

WJO 5<sup>th</sup> Anniversary Special Issues (5): Knee**Cytokines as biochemical markers for knee osteoarthritis**

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considered an important part of OAs pathophysiology, cytokines are being assessed as possible candidates for biochemical markers. Cytokines, both pro- and anti-inflammatory, as well as angiogenic and chemotactic, have in recent years been studied for relevant characteristics. Biochemical markers show promise in determination of the severity of disease in addition to monitoring of the efficacy and safety of disease-modifying OA drugs, with the potential to act as diagnostic and prognostic tools. Currently, the diagnostic power of interleukin (IL)-6 and the relationship to disease burden of IL-1 $\beta$ , IL-15, tumor necrosis factor- $\alpha$ , and vascular endothelial growth factor make these the best candidates for assessment. Grouping appropriate cytokine markers together and assessing them collectively alongside other bone and cartilage degradation products will yield a more statistically powerful tool in research and clinical applications, and additionally aid in distinguishing between OA and a number of other diseases in which cytokines are known to have an involvement. Further large scale studies are needed to assess the validity and efficacy of current biomarkers, and to discover other potential biomarker candidates.

**Key words:** Biomarker; Cytokines; Interleukin; Knee osteoarthritis

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**Abstract**

Osteoarthritis (OA) is a debilitating degenerative joint disease particularly affecting weightbearing joints within the body, principally the hips and knees. Current radiographic techniques are insufficient to show biochemical changes within joint tissue which can occur many years before symptoms become apparent. The need for better diagnostic and prognostic tools is heightened with the prevalence of OA set to increase in aging and obese populations. As inflammation is increasingly being

**Core tip:** This review discusses the role and significance of cytokines implicated in the pathophysiology and development of knee osteoarthritis. We aim to describe and summarize the current knowledge and advancement of the use of cytokines as biochemical markers in diagnosis and management of knee osteoarthritis (OA). Cytokines play an important role in the pathogenesis of OA. A better understanding of the biological mechanisms involved in this process may result in better treatment for OA patients. Biomarker investigation for OA diagnosis is still in the forefront of the research repertoire in OA. This review highlights some biomarker studies published in the PubMed database.

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## INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease characterized by articular cartilage degradation which can affect many joints in the body, but is particularly common in weight-bearing joints such as the knee and hip. The loss of cartilage can lead to joint space narrowing (JSN), pain, and loss of function and ultimately leads to the need for total joint replacement. There are a number of risk factors associated with OA, including genetic predisposition, obesity, age, and previous joint trauma. With obesity set to rise in future years<sup>[1]</sup>, combined with OA being a frequent condition among the elderly and an ageing population<sup>[2]</sup>, the prevalence of OA is expected to increase. An effective and reliable method for diagnosis and prognosis is needed, with increased demands on health services around the world.

Radiography is routinely used to aid in the diagnosis of OA. The Kellgren-Lawrence (KL) grading system of radiographic OA is one method commonly used to assess the severity of cases<sup>[3]</sup>. However, radiographic imaging is ineffective at detecting and monitoring the biochemical changes within joint tissue which can occur long before symptoms present. Because OA can take years and even decades to develop, finding biochemical markers associated with OA is an attractive idea. As they can be used to diagnose and predict prognosis in patients in ways that radiography cannot, they can therefore be early indicators of patients at risk of developing the disease. This would prove beneficial as preventative or mitigating measures could be taken.

In recent years, there has been a considerable effort to find biochemical markers which could aid in the monitoring of OA. Research has predominantly looked at two main candidates. The first are products of bone and cartilage degradation such as C-terminal telopeptide of type II collagen, cartilage oligomeric matrix protein, a collagen type II specific neoepitope, an aggrecan neoepitope, a number of matrix metalloproteinases, and procollagen type I amino-terminal propeptide<sup>[4-9]</sup>.

The second group of possible candidates has come to light with the increased understanding that inflammation plays a key role in OA, which is a shift from the historic opinion that it was solely a “wear and tear” disease. Pro- and anti-inflammatory agents, particularly cytokines, have been studied for their associations with the development and progression of OA in both human and animal models. As well as pro- and anti-inflammatory roles (for example, interleukin (IL)-6, IL-1 $\beta$ , tumor necrosis factor (TNF)- $\alpha$ , IL-10, IL-13 and IL-4)<sup>[10-15]</sup>, cytokines also contribute to the pathophysiology of OA through angiogenesis and chemotaxis<sup>[16-21]</sup>.

Different compounds may show different biochemical marker properties at different stages of the disease, reflecting the pathophysiological changes occurring within the joint tissue. Therefore, characterization of potential biomarkers is important to ensure their appropriate and optimal use. The characterization method used to assess biochemical markers in OA is BIPEDS; which stands for: Burden of disease, Investigative, Prognostic, Efficacy of intervention, Diagnostic, and Safety<sup>[22,23]</sup>. Diagnostic markers, as their name suggests, would aid in the diagnosis of OA. Early and reliable detection of the disease in a patient is obviously beneficial. Potential prognostic markers aid in the prediction of disease progression within OA patients, but also identify individuals who are at a higher risk of developing OA in the future. Due to the slow progression of the disease, which can take a number of decades to develop, preventative or mitigating measures could be taken before any symptoms became apparent to the patient or clinician. Additionally, identifying groups of patients in which the disease will progress at different rates can help physicians assign patients to a more appropriate and tailored treatment program. A burden of disease marker would reflect the severity of the disease in a patient and help in the administration of the appropriate treatment. Efficacy of intervention and safety biochemical markers assist in the ongoing hunt for disease modifying osteoarthritis drugs. Cytokines have also become targets themselves for therapeutic agents and as therapeutic agents<sup>[24-27]</sup>. Investigative markers are those for which there is insufficient data to assign them to another category.

