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Inflammatory markers in early knee joint osteoarthritis differ from well-matched controls and are associated with consistent, rather than intermittent knee pain

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ABSTRACT

Background: Osteoarthritis (OA) is characterised by the failure of normal biological processes to repair following damage. Traditionally, OA was considered a "wear and tear" disorder; however, it is now a recognised inflammatory condition, preceded by molecular modifications. The aim of this study was to evaluate inflammatory markers among individuals with early knee OA (eKOA) and well-matched asymptomatic controls.

Methods: Twenty six eKOA (females, n = 13; age = 60.2 ± 5.4 yrs, height = 1.73 ± 0.11 m, body mass = 77.8 ± 12.8 kg, body fat = 33.9 ± 8.5%) and twenty-three asymptomatic individuals (females, n = 14; age = 59.9 ± 5.5yrs, height = 1.71 ± 0.09 m, body mass = 72.6 ± 11.3 kg, body fat = 30.4 ± 8.2%) were recruited. The Timed Up and Go, and the 6 Minute Walk Tests evaluated physical function in addition to pain specific questionnaires (KOOS and ICOAP). Serum levels of IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8(CXCL8), IL-10, hsCRP and TNF-α were quantified using a multiplex assay via V-plex®Sector Imager 2400.

Results: As hypothesised, only KOOS and EQ-5D-5L metrics differed between the groups for non-blood derived measures (p < 0.04). Only IL-6 was higher in eKOA (P = 0.02; 95% CI = 0.202; by 0.197 pg/mL; 34.5%). Among eKOA, IL-6 did not relate to severity of KOOS pain (P = 0.696, r = −0.088), but had a positive relationship with ICOAP consistent (r = 0.469, P = 0.045) rather than intermittent pain. There was a moderate correlation between 6MWD and IL-8 (r = 0.471, P = 0.012).

Conclusion: Our results illustrate the potential for IL-6 as a biomarker for eKOA, and introduce the proposition for particular consideration in those with consistent pain. Further, for the first time the present data showed greater walking distance in eKOA with lower circulating IL-8. Future work should seek to verify these results and further investigate IL-6 and IL-8 related molecular pathways in eKOA, and their potential relationships with consistent knee pain and physical function.

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1. Introduction

Osteoarthritis (OA) is a condition of synovial joints that is characterised by deleterious morphological change and is one of the most prevalent chronic diseases today [1]. OA is a progressive condition caused by failure of normal biological processes to repair following damage due to abnormalities in synovial joint structures [2]. Previously, OA was considered to be a "wear and tear" condition, however it is now recognised as an inflammatory/biomechanical disease preceded by molecular (often inflammatory) modifications [3]. OA contributes significantly to the global and local healthcare disease burden and it is estimated that the economic cost could rise to 2.5% of gross domestic product in westernised countries [4].

Knee (K)OA accounts for 16–27% of all OA cases, approximately 80% of the OA disease burden [5] and there is a known relationship between inflammatory biomarkers and knee pain/function [6]. It is now accepted that KOA affects a younger adult populations than historically thought, reflected by studies now including participants from 45 years, the mean age of medical diagnosis approximately 57 years and the prevalence in those 25–45 years increasing annually by 2.8% (data up to 2015) [7]. However, clinical diagnosis of OA relies heavily upon symptomatic phenotypes (e.g. pain, swelling, stiffness) and confirmatory radiographic assessment. As a result, it is common for patients with suspected OA to only seek medical attention when these symptoms become difficult to manage independently i.e. at the advanced stages, when joint damage is already significant and later in the disease state. At this stage, treatments are often pharmaceutical, where persistent use carries significant adverse health consequences [8] and exploratory surgical interventions, prior to total joint replacement, that have little impact on the patients outcomes [9]. Therefore, it is important to identify and intervene early with potential disease modifying treatments; however, confirmatory diagnostic tests in tandem with clinical assessment would improve early OA classification [10], of which there is no universally accepted definition/criteria [11]. In fact, early identification of OA has been described as a "window of opportunity" to delay disease process and restore joint homeostasis [12].

Currently, expensive imaging methods that are often accompanied with significant and growing waiting lists in public health systems [13], are the clinical "go-to" as there are currently no accepted confirmatory blood tests to support the diagnosis of early-stage (e)KOA. This is despite the suggestion that the most pressing need for biomarkers in OA is to identify reliable markers 'early' in the disease [14]. Some promising data have emerged using advanced molecular techniques [15]. However, considering the classification of KOA as an inflammatory disease and that inflammation plays a greater role early in the disease progression [16], there is still limited knowledge of eKOA inflammatory biomarkers that if identified would aid in determining eKOA diagnostic criteria [12].

There have now been a number of investigations specifically considered inflammatory biomarkers of eKOA [17–19]. For example, in large samples, IL-6 and IL-1Ra (n = 470; n = 180) were shown to be discriminators of KOA severity (KL grade $\leq$ 2 vs KL grade $\geq$ 3) and capable of partially predicting eKOA pain and radiographic severity/progression, respectively [19,20]. Recently, IL-6 and IL-8 have been associated with MRI in "earlier stage" KOA (KL < 4 and experiencing pain on "most days" in the last month; n = 139), which differed from end stage disease [18], and identified at varying quantities in a number of knee joint tissues [21]. However, these studies did not quantify or detail anthropometrics (BMI, fat mass, lean muscle mass etc.) that can affect inflammatory status [22] (and are impacted by metabolic syndrome in KOA [21]). Nor to a matched control sample with no evidence of KOA pain – as others interested in non-inflammatory biomarkers of eKOA have done [23]. While the data are of great interest, due to the known effects of anthropometric variables on inflammation in older adults [24], these cannot be ruled out as potential confounders to the presented results. In fact, the current OARSI guidelines for assessing physical function suggest that the current assessments are "less generalizable" for eKOA [25]. Therefore, identification of biomarkers early in the disease progression would improve our understanding of the influence of inflammation [24].

The primary aim of this study was to compare the inflammatory status of eKOA to an age, sex and anthropometrically matched sample of healthy controls and identify biomarkers that could be used as early identifiers of KOA progression to pathology. The secondary aim was to investigate any relationships that identified markers may have with physical functional and specific pain metrics.

