ligamentous status seem unlikely to be potential confounders for the associations between inflammatory cytokines and BMLs. Lastly, as inclusion and exclusion criteria were applied in the original RCT design, the generalizability to the general knee OA population needs to be confirmed.

In conclusion, while serum IL-6 is associated with increased knee BMLs in both female and male OA patients, serum IL-17F and IL-23 only predict increased knee BML scores in females. These suggest that inflammation is involved in BML pathogenesis in knee OA, with the IL-6/IL-23/IL-17 axis having a role particularly in women.

**Table 1** Characteristics of participants at baseline, by presence or absence of BMLs at baseline

|  |  |  |  |
| --- | --- | --- | --- |
|  | Without Baseline BMLs  (N=36) | With Baseline BMLs  (N=156) | p value |
|  |  |  |  |
| Age (years) | 62.2 ± 7.3 | 63.3 ± 7.0 | 0.32 |
| Females (%) | 54% | 49% | 0.42 |
| BMI (kg/m2) | 30.1 ± 6.0 | 29.5 ± 4.8 | 0.38 |
| 25-(OH) D (nmol/L) | 44.4 ± 13.1 | 45.6 ± 13.6 | 0.47 |
| IL-6 (pg/ml) | 1.33 (0.4, 3.7) | 1.16 (0.4, 2.5) | 0.65 |
| IL-17A (%) \* | 20% | 25% | 0.48 |
| IL-17F (%) \* | 36% | 39% | 0.97 |
| IL-23 (%) \* | 17% | 28% | 0.08 |

Two-tailed Student’s t tests were used for differences between means, χ2 tests were used for proportions (percentages) and Wilcoxon rank-sum tests were used for differences between medians

Results are mean ± SD or median (IQR) except for percentage. BMI, body mass index. 25-(OH)D: 25-hydroxyvitamin D; IL: interleukin. \* Proportions of measurements above their limits of detection.

Limits of detection: IL-6, 0.49 (pg/ml); IL-17A, 1.84 (pg/ml); IL-17F, 14.6 (pg/ml); IL-23, 34.4 (pg/ml).

**Table 2** Cross-sectional and longitudinal associations between serum IL-6 and total knee BMLs/an increase in BMLs over 2 years

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Univariable** | | | **Multivariable** | | |
| ***Total knee BML scores*** | RM† | 95% CI |  | RM† | 95% CI |  |
| Baseline IL-6 (quarter)=1 |  | Ref |  |  | Ref |  |
| Baseline IL-6 (quarter)=2 | 1.23 | (0.83, 1.83) |  | 1.24 | (0.83, 1.86) |  |
| Baseline IL-6 (quarter)=3 | **1.63** | **(1.11, 2.40)** |  | **1.68** | **(1.12, 2.52)** |  |
| Baseline IL-6 (quarter)=4 | **1.61** | **(1.08, 2.39)** |  | **1.62** | **(1.09, 2.42)** |  |
| p for the trend |  | **p<0.01** |  |  | **p\*<0.01** |  |
|  |  |  |  |  |  |  |
| ***An increase in BMLs*** | RR | 95% CI |  | RR | 95% CI |  |
| Baseline IL-6 (quarter)=1 |  | Ref |  |  | Ref |  |
| Baseline IL-6 (quarter)=2 | 1.21 | (0.60, 2.43) |  | 1.25 | (0.62, 2.50) |  |
| Baseline IL-6 (quarter)=3 | 1.36 | (0.70, 2.67) |  | 1.58 | (0.80, 3.12) |  |
| Baseline IL-6 (quarter)=4 | 1.74 | (0.92, 3.29) |  | **1.90** | **(1.01, 3.58)** |  |
| p for the trend |  | p=0.07 |  |  | **p\*\*=0.05** |  |

The dependent variable: baseline total knee BML score in negative binominal models, or an increase in total knee BMLs in log binominal models. The independent variable: quarters of IL-6.

†Ratio of means presented from negative binomial model;

\*Adjustment for age, sex, BMI and vitamin D levels;

\*\* Adjusted for age, sex, BMI, vitamin D levels and baseline total knee BMLs.

Bold denoted statistical significance (p<0.05).

**Table 3** Cross-sectional and longitudinal associations between Th17 cytokines and total knee BMLs/an increase in BMLs over 2 years, by sex

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Multivariable analysis in Males** | | | **Multivariable analysis in Females** | | |
| ***Total knee BML scores*** | RM† | 95% CI\* |  | RM† | 95% CI**\*** |  |
| Baseline IL-17A | 1.28 | (0.68, 1.89) |  | 0.95 | (0.57, 1.57) |  |
| Baseline IL-17F | 1.31 | (0.72, 1.89) |  | 1.05 | (0.65, 1.68) |  |
| Baseline IL-23 | 1.34 | (0.74, 1.94) |  | 0.92 | (0.55, 1.54) |  |
|  |  |  |  |  |  |  |
| ***An increase in BMLs*** | RR | 95% CI\*\* |  | RR | 95% CI\*\* |  |
| Baseline IL-17A | 0.81 | (0.13, 1.50) |  | 1.53 | (0.79, 2.94) |  |
| Baseline IL-17F | 0.83 | (0.18, 1.48) |  | **1.85** | **(1.04, 3.28)** |  |
| Baseline IL-23 | 0.88 | (0.19, 1.57) |  | **2.10** | **(1.16, 3.79)** |  |

The dependent variable: baseline total knee BML score in negative binominal models, or an increase in total knee BMLs in log binominal models. The independent variables: baseline IL-17A, IL-23 (dichotomised at cut-off points of limits of detection), and IL-17F (highest quarter vs others).

†Ratio of means presented from negative binomial model;

\*Adjusted for age, BMI and vitamin D levels;

\*\* Adjusted for age, BMI, vitamin D levels and baseline total knee BMLs;

Bold denoted statistical significance (p<0.05).

**Figure legends:**

**Figure 1. Association between IL-6 and knee BMLs in total sample**. (**a**) Baseline total knee BMLs (mean +/- 95%CI), by quarters of baseline IL-6; (**b**) An increase in total knee BMLs over 2 years by quarters of baseline IL-6. BMLs: bone marrow lesions.

**Figures 2. Association between IL-17F, IL-23 and an increase in total knee BMLs over 2 years in females.**

**Figure 1(a)**

**Figure 1 (b)**

**Figure 2**

**AUTHOR CONTRIBUTIONS**

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Zhaohua Zhu had full access to all the data in this study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Patient Consent**.

Obtained.

**Ethics Approval.**

This study was approved by the Tasmania Health and Human Medical Research Ethics Committee (reference number H1040) and Monash University Human Research Ethics Committee (reference number CF10/1182-2010000616).

**Conflict of interest.**

The authors declare that they have no competing interests.

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