The ideal scenario, in terms of biochemical markers of OA, would be to have a non-invasive, reliable and valid biochemical marker or cluster of markers that could be measured to aid in the diagnosis and predict the development of OA in patients at an early stage before the disease becomes symptomatic. The ability to reduce the long-term effects of the disease could considerably reduce the substantial socioeconomic costs of OA<sup>[28-30]</sup>.

This review aims to examine and summarize current knowledge of the use of cytokines as biochemical markers in the diagnosis and management of knee OA. Table 1 shows a summary of a number of articles in which possible biochemical markers for OA have been studied.

## PROINFLAMMATORY CYTOKINES

Inflammation is increasingly being regarded as an important part of OA. Inflammation can occur locally, within the synovium, and systemically, with inflammatory agents circulating in the blood. In the pathophysiology of OA, proinflammatory cytokines have been shown to play important roles in the destruction of cartilage, synovitis, and pain<sup>[31-34]</sup>. The severity and form of inflammation appears to change with disease progression, with different cytokine signatures being present in early and advanced stages of the disease.

A number of proinflammatory cytokines have been, and continue to be, studied as potential biochemical markers with possible candidates being found for burden

**Table 1 Summary of studies in which cytokines have shown biochemical marker characteristics**

Ref.	Year	Cytokines	Joint	Tissue	Condition of samples	Samples	Controls	Assay	Follow-up	Results
Sun <i>et al</i> <sup>[65]</sup>	2013	IL-15	Knee	Serum	Primary Knee OA	226	106	ELISA	N/A	IL-15 Serum OA ↑ Control IL-15 positively correlated with WOMAC (Pain) IL-15 not correlated with KL
Shimura <i>et al</i> <sup>[65]</sup>	2013	IL-6	Knee	Serum	Postmenopausal females with medial knee OA	160	N/A	CLIA	N/A	Serum IL-6 = association with pain severity (VAS) in early stage Serum IL-6 = association with pain severity (JKOM) in early stage
Wang <i>et al</i> <sup>[66]</sup>	2013	IL-18	Knee	Plasma, SF and Articular Cartilage	Primary Knee OA	33	15	ELISA + Immunofluorescence Staining	N/A	IL-18 Plasma +SF OA ↑ Controls IL-18 positive cells AC OA ↑ Controls ↑ KL = ↑ IL-18 Plasma, SF + AC + ↑ IL-18 positive cells IL-18 Plasma, SF + AC = Positive correlation ↓ Pain severity (NRS-pain) = ↓ IL-1β ↓ IL-1β, IL-8 + IL-12 (Baseline - 6 mo) Adults vs Elderly (not significant) ↓ TNF-α (Baseline - 6 mo) in Adults vs Elderly ↑ SF IL-7 = ↑ KL SF VEGF 2 × ↑ KL=3/4 vs KL=0 IL-7 = positive correlation with age IL-7 2 × ↑ > 60 years old vs < 60 years old IL-1Ra, IL-6, IL-8, IL-10, IL-17, MCP-1, IL-13, IL-18 + HGF ≠ association with KL
Vincent <i>et al</i> <sup>[69]</sup>	2013	IL-1β, IL-6, IL-8, IL-12, TNF-α, IL-4, IL-10 + IL-13	Knee	SF	Chronic OA	Adult (50-64 yr): 14 Elderly (≥ 65 yr): 14	N/A	Multiplex	6 mo	
Rubenhagen <i>et al</i> <sup>[62]</sup>	2012	IL-1Ra, IL-6, IL-8, IL-10, IL-17, VEGF, MCP-1, IL-7, IL-13, IL-18 + HGF	Knee	SF	Total knee replacements or cruciate ligament, cartilage, or meniscal reconstruction surgery	82	N/A	Multiplex + ELISA	N/A	
Chadjichristos <i>et al</i> <sup>[62]</sup>	2012	IL-1β + TNF-α	Knee	SF	OA Established (Medial meniscectomy)	25	5	ELISA	N/A	↑ IL-1β + TNF-α = ↑ Degree of Inflammation IL-1β + TNF-α ↑ in weeks 2 + 4 IL-1β ↓ in week 12 TNF-α ↓ in weeks 8 + 12 OA SF IP10 2.5 × ↓ than plasma OA SF IP10 3 × ↓ than control plasma ↑ KL = ↓ Plasma + SF IP-10 Plasma + SF IP10 inversely correlated with KL (SF less so) ↑ IL-2 + IL-5 = ↑ Disease severity (ICRS) ↑ IL-1β, IL-6, IL-8, IL-12p70 + IPN-γ = ↑ Disease Severity (ICRS) (Borderline significance; P < 0.10) IL-1β, IL-6, IL-8, IL-12p70, IPN-γ, IL-2 + IL-5 ≠ association with KL
Saetan <i>et al</i> <sup>[63]</sup>	2011	IP-10	Knee	Plasma + SF	Knee OA	40	15	ELISA	N/A	
Vangness <i>et al</i> <sup>[61]</sup>	2011	IL-1β, IL-6, IL-8, IL-12p70, IFN-γ, IL-2 + IL-5	Knee	SF	Patients underwent an arthroscopy of the knee for a meniscal tear	12	N/A	Immunoassay high-throughput flow cytometry	N/A	
Orita <i>et al</i> <sup>[65]</sup>	2011	IL-6, TNF-α + NGF	Knee	SF	Adult patients with knee pain - No previous OA treatment	47	N/A	ELISA	N/A	TNF-α ≠ correlation with KL TNF-α ↓ KL (2-4) vs KL (1) TNF-α = P positive correlation with WOMAC + WOMAC (Pain, stiffness + physical function) IL-6 = Negative correlation with KL IL-6 ↓ KL (3 + 4) vs KL (1 + 2) IL-6 = Negative correlation with WOMAC (Stiffness) IL-6 ≠ correlation with WOMAC or WOMAC (Pain or physical function)