2. Materials and methods

2.1. Study design

This case-control design study was performed at the University College Dublin, Institute for Sport and Health between April 2017 and October 2018. The study was conducted in accordance with the declaration of Helsinki (2013), was approved by the local University Research Ethics Committee and all participants gave written informed consent.

2.2. Participants

Participants attended the laboratory fasted (12 h) between 07:00 and 10:00, having performed no strenuous exercise 24 h prior to the visit. All participants were Caucasian and free from any known comorbidities (such as diabetes, immune disorders etc.). Baseline participants characteristics, stratified by sex, are presented in Table 1.

Table 1
Participant characteristics at baseline, presented as means ± standard deviations.

	Controls			eKOA		
	Males (n = 12)	Females (n = 14)	All (n = 26)	Males (n = 10)	Females (n = 13)	All (n = 23)
Years with eKOA pain	–	–	–	15.0 ± 10.3	6.3 ± 5.8	9.6 ± 9.0
KL Grade (%)						
0	–	–	–	40.0	23.1	30.4
1	–	–	–	60.0	61.5	60.9
3	–	–	–	0.0	15.4	8.7
Age (y)	61.2 ± 6.1	59.6 ± 5.1	59.9 ± 5.5	61.5 ± 4.3	59.9 ± 6.0	60.2 ± 5.4
Height (m)	1.78 ± 0.05	1.65 ± 0.06	1.71 ± 0.09	1.84 ± 0.05	1.65 ± 0.06	1.73 ± 0.11
Mass (kg)	80.5 ± 7.4	65.8 ± 9.6	72.6 ± 11.3	86.4 ± 10.0	71.3 ± 10.9	77.8 ± 12.8
BMI (kg·m^2)	25.4 ± 2.6	24.3 ± 3.8	24.8 ± 3.3	25.6 ± 2.1	26.1 ± 3.5	25.8 ± 2.9
Body fat (%)	25.71 ± 6.9	35.0 ± 6.4	30.4 ± 8.2	27.3 ± 6.5	39.0 ± 6.1	33.9 ± 8.5
Lean muscle mass (kg)	57.4 ± 3.3	40.7 ± 3.8	48.4 ± 3.8	60.0 ± 6.8	41.3 ± 4.0	49.5 ± 10.8
Appendicular Lean Mass (kg)	26.3 ± 1.7	17.4 ± 1.7	21.5 ± 4.8	27.7 ± 3.2	17.9 ± 2.0	22.1 ± 5.6
Total BMD (g·cm^2)	1.263 ± 0.106	1.107 ± 0.117	1.179 ± 0.135	1.222 ± 0.089	1.115 ± 0.089	1.162 ± 0.109
KOOS (A.U.)						
Pain	98.6 ± 2.5	99.6 ± 1.5	99.2 ± 2.0	69.4 ± 10.0	69.0 ± 13.8	69.2 ± 13.8#
Symptoms	96.5 ± 5.6	98.6 ± 2.3	97.7 ± 4.2	76.4 ± 13.3	70.3 ± 15.5	73.0 ± 14.6#
ADL	99.5 ± 1.2	99.5 ± 1.6	99.5 ± 1.4	79.3 ± 12.6	73.5 ± 14.3	76.0 ± 13.6#
SR	98.3 ± 3.3	100.0 ± 0.0	99.2 ± 2.3	69.0 ± 20.4	61.5 ± 22.4	64.8 ± 20.4#
QoL	99.0 ± 2.3	100.0 ± 0.0	99.5 ± 1.6	63.4 ± 12.9	53.5 ± 12.7	57.8 ± 13.5#
ICOAP (A.U.)						
Constant	–	–	–	21.3 ± 12.8	21.4 ± 20.4	21.3 ± 18.5
Intermittent	–	–	–	29.2 ± 10.0	34.0 ± 17.5	31.9 ± 14.2
Total	–	–	–	23.6 ± 10.9	23.8 ± 17.8	23.7 ± 14.4
EQ-5D-5L (A.U.)						
Index	0.986 ± 0.047	0.971 ± 0.058	0.986 ± 0.053	0.773 ± 0.059	0.762 ± 0.059	0.766 ± 0.126#
VAS	83.4 ± 10.6	91.1 ± 7.4	87.6 ± 9.7	83.3 ± 11.9	76.7 ± 15.4	79.7 ± 14.1#
PASE (A.U.)	222.3 ± 101.9	220.5 ± 94.9	221.3 ± 96.2	208.0 ± 66.9	214.0 ± 109.3	211.4 ± 91.4
TuG (s)	6.57 ± 0.98	6.88 ± 0.45	6.74 ± 0.75	6.59 ± 1.41	7.45 ± 1.68	7.07 ± 1.60
6MWD (m)	567.4 ± 71.7	538.8 ± 55.2	552.0 ± 63.7	567.5 ± 78.7	503.3 ± 67.0	531.2 ± 82.1

$_e$KOA symptomatic osteoarthritis; KL, Kellgren and Lawrence; BMI, body mass index; BMD, bone mineral density; KOOS, knee injury and osteoarthritis outcome score; ICOAP, Intermittent and Constant Osteoarthritis Pain score; ADL, activities of daily living; SR, sport and recreation; QoL, quality of life; VAS, visual analogue scale; PASE, physical activity scale for the elderly; TuG, timed up and go; 6MWD, six minute walking distance. # indicates variables that differed significantly from controls (P < 0.02).

2.2.1. Early-stage Knee Osteoarthritis (eKOA)

As part of a previous recruitment [26], participants responded to the initial call and completed a secure online questionnaire to determine broad eligibility (e.g. age, height and mass, medical history etc.). Eighty-two participants fulfilled the inclusion criteria and were then stratified by sex, and randomly selected for interview to ascertain specific inclusion and clinical features. Case reports were reviewed by a consultant rheumatologist (CM) and appropriate candidates progressed to X-ray phenotyping. X-rays were performed on thirty one participants and following application of the inclusion criteria twenty three eKOA participants were included.

