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

Z. Zhu † ‡, P. Otahal †, B. Wang §, X. Jin †, L.L. Laslett †, A.E. Wluka §, B. Antony †, W. Han †, X. Wang † ‡, T. Winzenberg † ‖, F. Cicuttini §, G. Jones †, C. Ding † ‡ § *

† *Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia*
‡ *Arthritis Research Institute, 1st Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China*
§ *Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia*
‖ *Faculty of Health, University of Tasmania, Hobart, Tasmania, Australia*

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SUMMARY

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-suppressed 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]. 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], albeit at much lower levels than the recognised inflammatory arthropathies[5,6].

IL-6 is an inflammatory cytokine with pro- and anti-inflammatory effects, both inside and outside of the joints[7–9]. Although emerging evidence suggests that low-level systemic inflammation is involved in OA pathogenesis[2,10], 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]. Circulating levels of IL-6 have been associated with the prevalence of knee OA[5].

* Address correspondence and reprint requests to: C. Ding, Menzies Institute for Medical Research, University of Tasmania, Private Bag 23, Hobart, Tasmania 7000, Australia. Fax: 61-3-62267704.
E-mail address: Changhai.Ding@utas.edu.au (C. Ding).

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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]. IL-17A and IL-17F share the most homology at the amino acid level[12], have overlapping but also distinct effector functions in a range of autoimmune diseases[13]. The development of CD4 (+) Th-17 cells is differentiated by IL-6, and stabilised by IL-23[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[14,15]. 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].

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[20,21], and OA incidence[22] and progression[23,24]. Additionally, BMLs predict knee joint space loss on X-ray[23], cartilage defect progression[25] and cartilage loss on MRI[26], as well as knee replacement surgery[27,28]. However, thus far, the aetiology 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]. BMLs could be caused by an inflammatory reaction to cartilage breakdown products, or other factors in intruded synovial fluid[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].

Although IL-6/IL-23/IL-17 axis has been implicated in the pathogenesis of many inflammatory conditions[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

Study sample

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]. Briefly, eligible subjects met the American College of Rheumatology (ACR) criteria for clinical knee OA[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], and relatively good health, with a score of 0–2 out of a maximum score of 4 on a global investigator assessment of disease status[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 2 years. Exclusion criteria included grade 3 radiographic changes according to Altman's atlas[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]. 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 BMLs

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 90°, 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]. 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].

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, 25–50% of the subregion; grade 3, >50% of the subregion[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 0–45. Baseline and follow-up MRIs were scored in pairs in chronological order to minimise measurement error[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)/height^2 (m^2)] was calculated.

25OHD assays

Serum 25OHD was assayed by the Liaison method at baseline, utilizing a direct competitive chemiluminescent immunoassays