Author	Year	Study Design	Location	Sample Size	RA: 22 Donor: 20	Method	Duration	Findings
Kokébie <i>et al</i> <sup>[86]</sup>	2011	Fulfilled ACR criteria	Knee	45	N/A	ELISA	N/A	IL-8 RA ↑ OA + Donors IL-11 RA = OA IL-11 OA ↑ Donors LIF Donors ↑ OA + RA LIF OA = RA SF WBC positive correlation with IL-6 + IL-1 IL-11, IL-8 + LIF no correlation with SF WBC No biomarkers correlated with KL or disease activity (WOMAC) IL-6 = inverse correlation with muscle resistance + muscle balance (hamstring) IL-6 ≠ correlation with functionality IL-6 + TNF-α Serum = association with JSN (medial tibiofemoral) IL-6 + TNF-α Serum ≠ association with cartilage volume (tibial) IL-6 Serum predicted ↓ cartilage volume (medial + tibial) IL-6 + TNF-α associated with ↓ cartilage volume (medial + lateral) TNF-α associated with JSN (older patients) IL-6 Male = Female ↑ IL-6 = ↑ JSN (female) IL-6 ≠ association with JSN (Male) Trunk-fat + Total Fat ratios associated with IL-6 IL-6 ≠ association with presence or severity of osteophytes SF IL-15 ↑ early vs advanced Expression of IL-15 early = advanced Synovial membrane IL-1β, IL-6 + TNF-α early = advanced SF IL-1β, IL-6 + TNF-α early = advanced Synovial membrane IL-21 + IL-2 ↓ early vs advanced SF IL-21 + IL-2 early = advanced IL-15 predicted OA at Initial X-ray IL-1α, IL-2, IL-15 + 6Ckine OA ≠ Control IL-15 OA ↑ Control (at Initial + Classifying X-rays)
Santos <i>et al</i> <sup>[87]</sup>	2011	Knee OA (ACR)	Knee	80	N/A	ELISA	N/A	IL-6 = inverse correlation with muscle resistance + muscle balance (hamstring) IL-6 ≠ correlation with functionality IL-6 + TNF-α Serum = association with JSN (medial tibiofemoral) IL-6 + TNF-α Serum ≠ association with cartilage volume (tibial) IL-6 Serum predicted ↓ cartilage volume (medial + tibial) IL-6 + TNF-α associated with ↓ cartilage volume (medial + lateral) TNF-α associated with JSN (older patients) IL-6 Male = Female ↑ IL-6 = ↑ JSN (female) IL-6 ≠ association with JSN (Male) Trunk-fat + Total Fat ratios associated with IL-6 IL-6 ≠ association with presence or severity of osteophytes SF IL-15 ↑ early vs advanced Expression of IL-15 early = advanced Synovial membrane IL-1β, IL-6 + TNF-α early = advanced SF IL-1β, IL-6 + TNF-α early = advanced Synovial membrane IL-21 + IL-2 ↓ early vs advanced SF IL-21 + IL-2 early = advanced IL-15 predicted OA at Initial X-ray IL-1α, IL-2, IL-15 + 6Ckine OA ≠ Control IL-15 OA ↑ Control (at Initial + Classifying X-rays)
Stannus <i>et al</i> <sup>[88]</sup>	2010	Selected randomly from the roll of electors	Knee	172	N/A	CLIA	3 yr	IL-6 = inverse correlation with muscle resistance + muscle balance (hamstring) IL-6 ≠ correlation with functionality IL-6 + TNF-α Serum = association with JSN (medial tibiofemoral) IL-6 + TNF-α Serum ≠ association with cartilage volume (tibial) IL-6 Serum predicted ↓ cartilage volume (medial + tibial) IL-6 + TNF-α associated with ↓ cartilage volume (medial + lateral) TNF-α associated with JSN (older patients) IL-6 Male = Female ↑ IL-6 = ↑ JSN (female) IL-6 ≠ association with JSN (Male) Trunk-fat + Total Fat ratios associated with IL-6 IL-6 ≠ association with presence or severity of osteophytes SF IL-15 ↑ early vs advanced Expression of IL-15 early = advanced Synovial membrane IL-1β, IL-6 + TNF-α early = advanced SF IL-1β, IL-6 + TNF-α early = advanced Synovial membrane IL-21 + IL-2 ↓ early vs advanced SF IL-21 + IL-2 early = advanced IL-15 predicted OA at Initial X-ray IL-1α, IL-2, IL-15 + 6Ckine OA ≠ Control IL-15 OA ↑ Control (at Initial + Classifying X-rays)
Stannus <i>et al</i> <sup>[84]</sup>	2010	Selected randomly from the roll of electors	Hip	193	N/A	ELISA	N/A	IL-6 = inverse correlation with muscle resistance + muscle balance (hamstring) IL-6 ≠ correlation with functionality IL-6 + TNF-α Serum = association with JSN (medial tibiofemoral) IL-6 + TNF-α Serum ≠ association with cartilage volume (tibial) IL-6 Serum predicted ↓ cartilage volume (medial + tibial) IL-6 + TNF-α associated with ↓ cartilage volume (medial + lateral) TNF-α associated with JSN (older patients) IL-6 Male = Female ↑ IL-6 = ↑ JSN (female) IL-6 ≠ association with JSN (Male) Trunk-fat + Total Fat ratios associated with IL-6 IL-6 ≠ association with presence or severity of osteophytes SF IL-15 ↑ early vs advanced Expression of IL-15 early = advanced Synovial membrane IL-1β, IL-6 + TNF-α early = advanced SF IL-1β, IL-6 + TNF-α early = advanced Synovial membrane IL-21 + IL-2 ↓ early vs advanced SF IL-21 + IL-2 early = advanced IL-15 predicted OA at Initial X-ray IL-1α, IL-2, IL-15 + 6Ckine OA ≠ Control IL-15 OA ↑ Control (at Initial + Classifying X-rays)
Scanzello <i>et al</i> <sup>[88]</sup>	2009	Early: degenerative meniscal tears Adv: Knee replacement surgery	Knee	Early: 19 Adv: 15	N/A	ELISA + Quantitative PCR	N/A	IL-6 = inverse correlation with muscle resistance + muscle balance (hamstring) IL-6 ≠ correlation with functionality IL-6 + TNF-α Serum = association with JSN (medial tibiofemoral) IL-6 + TNF-α Serum ≠ association with cartilage volume (tibial) IL-6 Serum predicted ↓ cartilage volume (medial + tibial) IL-6 + TNF-α associated with ↓ cartilage volume (medial + lateral) TNF-α associated with JSN (older patients) IL-6 Male = Female ↑ IL-6 = ↑ JSN (female) IL-6 ≠ association with JSN (Male) Trunk-fat + Total Fat ratios associated with IL-6 IL-6 ≠ association with presence or severity of osteophytes SF IL-15 ↑ early vs advanced Expression of IL-15 early = advanced Synovial membrane IL-1β, IL-6 + TNF-α early = advanced SF IL-1β, IL-6 + TNF-α early = advanced Synovial membrane IL-21 + IL-2 ↓ early vs advanced SF IL-21 + IL-2 early = advanced IL-15 predicted OA at Initial X-ray IL-1α, IL-2, IL-15 + 6Ckine OA ≠ Control IL-15 OA ↑ Control (at Initial + Classifying X-rays)
Ling <i>et al</i> <sup>[64]</sup>	2009	Radiographic OA in one or both knees and one or both hands	Hand and Knee	Initial: 21 Classifying: 19	Initial: 61 Classifying: 66	RCA enhanced antibody-based protein microarray	OA: 10.03 ± 0.31 Controls: 9.88 ± 0.22 (Years)	IL-6 = inverse correlation with muscle resistance + muscle balance (hamstring) IL-6 ≠ correlation with functionality IL-6 + TNF-α Serum = association with JSN (medial tibiofemoral) IL-6 + TNF-α Serum ≠ association with cartilage volume (tibial) IL-6 Serum predicted ↓ cartilage volume (medial + tibial) IL-6 + TNF-α associated with ↓ cartilage volume (medial + lateral) TNF-α associated with JSN (older patients) IL-6 Male = Female ↑ IL-6 = ↑ JSN (female) IL-6 ≠ association with JSN (Male) Trunk-fat + Total Fat ratios associated with IL-6 IL-6 ≠ association with presence or severity of osteophytes SF IL-15 ↑ early vs advanced Expression of IL-15 early = advanced Synovial membrane IL-1β, IL-6 + TNF-α early = advanced SF IL-1β, IL-6 + TNF-α early = advanced Synovial membrane IL-21 + IL-2 ↓ early vs advanced SF IL-21 + IL-2 early = advanced IL-15 predicted OA at Initial X-ray IL-1α, IL-2, IL-15 + 6Ckine OA ≠ Control IL-15 OA ↑ Control (at Initial + Classifying X-rays)
Livshits <i>et al</i> <sup>[87]</sup>	2009	White females	Knee	Year 5: 430 (IL-6: 429) Year 8: 473 Year 15: 322	N/A	TNF-α: High-sensitivity ELISA IL-6: Ultra-Sensitivity ELISA	Year 5, 8 + 15	IL-6 = inverse correlation with muscle resistance + muscle balance (hamstring) IL-6 ≠ correlation with functionality IL-6 + TNF-α Serum = association with JSN (medial tibiofemoral) IL-6 + TNF-α Serum ≠ association with cartilage volume (tibial) IL-6 Serum predicted ↓ cartilage volume (medial + tibial) IL-6 + TNF-α associated with ↓ cartilage volume (medial + lateral) TNF-α associated with JSN (older patients) IL-6 Male = Female ↑ IL-6 = ↑ JSN (female) IL-6 ≠ association with JSN (Male) Trunk-fat + Total Fat ratios associated with IL-6 IL-6 ≠ association with presence or severity of osteophytes SF IL-15 ↑ early vs advanced Expression of IL-15 early = advanced Synovial membrane IL-1β, IL-6 + TNF-α early = advanced SF IL-1β, IL-6 + TNF-α early = advanced Synovial membrane IL-21 + IL-2 ↓ early vs advanced SF IL-21 + IL-2 early = advanced IL-15 predicted OA at Initial X-ray IL-1α, IL-2, IL-15 + 6Ckine OA ≠ Control IL-15 OA ↑ Control (at Initial + Classifying X-rays)
Botha-Scheepers <i>et al</i> <sup>[87]</sup>	2007	Symptomatic knee OA in at least one knee at baseline	Knee	86	N/A	ELISA	2 yr	IL-6 = inverse correlation with muscle resistance + muscle balance (hamstring) IL-6 ≠ correlation with functionality IL-6 + TNF-α Serum = association with JSN (medial tibiofemoral) IL-6 + TNF-α Serum ≠ association with cartilage volume (tibial) IL-6 Serum predicted ↓ cartilage volume (medial + tibial) IL-6 + TNF-α associated with ↓ cartilage volume (medial + lateral) TNF-α associated with JSN (older patients) IL-6 Male = Female ↑ IL-6 = ↑ JSN (female) IL-6 ≠ association with JSN (Male) Trunk-fat + Total Fat ratios associated with IL-6 IL-6 ≠ association with presence or severity of osteophytes SF IL-15 ↑ early vs advanced Expression of IL-15 early = advanced Synovial membrane IL-1β, IL-6 + TNF-α early = advanced SF IL-1β, IL-6 + TNF-α early = advanced Synovial membrane IL-21 + IL-2 ↓ early vs advanced SF IL-21 + IL-2 early = advanced IL-15 predicted OA at Initial X-ray IL-1α, IL-2, IL-15 + 6Ckine OA ≠ Control IL-15 OA ↑ Control (at Initial + Classifying X-rays)