Participants were excluded from the eKOA group if diagnosed with rheumatoid arthritis, had surgery in affected limb, injection therapy, any muscle disorder, serious medical comorbidities or contraindications to dependent variables (e.g. trypanophobia, unable to perform physical function assessments etc.). Participants were included if their reported mild-knee pain was confirmed on examination, a Knee Injury and Osteoarthritis Outcome Score (KOOS) pain score of $\geq$50 [27]; aged 50–70 years, a BMI < 35 kg·m^2, Kellgren and Lawrence (KL) grade $\leq$ 2 (i.e. little to no structural damage) [28] and had not yet undergone any medically prescribed pharmacological or physical therapies (using a combination of suggested criteria for eKOA [11]).

2.2.2. Controls

Sixty-seven participants responded to the initial recruitment call and were contacted by phone or email to assess inclusion criteria (n = 15 declined at this point). The remaining (n = 52) were interviewed and 26 respondents were excluded as described below. The remaining (n = 26) participants were invited to participate.

Participants were included if they were aged 50–70 years, reported no knee or joint pain at interview, no history of intermittent knee pain and a BMI < 35 kg·m^2. Participants were excluded if diagnosed with rheumatoid arthritis, had any muscle disorder, serious medical comorbidities, contraindications to dependent variables, undergoing pharmacological or physical therapies for any reason or have had a previous lower limb injury that required medical attention/surgical intervention.

2.3. X-ray procedure

Selected eKOA participants (due to ethical considerations, controls did not undergo X-ray assessment) underwent X-ray radiography to determine the degree structural OA, as recommended by [OARSI; 2,29]. Both knees were X-rayed in a weight-bearing, semi-flexed position (~10–15°) using a posterior-anterior beam direction (film focus distance 110 cm, 60 kV and 10 mA) with the aid of fluoroscopy to optimally align the tibia plateau. An independent consultant radiologist (SE) specializing in musculoskeletal radiology, blinded to the clinical and biochemical data assessed joint space narrowing and osteophytes. To approximate Kellgren and Lawrence (KL) grade, one or more of the following criteria were fulfilled in either the medial or lateral tibiofemoral compartment: joint space narrowing or the sum of marginal osteophyte grades in the same compartment.

2.4. Knee Injury and Osteoarthritis Outcome Score (KOOS)

For assessment of pain and symptoms, KOOS questionnaire was used. The KOOS consists of 42 items on 5 dimensions (pain, symptoms, activities of daily living (ADL), sport and recreational activity and quality of life) concerning the last 7 days [27]. A Likert scale, scored from 0 (no problems) to 4 (extreme problems) and each of the five dimensions were calculated as the sum of the items. Scores were then transformed to a 0–100 scale, with zero representing extreme and 100 representing no knee problems [27].

2.5. Intermittent and Constant Osteoarthritis Pain (ICOAP)

In the eKOA group (only), the ICOAP identified pain patterns [30]. This tool has 11 items and 2 dimensions, 'constant pain' with score ranges 0–20 and 'intermittent pain' ranges 0–24, assessing symptom type and severity over the prior 7 days. It uses a 5-point Likert scale, where higher scores are indicative of greater severity and each dimension is transformed to a score of 100. Pain patterns were defined as follows: (1) no intermittent or constant pain, (2) intermittent pain only, (3) constant pain only and (4) a combination of constant and intermittent pain.

2.6. Physical Activity Scale for the Elderly (PASE)

PASE was used to assess recent physical activity with 12 items in three dimensions (leisure activity, household activity and work-related activity). The frequency, duration, and intensity of activity over the previous 7 days were used to assign a score, ranging from 0 to 793. A higher total score represents greater achieved physical activity.

2.7. Anthropometrics

Height and Mass were measured by standard methods and used to calculate BMI ($kg \cdot m^2$). Dual Energy X-Ray Absorptiometry (DXA; Lunar iDXA; GE Healthcare, Buckinghamshire, UK) measured body composition following a 12 h overnight fast. Participants lay in a supine position, avoiding contact between the trunk and limbs during the procedure (effective dose, <6 μSv). Participants remained completely still for the duration of the scan. To ensure measurement accuracy and reliability, the scanner was 'quality assured' before each test session (densitometry block supplied by the manufacturers). All scanning and subsequent analyses procedures were undertaken by trained DXA operator on the same scanner.

2.8. Functional performance

2.8.1. Timed up and Go (TuG)

The TuG was performed using a standard high-back adjustable orthopaedic armchair, seat height 46 cm and arm height 67 cm [25]. Participants sat against the back of the seat, feet maintaining full contact with the floor. They were instructed to rise from the chair without the aid of the armrests and walk three metres from the anterior surface of the armchair legs, then return to the start position. Participants were timed from when their buttocks left the chair until their buttocks returned to the seat. This was repeated three times and an average value was calculated. Participant were instructed to use their "comfortable and safe walking speed". Faster time represent better functional performance.

2.8.2. Six Minute Walk Distance (6MWD)

Participants performed the 6MWD, often used in arthritic and elderly populations [25]. A 25 m course was laid out with both ends visibly marked. Participants began walking with the instruction to "walk at their regular comfortable walking pace" for six minutes. During the test, participants received verbal encouragement and their maximal distance over the six minute period was recorded.

2.9. Biochemical analysis

Blood was drawn from a superficial forearm vein (10 mL) and collected into serum separator vacutainer tube. Samples were kept on an ice bed for 2 h and then centrifuged at 4 °C for 10 min at 4800 rpm, the supernatant was removed and stored in 2 mL aliquots at −80 °C until subsequent analysis.

2.10. Inflammatory cytokines

Serum levels of select Interleukins (IL-1β, IL-2, IL-4, IL-6, IL-8(CXCL8), IL-10) and Tumour Necrosis Factor alpha (TNF-α) were measured using the human V-plex-Proinflammatory Panel 1. Interleukins-1 alpha (IL-1α) was measured using the human V-plex-Cytokine Panel 1 and high sensitivity C-reactive protein (hsCRP) were measured using the V-plex-Vascular Injury Panel 2 (Meso Scale Discovery, Rockville, MD, USA).

