

Osteoarthritis and Cartilage



Review

Osteoarthritis year in review 2018: biomarkers (biochemical markers)

F. Saberi Hosnijeh †‡*, S.M. Bierma-Zeinstra §, A.C. Bay-Jensen ||



† Immunology Department, Erasmus University Medical Center, Rotterdam, the Netherlands

‡ Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, the Netherlands

§ Department of General Practice, Erasmus University Medical Center, Rotterdam, the Netherlands

|| Biomarker and Research, Nordic Bioscience, Herlev, Denmark

ARTICLE INFO

Article history:

Received 1 August 2018

Accepted 5 December 2018

Keywords:

Osteoarthritis

Biomarker

Outcome

Prognosis

Diagnosis

SUMMARY

Objective: The aim of this narrative review is to summarize important findings from biochemical marker studies relevant to osteoarthritis (OA) in the context of new discoveries and clinical and scientific need.

Design: We conducted a systematic search of electronic medical databases (Embase, Medline, Web of Science, Cochrane central) between 01-03-2017 and 31-03-2018. The search was restricted to human studies, English language and full text available publications while reviews were excluded. Only papers describing protein based biomarkers measured in human body fluids (blood, urine and synovial fluid (SF)) were included. Of the 992 papers, 86 were reviewed here, with inclusion primarily based on relevance to OA biochemical markers.

Results: This review highlights a selection of studies based on their quality and perceived importance to the field mainly including those that¹ evaluate prognostic value of biomarkers for OA progression (i.e., biomarkers reflecting change in composition of joint tissues and biomarkers of inflammation)², help in assessment of intervention efficacy, and³ are innovative and uncover new candidate biomarkers, or use new approaches in biomarker discovery.

Conclusions: Key findings and implications for possible clinical utility of biochemical markers are summarized and discussed. Given the paucity of robust biomarkers within the field, and the heterogeneity of the condition, enormous works are needed for development and validation of novel and clinically applicable biomarkers to reduce the impact of this highly prevalent and debilitating condition.

© 2018 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

Introduction

Osteoarthritis (OA), the most common form of arthropathy, is a leading cause of chronic pain and impaired mobility in older individuals. Structurally, it is characterized by alteration of joint structure including progressive cartilage destruction, synovial inflammation, and changes to the subchondral bone¹.

It is increasingly recognized that OA affects all structures within the joint via multiple causal pathways. Recent efforts are now focused on identifying subgroups of patients with distinct disease pathology, which will allow the development of new targeted therapies¹. Biochemical markers (biomarkers) have the potential to

serve as a measure of the different pathological processes linked to OA. Both effector molecules, such as cytokines and enzymes, and extracellular matrix constituents, such as precursors or degradation products of collagen and proteoglycan, have potential as biomarkers. Their concentrations are linked to tissue metabolism and can be measured in blood, urine, or synovial fluid (SF)². Moreover, biomarkers can be used in the process of drug development as well as in assessment of an individual patient's response to treatment or intervention.

The aim of this review is to summarize the OA related soluble biomarker publication recently examined in observational and interventional studies.

Methods

Search strategy and inclusion criteria

We conducted a systematic search of electronic medical databases from 03-2017 by 03–2018 to identify scientific articles

* Address correspondence and reprint requests to: F. Saberi Hosnijeh, Department of Immunology, Erasmus University Medical Center, P.O. Box 2040, 3000 CA Rotterdam, the Netherlands. Tel: 31-10-703-84-21.

E-mail addresses: f.saberihosnijeh@erasmusmc.nl (F. Saberi Hosnijeh), s.bierma-zeinstra@erasmusmc.nl (S.M. Bierma-Zeinstra), acbj@NordicBio.com (A.C. Bay-Jensen).

assessing OA related biomarkers. The search strategy in "Appendix" was applied. Only original studies carried out in humans, with English language and full text available publication were included. OA defined according to validated structural or clinical criteria including Kellgren and Lawrence score (KL ≥ 2), joint space narrowing (JSN), American College of Rheumatology criteria, Croft classification system, and total joint replacement (TJR) due to primary OA were used. Studies reported markers for OA, but without information on the joint site were excluded given the heterogeneous nature of the disease. Selection was restricted to protein based biomarkers measured in blood, urine and SF. Moreover, publications on biomarkers from cartilage, bone, or tissue extracts were not included, given the restricted availability and accessibility of these tissues. Additionally, to further limit inclusion to high quality studies, publications need to present associations between biomarkers and OA adjusted for major risk factors such as age, sex, and body mass index or body weight in order to be eligible. This resulted in 86 publications, which are referenced in this study (Fig. 1).