Author	Year	IL-6	Knee + Hip	Serum + SF	End-stage OA (Hip or Knee)	49 (Knee: 32, Hip: 17)	N/A	ELISA	16% OA ↑ IL-6 vs 84% OA (Possible sub-groups) IL-6 SF = in Knee vs Hip OA, age, sex, stage of disease or course
Doss <i>et al</i> <sup>[60]</sup>	2007	IL-6	Knee + Hip	Serum + SF	End-stage OA (Hip or Knee)	49 (Knee: 32, Hip: 17)	N/A	ELISA	16% OA ↑ IL-6 vs 84% OA (Possible sub-groups) IL-6 SF = in Knee vs Hip OA, age, sex, stage of disease or course
Riyazi <i>et al</i> <sup>[61]</sup>	2005	TNF- $\alpha$ , IL-1 $\beta$ , IL-1Ra, + IL-10	Hand, Spine, Hip or Knee	Whole Blood	OA at multiple sites	305	137	ELISA	↑ IL-1 $\beta$ + IL-1Ra + ↓ IL-10 ( <i>ex vivo</i> production with LPS stimulation) = ↑ Multiple site OA familial risk ↑ TNF- $\alpha$ ( <i>ex vivo</i> production with LPS stimulation) ≠ ↑ OA risk
Pheminx <i>et al</i> <sup>[61]</sup>	2004	IL-6, CRP, TNF- $\alpha$ , IL-6sR, IL-2sR, TNF-sR1, + TNF-sR	Knee	Serum	Over-weight, older ( $\geq$ 60 yr) + knee OA	274	N/A	ELISA	↑ TNF-sR1 and TNF-sR2 = ↑ WOMAC (Physical Function) ↑ IL-6 = ↓ Walking speed ↑ TNF-sR = ↑ Pain, Stiffness + KL IL-1 ≠ association with KL or WOMAC (Physical Function)