All assays were performed according to the manufacturer's instructions and with the assistance of an MSD applications scientist. The serum samples were diluted according to assay requirements and all samples were run in duplicate. A total of 6 plates were used to measure the serum inflammatory concentrations and included a control sample, in quadruplet, to quantify plate-plate variability. The data were acquired on the V-plex®Sector Imager 2400 and analysed using the Discovery Workbench 3.0 software (Meso Scale Discovery, Rockville, MD, USA). The standard curves for each cytokine were generated using the premixed lyophilized standards provided in the kits. Serial assay specific dilutions of the standards were run to generate a 7 point standard concentration set and the diluent alone was used as a blank. The assay concentrations were determined from the standard curve using a 4-parameter logistic fit curve to transform the mean light intensities into concentrations.

2.11. Statistical analysis

A priori Power calculations were derived from Min et al. [31] TNF- α data that determined the required sample size, accounting for 10% dropout, of n = 48 to achieve an effect size 0.44 and 1-β 0.8 at a Type 1 error of 5% (G* Power v3.1). If parametric assumptions were satisfied, one way ANOVA compared group baseline anthropometric characteristics, otherwise Kruskal-Wallis was used. Biomarkers that violated parametric assumptions, were compared using Mann-Whitney U tests and Bonferroni corrected for multiple comparisons, adjusted p values are presented. Effect estimates are presented as 95% confidence intervals (95% CI). Depending on variable violation of parametric assumptions, either Pearson or Spearman's correlations coefficients were used to assess relationships between selected variables, interpreted as 0.1, small; 0.3, moderate and 0.5, large. Intra and inter-class coefficients of variations (CV) and lower limit of quantification (LLOQ) were calculated for each biomarker (Supplementary Table 1). ROC area under the curve was used to quantify specificity and sensitivity of identified analytes, interpreted as of Fischer et al. [32]. All statistics were performed using SPSS 24 (SPSS Inc., Chicago, IL) with alpha set at P = 0.05.

3. Results

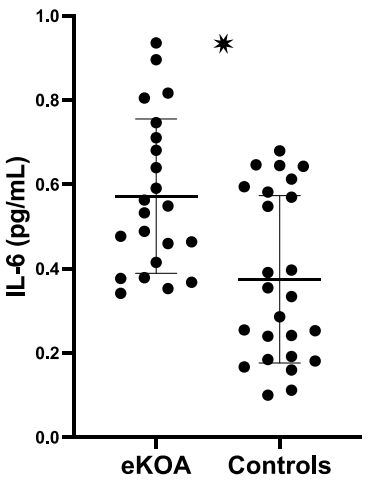
There were no differences between the eKOA group and controls for age, any anthropometric variables or physical function (P > 0.05). The eKOA group had worse KOOS pain and all other symptomatic measures than controls (P < 0.04; Table 1).

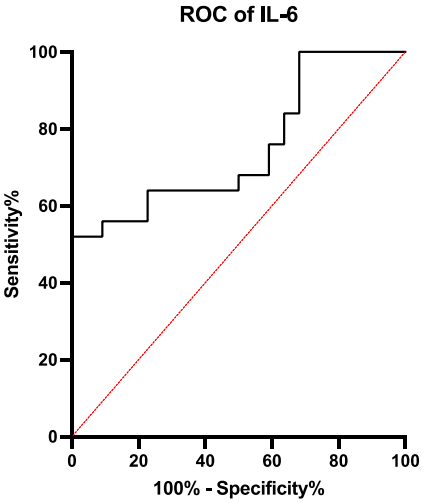
Four analytes produced insufficient data due to the majority of samples (mainly controls) cytokine levels being outside of the detectable ranges, thus it was not possible to perform statistical analysis. Of the remaining analytes, there were no statistical differences between groups for IL-8 (P = 1.00; 95% CI = −1.10), TNF-α (P = 0.35; 95% CI = −0.244), hsCRP (P = 0.25; 95% CI = 0.269) and IL-10 (P = 1.00; −0.011). However, controls had lower levels of IL-6 (P = 0.02; 95% CI = 0.202; by 0.197 pg/mL; 34.5%) compared to eKOA (Figure 1). Subgroup analysis excluding participants with KL grade 0 (n = 7) conformed these results (IL-6, P = 0.03, 95% CI = 0.210; all other analytes p > 0.07; data not shown). ROC analysis identified a moderate discrimination accuracy (AUC = 0.751, 95% CI 0.610–0.891, P = 0.003; Figure 2). With an accurate positive rate (APR) of 84% (false positive rate (FPR) = 64%) at an IL-6 cut off below 0.627 pg/mL.

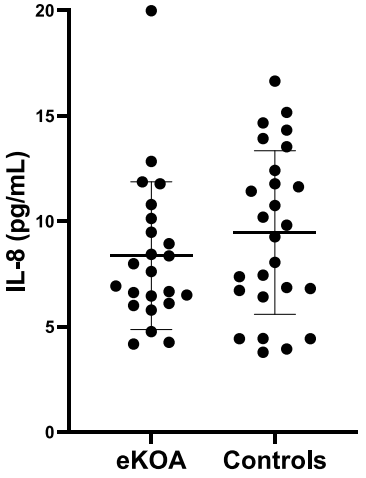
In the eKOA group, there was no relationship between circulating IL-6 level and severity of KOOS pain (P = 0.696, r = -0.088, data not shown); however there was a moderate relationship between IL-6 and those reporting constant (n = 14, r = 0.469, P = 0.045) rather than intermittent pain (n = 23, r = 0.101, P = 0.328) or total pain (n = 22, r = 0.158, P = 0.241).

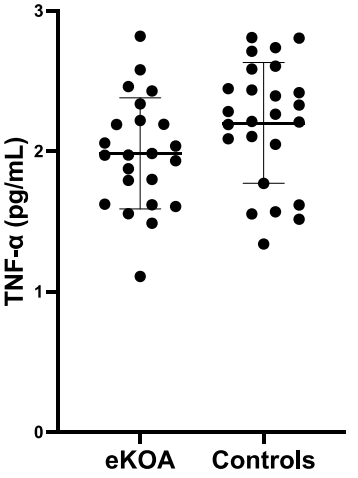
There was a moderate correlation between 6MWD and serum levels of IL-8, showing a greater walking distance in those with lower circulating IL-8 (r = 0.471, P = 0.012). This relationship was not evident in the control sample (P = 0.420; Figure 3). There were no relationships between physical function and any other biomarkers in either group (P > 0.06).

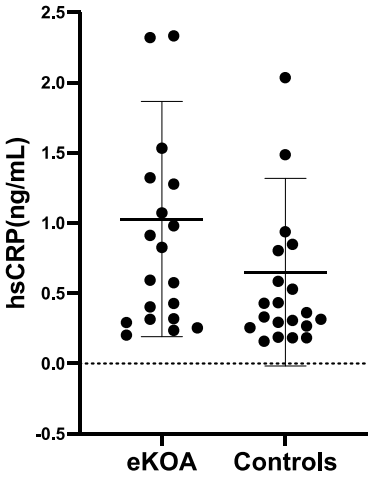
The only identified biomarker interactions were weak relationships between IL-10 and hsCRP in eKOA and controls (r = 0.368, P = 0.046; r = 0.385; P = 0.029, respectively) and IL-10 and TNF-α (r = 0.380, P = 0.030,) in controls, but not in eKOA (P > 0.082; data not shown).











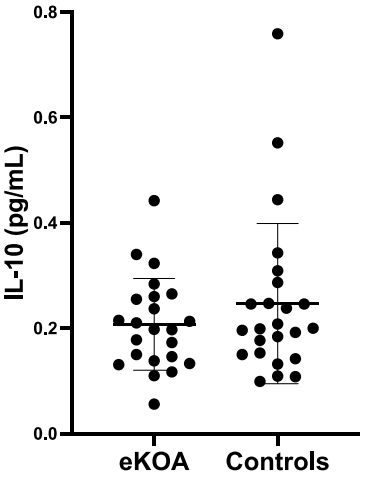


Figure 1. Inflammatory markers between eKOA and matched controls. The top right panel, Receiver Operator Curve (ROC) showing the accuracy of identifying eKOA with circulating Interleukin-6. '"*", post-hoc Null-hypotheses significance; IL, Interleukins; CRP, C reactive Protein; TNF-α, tumour narcosis factor alpha.

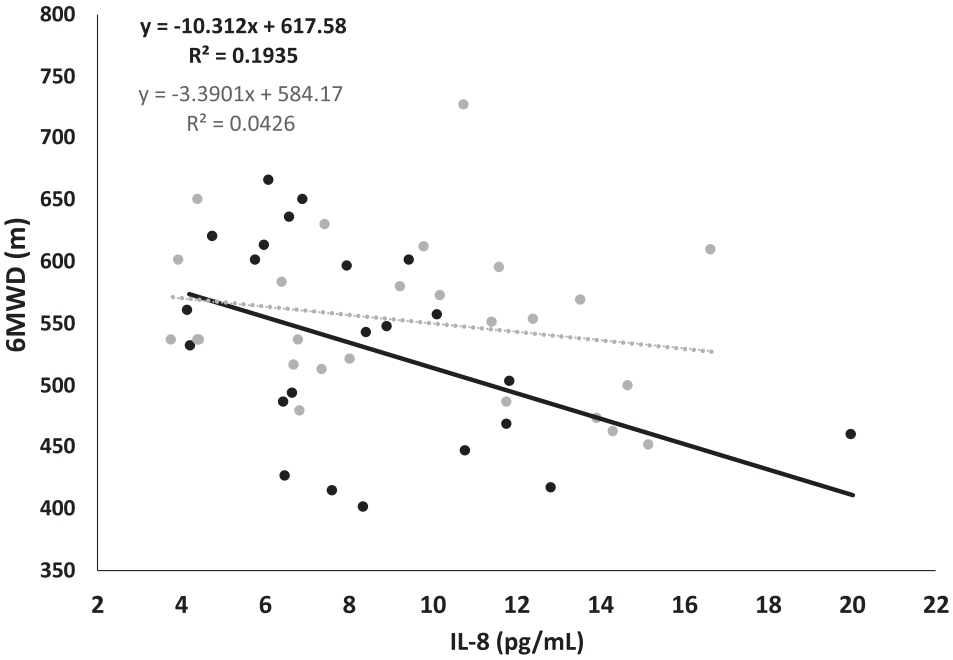


Figure 2. Relationship between Interleukin-8 (IL-8) and six-minute walking distance (6MWD) of eKOA (black line/equation text) and controls (grey line/equation text).

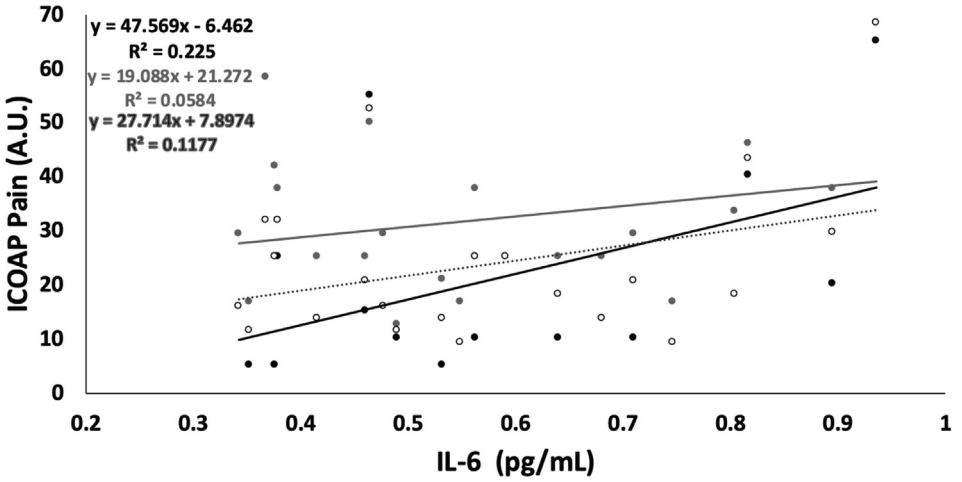


Figure 3. Relationship between Interlukin-6 (IL-6) and Intermittent and Constant Osteoarthritis Pain (ICOAP) pain in eKOA. The black line/text box shows constant pain, solid grey line/text shows intermittent pain and dashed grey line/text box represents ICOP total.