ACR: American College of Rheumatology; Adv: Advanced; CLIA: Chemiluminescent enzyme immunoassay; COMP: Cartilage oligomeric matrix protein; ELISA: Enzyme link immunosorbent assay; GF: Growth factor; HGF: Hepatocyte growth factor; hsCRP: High-sensitivity C reactive protein; ICRS: International cartilage repair society; IFN: Interferon; IL-1Ra: IL-1 Receptor antagonist; IP-10: Interferon- $\gamma$  inducible protein-10; KOM: Japanese knee osteoarthritis measure; JSN: Joint space narrowing; KL: Kellgren-lawrence grading; LIF: Leukemia inhibitory factor; LPS: Lipopolysaccharides; MCP: Monocyte chemoattractant protein; MIP: Macrophage inflammation protein; NGF: Nerve growth factor; NRSpain: Numerical pain rating scale; OA: Osteoarthritis; PCR: Polymerase chain reaction; RA: Rheumatoid arthritis; RCA: Rolling circle amplification; RKO: Radiographic knee osteoarthritis; SF: Synovial fluid; sR: Soluble receptor; TNF: Tumor necrosis factor; VAS: Visual analog scale; VEGF: Vascular endothelial growth factor; WBC: White blood cell; WOMAC: Western ontario mcMaster university osteoarthritis index.