4. Discussion

Proinflammatory and anti-inflammatory cytokines play a pivotal role in the progression of KOA, however, until recently they have been sparsely investigated in early symptomatic individuals. Results from the present study demonstrate that circulating IL-6 differed between eKOA and matched controls (by 35%), and may be a more relevant biomarker for those experiencing constant rather than intermittent pain. This adds to the evidence that elevated IL-6 may be a potential early identifying biomarker to differentiate eKOA from asymptomatic well-matched controls.

The present data show that at the earliest stages of KOA (i.e. initial presentation of pain with little radiographic change), IL-1α, IL-1β, IL-2, IL-4, IL-10, TNF-α and hsCRP had no differentiating value from asymptomatic controls. The results for TNF-α and hsCRP were initially surprising given their mechanistic roles in pain and OA [33]. However, considering the relative

health and healthful anthropometric status of both groups, this result is logical in that positive alterations in anthropometrics can return CRP back to normal levels [34]. Further, elevated circulating TNF-α is known to be associated with obesity (rather than the inverse) and any potential effect of TNF-α in eKOA pain may not be evident when patients present with normal anthropometric and physical function values [35], rather than because of KOA itself [33].

Interleukin-6 has been identified in KOA joints [21], and as one of the main biomarkers of low-level systemic inflammation in OA pathogenesis [36,37]. While IL-6 is higher in later stage OA patients compared to controls [19] and positively related to radiographic severity [38], the relationship between IL-6 and early structural KOA (KL $\leq$ 2) has shown inconsistent findings. This is particularly evident in IL-6′s ability to discriminate from more progressive radiographic KOA (K-L grades 3 and 4) [19]. In OA pathophysiology, IL-6 production can be directly stimulated by a number of other elevated cytokines [37] and therefore during the early stages (i.e. before these other biomolecules are detectible systemically) their accumulative stimulation may lead to IL-6 being one of the earliest markers of a heightened inflammatory state, potentially manifesting symptomatically before all else. As such, there appears to be growing evidence that IL-6 is expressed higher in early stage compared to later stage KOA [33] and is modestly correlated with pain (r = 0.27) [33,39], consistent pain in the present results (r = 0.47). In agreement, a longitudinal analysis of pain outcomes over five-year changes showed higher IL-6 was associated with worsening pain [40]. The present data adds to these findings by demonstrating that pain pattern (i.e. constant rather than intermittent pain) may be a contributing factor to the IL-6 literature.

As IL-6 is so heavily involved in osteogenesis it may be that early osteogenic assault plays a role in the early detection of OA pain. It is know that bone marrow lesions (BMLs) can play a large role in KOA pain because of the presence/abundance of nociceptors [41] and their role as precursors of cartilage depletion [42]. This is less common in asymptomatic patients without OA [43]. In agreement with this hypothesis, IL-6 was recently found to be associated with BMLs in both male and female KOA patents [36] with higher levels associated with a greater prevalence of osteophytes [44]. Over two years, eKOA patients had higher baseline serum IL-6 which was associated with a greater likelihood of increased BMLs and progressing the disease [36]. In fact, some have argued that the ratio between IL-6 and other inflammatory cytokines (IL-10) known to have an opposing oestrogenic action can increase risk factors for OA [45]. This may be logically through the mechanistic crosstalk and regulation of IL-6 on other T cell stimulated cytokines, such as IL-1β, -8, -17 and others [46]. If it is the case that the elevated IL-6 in eKOA could be a result of early damage to subchondral bone before it is evident on imaging, but not specifically associated with pain severity (but maybe pain type i.e. consistent) at the earliest stages of the disease progression (results from the present study), further work is needed to examine this theory. As the ROC FPR was high in the present sample, replication in a larger sample with the inclusion of MRI imagery to investigate the specific relationship with BMLs at early in the disease is warranted.

While there was no relationships between IL-6 and physical function in the present study, there was a moderate positive relationship for lower serum IL-8 (a major inflammatory mediator [47]) and greater 6MWD in the eKOA sample. This finding is partially supported by data showing an association between IL-8 and self-reported WOMAC physical dysfunction in a large sample of KOA patients (n = 160) [47]. Interlukin-8 has been shown to be sensitive to alterations in physical activity i.e. 10% lower than controls after a 12 month intervention [48], to the authors knowledge, a direct link with eKOA specific physical function (6MWD) had not yet been established before now. Nonetheless, there is a plausible rationale. In end-stage KOA patients (i.e. scheduled for surgery) both higher plasma and synovial IL-8 is evident compared to non-KOA [49]. Whereas in less severe KOA, associations seem to be limited to synovial fluid and not circulating levels [21]. This, and the present findings, suggest that circulating IL-8 could be a marker for altered physical function in eKOA, due to its sensitivity to physical activity and early elevated synovial concentration, until later in the disease progression. Nonetheless, confirmatory studies and replication, potentially with extracted synovial fluid, are needed therefore this finding should be viewed with caution.

In conclusion, the present study has shown IL-6 to be a potential inflammatory identifier of early symptomatic KOA when compared to age, sex, anthropometrically and functionally matched controls. Further, the present study has shown, for the first time, that this could possibly be more relevant for those experiencing constant rather than intermittent pain. The results also introduce IL-8 as a potential marker associated with physical function in eKOA. It is possible that these markers are early circulating indicators of local subcentral injury, potentially before the onset cartilage/subchondral bone damage. However, additional work is needed for the present findings to be considered in clinical practice.

CRediT authorship contribution statement

Shane M. Heffernan: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Gillian E. Conway:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Conor McCarthy:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Stephen Eustace:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Formal analysis, Data curation. **Mark Waldron:** Writing – review & editing, Writing – original draft, Resources, Methodology, Formal analysis. **Giuseppe De Vito:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Eamonn Delahunt:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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References

[1] Cui A, Li H, Wang D, Zhong J, Chen Y, Lu H. Global, regional prevalence, incidence and risk factors of knee osteoarthritis in population-based studies. EClinicalMedicine 2020;29:100587.
[2] Lane NE, Brandt K, Hawker G, Peeva E, Schreyer E, Tsuji W, et al. OARSI-FDA initiative: defining the disease state of osteoarthritis. Osteoarth Cartil 2011;19(5):478–82. doi: https://doi.org/10.1016/j.joca.2010.09.013.
[3] Thijssen E, Van Caam A, Van Der Kraan PM. Obesity and osteoarthritis, more than just wear and tear: pivotal roles for inflamed adipose tissue and dyslipidaemia in obesity-induced osteoarthritis. Rheumatology 2015;54(4):588–600.
[4] Hiligsmann M, Cooper C, Arden N, Boers M, Branco JC, Brandi ML, et al. Health economics in the field of osteoarthritis: an expert's consensus paper from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). Sem Arthritis Rheum 2013;43(3):303–13.
[5] Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380(9859):2163–96. doi: https://doi.org/10.1016/s0140-6736(12)61729-2.
[6] Wood MJ, Miller RE, Malfait A-M. The genesis of pain in osteoarthritis: inflammation as a mediator of osteoarthritis pain. Clin Geriatric Med 2022;38(2):221–38.
[7] Spitaels D, Mamouris P, Vaes B, Smeets M, Luyten F, Hermens R, et al. Epidemiology of knee osteoarthritis in general practice: a registry-based study. BMJ Open 2020;10(1):e031734.
[8] Varrassi G, Pergolizzi J, Peppin JF, Paladini A. Analgesic drugs and cardiac safety. Brain Heart Dyn 2020:649–70. doi: https://doi.org/10.1007/978-3-030-28008-6_43.
[9] O'connor D, Johnston RV, Brignardello-Petersen R, Poolman RW, Cyril S, Vandvik PO, Buchbinder R. Arthroscopic surgery for degenerative knee disease (osteoarthritis including degenerative meniscal tears). Cochrane Syst Rev 2022;3:CD014328. doi: https://doi.org/10.1002/14651858.CD014328.
[10] Hawker G, Lohmander L. What an earlier recognition of osteoarthritis can do for OA prevention. Osteoarth Cartil 2021;29(12):1632–4.
[11] Im GI. The concept of early osteoarthritis and its significance in regenerative medicine. Tissue Eng Regen Med 2022;19(3):431–6. doi: https://doi.org/10.1007/s13770-022-00436-6.
[12] Mahmoudian A, Lohmander LS, Mobasheri A, Englund M, Luyten FP. Early-stage symptomatic osteoarthritis of the knee – time for action. Nat Rev Rheumatol 2021;17(10):621–32. doi: https://doi.org/10.1038/s41584-021-00673-4.
[13] Nhs, NHSE. NHS diagnostic waiting times and activity data. 2022. https://www.england.nhs.uk/statistics/wp-content/uploads/sites/2/2022/02/DWTA-Report-December-2021_R3O2G.pdf.
[14] Watt FE. Osteoarthritis biomarkers: year in review. Osteoarth Cartil 2018;26(3):312–8.
[15] Ahmed U, Anwar A, Savage RS, Thornalley PJ, Rabbani N. Protein oxidation, nitration and glycation biomarkers for early-stage diagnosis of osteoarthritis of the knee and typing and progression of arthritic disease. Arthritis Res Ther 2016;18(1):1–11.
[16] Orlowsky EW, Kraus VB. The role of innate immunity in osteoarthritis: when our first line of defense goes on the offensive. J Rheum 2015;42(3):363–71.
[17] Ren G, Krawetz RJ. Biochemical markers for the early identification of osteoarthritis: systematic review and meta-analysis. Mol DiaTher 2018;22(6):671–82.
[18] Rajandran SN, Ma CA, Tan JR, Liu J, Wong SBS, Leung Y-Y. Exploring the association of innate immunity biomarkers with MRI features in both early and late stages osteoarthritis. Frontiers Med 2020;7:554669.
[19] Livshits G, Zhai G, Hart DJ, Kato BS, Wang H, Williams FM, et al. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: the Chingford study. Arthritis Rheum 2009;60(7):2037–45.
[20] Attur M, Statnikov A, Samuels J, Li Z, Alekseyenko A, Greenberg J, et al. Plasma levels of interleukin-1 receptor antagonist (IL1Ra) predict radiographic progression of symptomatic knee osteoarthritis. Osteoarthr Cartil 2015;23(11):1915–24.
[21] Christoforakis Z, Dermitzaki E, Paflioti E, Katrinaki M, Deiktakis M, T HT, et al. Correlation of systemic metabolic inflammation with knee osteoarthritis. Hormones 2022. doi: https://doi.org/10.1007/s42000-022-00381-y.
[22] Karczewski J, Śledzińska E, Baturo A, Jończyk I, Maleszko A, Samborski P, et al. Obesity and inflammation. Euro Cytokine Net 2018;29(3):83–94.
[23] Ahmed U, Anwar A, Savage RS, Costa ML, Mackay N, Filer A, et al. Biomarkers of early stage osteoarthritis, rheumatoid arthritis and musculoskeletal health. Sci Rep 2015;5(1):1–7.
[24] Greene MA, Loeser RF. Aging-related inflammation in osteoarthritis. Osteoarth Cartil 2015;23(11):1966–71.
[25] Dobson F, Hinman RS, Roos EM, Abbott JH, Stratford P, Davis AM, et al. OARSI recommended performance-based tests to assess physical function in people diagnosed with hip or knee osteoarthritis. Osteoarth Cartil 2013;21(8):1042–52. doi: https://doi.org/10.1016/j.joca.2013.05.002.