of disease assessment, prognostics and diagnostics. IL-1 $\beta$  and TNF- $\alpha$  are among the key players in terms of proinflammatory cytokines involved in OA<sup>[35]</sup>. IL-6 also plays a major pro-inflammatory role in OA. Alongside these, more minor players, for example IL-15 and IL-18, have also been investigated as potential candidates for biochemical markers in OA.

**IL-6**

IL-6 is a 184 amino acid residue protein<sup>[34]</sup> which has been shown in a number of studies to play a pro-inflammatory role in the pathophysiology of OA. Healthy chondrocytes produce low amounts of IL-6 without the presence of a stimulating agent<sup>[36]</sup> but when exposed to certain cytokines, including IL-1 $\beta$ , a key player in the inflammation of arthritic joints, chondrocytes increase production<sup>[10,37]</sup>. Likewise TNF- $\alpha$  and interferon- $\gamma$  have also been shown to induce IL-6 production<sup>[36]</sup>. IL-6 has been shown to inhibit the production of type II collagen in animal models<sup>[38]</sup>.

In animal models, higher levels of IL-6 have been found in osteoarthritic groups compared with controls<sup>[39-41]</sup>. This is mirrored in human patients with OA in a number of studies. However, synovial fluid (SF) levels of IL-6 have been shown not to correlate with body mass index (BMI), age, or OA severity (KL) in a study of 82 knee OA patients<sup>[42]</sup>. Conversely, a study of 47 knee OA patients with no previous OA treatment found that SF samples of IL-6 negatively correlated with KL grades and were not associated with WOMAC (Western Ontario McMaster University Osteoarthritis Index<sup>[43]</sup>) pain and function scores with the exception of the subset of stiffness with which IL-6 correlated slightly. In a cross-sectional study of hip OA patients, serum IL-6 levels in women were positively associated with hip JSN, but not with the presence of osteophytes<sup>[44]</sup>. Instead, in a recent study of 160 postmenopausal females by Shimura *et al*<sup>[45]</sup>, serum IL-6 levels were associated with pain severity in early stage knee OA but not advanced stages of the disease. Higher serum levels also tended to be associated with decreased walking speeds<sup>[46]</sup>.

A prospective population-based study of females by Livshits *et al*<sup>[47]</sup> revealed higher serum IL-6 levels associated with an increased chance of diagnosis of OA throughout a 15 year follow-up. This supports the potential of IL-6 to be a biomarker for early diagnosis of OA. This idea that IL-6 plays a role in early stage OA is supported by Stannus *et al*<sup>[48]</sup>, who conducted a study of 172 OA patients in which they found that circulating IL-6 was associated with JSN and knee cartilage loss and that, longitudinally, the baseline levels of IL-6 predicted both medial and lateral tibial cartilage volume. Higher IL-6 levels were also associated with an increased prevalence of osteophytes compared with lower IL-6 levels. The study suggested that IL-6 may play a role in cartilage loss in early stage OA. Because of this early stage role, IL-6 could be classed as a diagnostic and prognostic biomarker; however, further studies are required before a conclusive view can be formed.

Doss *et al*<sup>[49]</sup> suggested possible subgroups of OA patients with varying levels of IL-6. Patients found to produce relatively high levels of IL-6 showed a possible increase in the frequency of the <sup>-174</sup>C-allele of the IL-6 gene. Whilst the authors note the study size was insufficient to draw a significant result, it does raise an important point that the presence of subgroups could pose an obstacle in the hunt for a universal and specific biochemical marker for OA due to the varying levels of expression between groups. Another consideration to take into account with the use of IL-6 as a biochemical marker is that it appears to have a circadian rhythm<sup>[50]</sup>. In addition, plasma IL-6 levels have been seen to increase significantly during periods of modest sleep deprivation in healthy adults<sup>[51]</sup>. These could potentially interfere with measurements of biochemical markers. Another noteworthy point that should be taken into consideration when assessing IL-6 is that levels have been shown to increase with repeated catheter use when drawing blood samples. This is thought to be local production rather than a physiological process<sup>[20,52]</sup>.

### IL-1 $\beta$

One of the most important proinflammatory cytokines to play a role in the pathophysiology of OA is IL-1 $\beta$ . This 17.5 kDa protein<sup>[53]</sup> is a suppressor of type II collagen and aggrecan synthesis which are key constituents of cartilage<sup>[31,32]</sup>. With a decreased production of these components, cartilage degradation is worsened. Furthermore, IL-1 $\beta$  induces the production of a number of cytokines and chemokines which contribute to the state of inflammation; these include IL-6 and IL-8<sup>[36,54]</sup> (reviewed by Kapoor *et al*<sup>[10]</sup>). Due to its large involvement, IL-1 $\beta$  has been investigated in a number of studies as a potential candidate as a biochemical marker.