[26] Heffernan SM, Mccarthy C, Eustace S, Fitzpatrick RE, Delahunt E, De Vito G. Mineral rich algae with pine bark improved pain, physical function and analgesic use in mild-knee joint osteoarthritis, compared to Glucosamine: a randomized controlled pilot trial. Complement Ther Med 2020;50:102349. doi: https://doi.org/10.1016/j.ctim.2020.102349.
[27] Roos EM, Lohmander LS. The Knee injury and Osteoarthritis Outcome Score (KOOS): from joint injury to osteoarthritis. Health Qual Life Outcomes 2003;1:64. doi: https://doi.org/10.1186/1477-7525-1-64.
[28] Shimizu H, Shimoura K, Iijima H, Suzuki Y, Aoyama T. Functional manifestations of early knee osteoarthritis: a systematic review and meta-analysis. Clin Rheumatol 2022;41(9):2625–34. doi: https://doi.org/10.1007/s10067-022-06150-x.
[29] Mcalindon TE, Driban JB, Henrotin Y, Hunter DJ, Jiang GL, Skou ST, et al. OARSI Clinical Trials Recommendations: Design, conduct, and reporting of clinical trials for knee osteoarthritis. Osteoarth Cartil 2015;23(5):747–60. doi: https://doi.org/10.1016/j.joca.2015.03.005.
[30] Hawker GA, Davis AM, French MR, Cibere J, Jordan JM, March L, et al. Development and preliminary psychometric testing of a new OA pain measure–an OARSI/OMERACT initiative. Osteoarth Cartil 2008;16(4):409–14. doi: https://doi.org/10.1016/j.joca.2007.12.015.
[31] Min S, Wang C, Lu W, Xu Z, Shi D, Chen D, et al. Serum levels of the bone turnover markers dickkopf-1, osteoprotegerin, and TNF-α in knee osteoarthritis patients. Clin Rheumatol 2017;36(10):2351–8. doi: https://doi.org/10.1007/s10067-017-3690-x.
[32] Fischer JE, Bachmann LM, Jaeschke R. A readers' guide to the interpretation of diagnostic test properties: clinical example of sepsis. Intensive Care Med 2003;29(7):1043–51.
[33] Li L, Li Z, Li Y, Hu X, Zhang Y, Fan P. Profiling of inflammatory mediators in the synovial fluid related to pain in knee osteoarthritis. BMC Musculoskelet Disord 2020;21(1):1–10.
[34] Beavers KM, Beavers DP, Newman JJ, Anderson AM, Loeser Jr RF, Nicklas BJ, et al. Effects of total and regional fat loss on plasma CRP and IL-6 in overweight and obese, older adults with knee osteoarthritis. Osteoarth Cartil 2015;23(2):249–56.
[35] Peluso I, Palmery M. The relationship between body weight and inflammation: Lesson from anti-TNF-α antibody therapy. Hum Immun 2016;77(1):47–53.
[36] Zhu Z, Otahal P, Wang B, Jin X, Laslett LL, Wluka A, et al. Cross-sectional and longitudinal associations between serum inflammatory cytokines and knee bone marrow lesions in patients with knee osteoarthritis. Osteoarthr Cartil 2017;25(4):499–505.
[37] Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier J-P, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheum 2011;7(1):33–42.
[38] Mabey T, Honsawek S, Tanavalee A, Yuktanandana P, Wilairatana V, Poovorawan Y. Plasma and synovial fluid inflammatory cytokine profiles in primary knee osteoarthritis. Biomarkers 2016;21(7):639–44. doi: https://doi.org/10.3109/1354750x.2016.1171907.
[39] Azim S, Nicholson J, Rebecchi MJ, Galbavy W, Feng T, Rizwan S, et al. Interleukin-6 and leptin levels are associated with preoperative pain severity in patients with osteoarthritis but not with acute pain after total knee arthroplasty. Knee 2018;25(1):25–33.
[40] Stannus OP, Jones G, Blizzard L, Cicuttini FM, Ding C. Associations between serum levels of inflammatory markers and change in knee pain over 5 years in older adults: a prospective cohort study. Annals Rheum Dis 2013;72(4):535–40.
[41] Ivanusic JJ. Molecular mechanisms that contribute to bone marrow pain. Front Neurol 2017;8:458. doi: https://doi.org/10.3389/fneur.2017.00458.
[42] Shabestari M, Vik J, Reseland JE, Eriksen EF. Bone marrow lesions in hip osteoarthritis are characterized by increased bone turnover and enhanced angiogenesis. Osteoarth Cartil 2016;24(10):1745–52. doi: https://doi.org/10.1016/j.joca.2016.05.009.
[43] Li G, Yin J, Gao J, Cheng TS, Pavlos NJ, Zhang C, et al. Subchondral bone in osteoarthritis: insight into risk factors and microstructural changes. Arthritis Res Ther 2013;15:6 223. doi: https://doi.org/10.1186/ar4405.
[44] Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J, et al. Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. Osteoarth Cartil 2010;18(11):1441–7. doi: https://doi.org/10.1016/j.joca.2010.08.016.
[45] Suyasa IK, Kawiyana IK, Bakta IM, Widiana IG. Interleukin-6 and ratio of plasma interleukin-6/interleukin-10 as risk factors of symptomatic lumbar osteoarthritis. World J Orthop 2017;8(2):149–55. doi: https://doi.org/10.5312/wjo.v8.i2.149.
[46] Nograles KE, Zaba LC, Guttman-Yassky E, Fuentes-Duculan J, Suárez-Fariñas M, Cardinale I, et al. Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. Br J Dermatol 2008;159(5):1092–102. doi: https://doi.org/10.1111/j.1365-2133.2008.08769.x.
[47] Ruan G, Xu J, Wang K, Zheng S, Wu J, Bian F, et al. Associations between serum IL-8 and knee symptoms, joint structures, and cartilage or bone biomarkers in patients with knee osteoarthritis. Clin Rheumatol 2019;38(12):3609–17. doi: https://doi.org/10.1007/s10067-019-04718-8.
[48] Beavers KM, Hsu FC, Isom S, Kritchevsky SB, Church T, Goodpaster B, et al. Long-term physical activity and inflammatory biomarkers in older adults. Med Sci Sports Exerc 2010;42(12):2189–96. doi: https://doi.org/10.1249/MSS.0b013e3181e3ac80.
[49] Koh SM, Chan CK, Teo SH, Singh S, Merican A, Ng WM, et al. Elevated plasma and synovial fluid interleukin-8 and interleukin-18 may be associated with the pathogenesis of knee osteoarthritis. Knee 2020;27(1):26–35. doi: https://doi.org/10.1016/j.knee.2019.10.028.