Ning *et al*<sup>[55]</sup> examined the expression of IL-1 $\beta$  in 23 patients with medial knee OA. They found, through immunohistochemical analysis, that expression of IL-1 $\beta$  in both the lining and sublining of the medial perimeniscal synovial tissue samples collected had a significant positive correlation with joint space width. The levels were also negatively correlated with joint alignment (femoro-tibial angle). As well as joint alignment, the authors reported a significant negative correlation with physical disability. This study suggested that local expression levels of IL-1 $\beta$  are associated with the severity of disease and thus have potential as burden of disease markers.

Using lipopolysaccharides (LPS) to stimulate whole blood samples, Riyazi *et al*<sup>[56]</sup> investigated the production of IL-1 $\beta$ , as well as IL-1 receptor antagonist (IL-1Ra), IL-10, and TNF- $\alpha$  in OA patients. The patients had OA in various joints including hips, the spine, hands, and knees. High innate *ex vivo* production of IL-1 $\beta$  and IL-1Ra was associated with an increased risk of familial OA at multiple sites. However, in a separate study, both IL-1 $\beta$  and IL-1Ra failed to show a significant association between innate *ex vivo* production and the progression of knee OA (JSN) over a 2-year period<sup>[57]</sup>.

Very recently, mouse models have shown that IL-

1 $\beta$  plays important roles in pain sensitivity<sup>[58]</sup>. However, interestingly, IL-1 $\beta$  levels in guinea pig serum were statistically similar between OA-prone and OA-resistant strains<sup>[39]</sup>. In rabbits, the expression levels of IL-1 $\beta$  and TNF- $\alpha$  were suggested to reflect the severity of inflammation in experimental OA. Levels were increased in early stages of the disease but reduced with regression of synovitis<sup>[33]</sup>.

IL-1 $\beta$  has been used as a marker of efficacy of intervention in a study assessing the effects of intraarticular hyaluronic acid treatment in patients with knee OA. A moderate negative correlation between changes in synovial fluid IL-1 $\beta$  and a reduction in pain severity over a 6-mo period was observed<sup>[59]</sup>.

### Tumor necrosis factor- $\alpha$

TNF- $\alpha$  is a 17 kDa protein produced predominately by activated macrophages which effects the production of cytokines including IL-6 and IL-8 among others<sup>[36,54,60]</sup>.

In the same study as previously mentioned in which hyaluronic acid injections in OA patients were assessed through measuring IL-1 $\beta$ , TNF- $\alpha$  also showed a significant reduction from baseline to 6 mo in adults compared with elderly adults<sup>[59]</sup>.

Soluble TNF receptors in serum samples from OA patients showed a positive correlation with pain, joint stiffness and higher radiographic severity of disease<sup>[61]</sup>. However, in canine models, TNF- $\alpha$  and its receptors did not show an association with mild osteoarthritic changes when increased in articular cartilage<sup>[62]</sup>. In addition, no association was found between plasma TNF- $\alpha$  levels and OA characteristics in patients with hand OA<sup>[63]</sup>. Following LPS stimulation, high *ex vivo* production of TNF- $\alpha$  did not increase the risk of OA<sup>[56]</sup>.

TNF- $\alpha$  has shown characteristics as a marker of treatment efficacy, and mixed results as a burden of disease marker. Further study is needed to clarify its position and efficacy as a biochemical marker of OA.

### IL-15

IL-15 contributes to inflammation in OA as a proinflammatory cytokine. There have been relatively few studies examining its potential use as a biochemical marker. However, a few articles have suggested it could be a prognostic and burden of disease marker.

In a study of knee and hand OA patients and controls, IL-15 was found to predict the development of OA in patients who were asymptomatic for OA at baseline then assessed at a 10-year follow-up. At baseline and at follow-up, the levels of IL-15 in OA patients' serum were elevated compared with healthy controls. These two findings suggest IL-15 has potential as both a diagnostic and prognostic biochemical marker<sup>[64]</sup>.

IL-15 was found to be slightly, but significantly, elevated in serum samples of OA patients compared with those of controls. In the same study, IL-15 levels were found to have an independent positive correlation with WOMAC pain scores but showed no significant relationship with the severity of OA (KL)<sup>[65]</sup>.

It can be suggested from this that IL-15 is a possible burden of disease biomarker for assessing the pain associated with OA but not, however, for the assessment of the progression of cartilage destruction and severity. IL-15 also has potential as a diagnostic biochemical marker.

### IL-18

Whilst SF IL-18 levels have been shown to have no correlation with OA grade (KL), BMI or age<sup>[43]</sup>, IL-18 levels in plasma, SF and articular cartilage samples from knee OA patients have been shown to be significantly higher than in healthy controls. Patients with higher disease severity had significantly higher IL-18 in all three sample media<sup>[66]</sup>. This would suggest IL-18 has the potential to distinguish between healthy and OA sufferers and to assess the severity of the disease in OA patients.

## ANTI-INFLAMMATORY CYTOKINES

Countering the proinflammatory cytokines, anti-inflammatory cytokines also play a role in the pathophysiology of OA. In particular, IL-10 and IL-4 contribute to the suppression of inflammation of the synovial membrane<sup>[67,68]</sup>. By reducing inflammation, these mediators can support cartilage production, acting as anabolic effectors which can slow the progression of OA. In disease-free conditions, the balance between anabolic and catabolic cytokines enables stable levels of cartilage. In OA, an imbalance in this equilibrium contributes to the pathophysiology of the disease. Generally, however, anti-inflammatory cytokines have been less well studied in the search for biochemical markers of OA.

### IL-10 and IL-2

Low *ex vivo* production of IL-10 upon LPS stimulation was associated with an increased risk of familial OA at multiple sites<sup>[56]</sup>. In a similar study using LPS stimulation of knee OA samples, patients in the highest quartile of *ex vivo* IL-10 production had a 4-fold increased risk of radiological progression of JSN. This association was independent of age, sex or BMI<sup>[57]</sup>.

IL-2 was found by Ling *et al.*<sup>[64]</sup> to be higher in knee OA patients at the end of a 10-year follow-up period compared with healthy controls.

## CHEMOKINES AND ANGIOGENIC GROWTH FACTORS

Chemotactic cytokines, or chemokines, have been shown to influence inflammation in OA through their ability to influence the number of immune cells in the vicinity of the joint. They also stimulate IL-6 production and proteoglycan depletion<sup>[69,70]</sup>. Angiogenic growth factors contribute to synovitis and pain as well as cartilage destruction<sup>[16,18,71]</sup>.

### Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is a 46-48 kDa glycosylated polypeptide<sup>[72]</sup> and a potent angiogenic

cytokine that has been shown to play a role in OA<sup>[17,73]</sup>. It is produced by hypotrophic chondrocytes, macrophages and synovial fibroblasts<sup>[73-75]</sup>.

VEGF in SF has been shown to correlate with OA severity, and no correlation with BMI, with a 2-fold increase between grade 0 and grade 3-4 patients<sup>[42]</sup>. We recently found similar results in our laboratory. SF and plasma levels were both positively correlated with the severity of OA (KL), though SF samples presented a stronger correlation<sup>[76]</sup>.

### IL-7

IL-7 is a hemopoietic growth factor involved in the development of B and T cells. It has been found to increase with age in samples of SF from OA patients, with the median concentration in patients over 60 years old double that of those under 60 years old. However, in the same study, there was no reported association between OA severity and IL-7 levels, and levels were depressed in patients with severe 3-compartment OA. IL-7 levels were found to have a weak correlation with BMI<sup>[42]</sup>.

### IL-8

IL-8 (also known as CXXL8) is a potent chemokine in the immune system. Few studies have examined this chemokine in detail with respect to levels in both SF or circulating media, and its relationship with OA. Nevertheless, it has been shown not to correlate with OA grade, BMI or age<sup>[42]</sup> in SF samples from OA patients. Pierzchala *et al.*<sup>[77]</sup> found no correlation between IL-8 levels and OA severity (WOMAC) nor was there an association with bone remodeling<sup>[78]</sup>. Hitherto, there is insufficient evidence to suggest IL-8 possesses traditional characteristics of a biochemical marker. However, there have been few studies examining its potential. In contrast, it might be applicable as a housekeeping marker with levels not expected to change throughout the course of the disease or between healthy controls and OA patients.

## CONCLUSION

A number of cytokines have shown potential as different types of biochemical markers (Table 2). Currently, IL-6 shows potential as a diagnostic and prognostic biomarker of OA. Other cytokines, including IL-1 $\beta$ , TNF- $\alpha$ , IL-15 and VEGF, show promise as burden of disease markers.

Differences between circulating (systemic) and SF (local) sampling should be taken into consideration when designing future studies and clinical applications to assess cytokine levels.

Due to their increased statistical power, using clusters of markers will have more impact than individual biomarkers. Unfortunately, in the hunt for biochemical markers specific to OA, most cytokines can be associated with a number of diseases. IL-15, for example, plays roles in rheumatoid arthritis, diabetes mellitus type 1 and type 2, and cancers<sup>[79-82]</sup>. This is a hindrance shared with many cytokines being investigated and supports the need to assess multiple candidates together.

**Table 2** Classification of cytokines as biochemical markers in osteoarthritis

Cytokine	BIPEDS classification
IL-6	D, P
IL-1 $\beta$	B, E
TNF- $\alpha$	B, E
IL-15	B, D
IL-18	D
IL-10	P
IL-2	D
VEGF	B
IL-7	D

B: Burden of Disease; D: Diagnostic; E: Efficacy of Intervention; I: Investigative; P: Prognostic; S: Safety; TNF: Tumor necrosis factor; VEGF: Vascular endothelial growth factor.

Owing to the invasive nature of collecting SF samples, it is usually only collected from patients undergoing knee surgery. In future clinical applications of the use of biochemical markers, this may not be feasible. Less invasive sample mediums, for example serum, plasma, and urine should continue to be investigated and validated.

More large-scale studies are required to assess the use of groups of biochemical markers and their effectiveness. Different groups designed for different purposes, for example diagnostic or prognostic, may prove valuable. In addition to customization of groups for intended purposes, adjusting for the stage of disease to be assessed, particularly for burden of disease assessment, would yield a more finely tuned clinical tool.

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