Results

Predictors of OA progression

Table 1 shows prospective observational and interventional studies that reported biomarkers of progression. Traumatic knee injury, such as an anterior cruciate ligament (ACL) injury, is associated with increased release of different proteins of bone, cartilage, and synovium. Predictive SF biomarkers for risk of radiographic Knee OA (RKOA) after 16 years following an ACL injury were evaluated for 88 patients without KOA at baseline. Biomarkers were

assessed during the first 7.5 years after the ACL injury. SF collected early after injury had considerably higher concentrations of aggrecan and cartilage oligomeric matrix protein (COMP) than the levels in the knees of the healthy reference group and the levels decreased to reference values during follow-up. Concentrations of aggrecan, COMP, matrix metalloproteinase-3 (MMP-3) and tissue inhibitor of metalloproteinase-1 (TIMP-1) failed to predict KOA after ACL injury in this study³. Progression of articular cartilage damage determined by arthroscopic evaluation was evaluated for 62 ACL patients approximately 2 years post-operation⁴. The progression of high-grade cartilage damage was associated with baseline SF levels of Δ di-C6S, keratan sulfate (KS), and C6S/C4S ratio. Interestingly, subjects with progression showed a cartilage aggrecan metabolism similar to that observed in patients with advanced radiographic OA in previous studies⁵. In another study among 26 ACL subjects who underwent reconstruction at an average of 8 weeks after injury, articular cartilage degeneration was measured by T1 ρ and T2 relaxation times using quantitative magnetic resonance prior to surgery and during the 3 years after surgery⁶. At the time of ACL reconstruction, profiles of SF inflammatory cytokines, degradative enzymes, and cartilage breakdown products show promise as predictors of abnormal cartilage tissue integrity (increased T1 ρ and T2 values) throughout the first 3 years after surgery.

Changes in bone remodeling are frequently present early in the OA process⁷. Using data of the Osteoarthritis Initiative, association between serum (S) and urine (U) biochemical markers of bone turnover and bone features on magnetic resonance images (MRI) was evaluated. Although at baseline, most biomarkers, in particular urinary C-telopeptide of type II collagen (uCTX-II), were associated with bone marrow lesions (BMLs), the markers were not predictive of changes in BMLs or osteophytes at follow-up⁷. Serum biomarkers

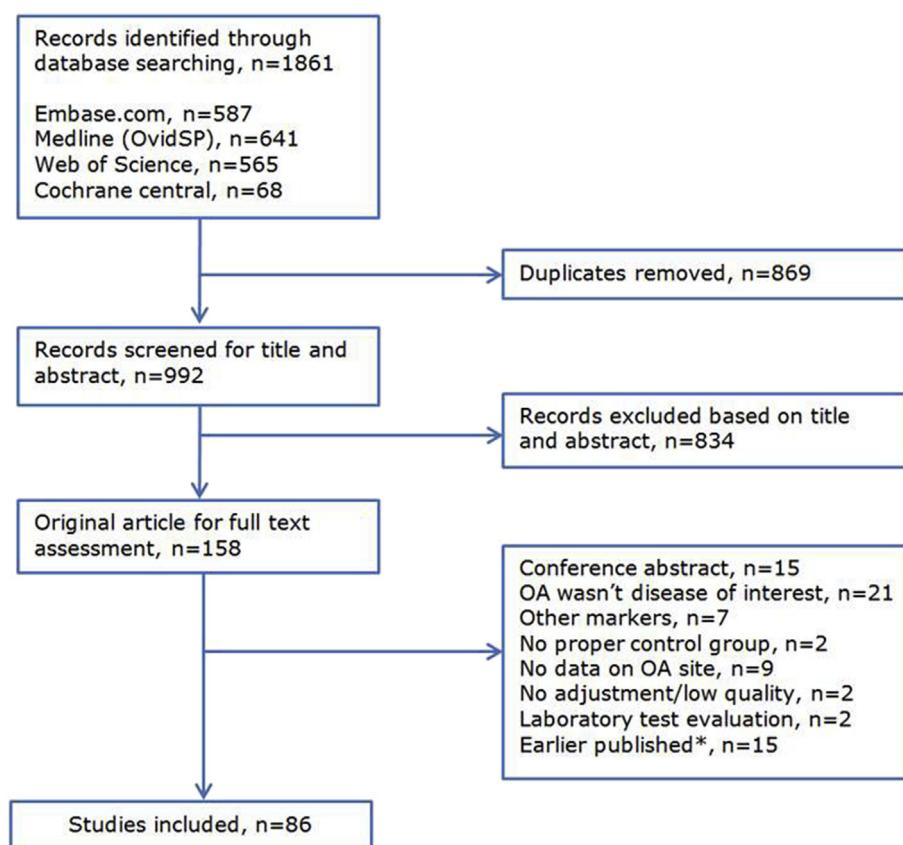


Fig. 1. Flow chart of the inclusion of the articles. *Earlier published before the first of March 2017.

Table I

Longitudinal observational and interventional studies on associations with changes of OA features

Biomarker	Sample	Outcome	Study size and results	Ref.
COMP, Aggrecan, MMP3, TIMP1	SF	RKOA	88 ACL and 12 controls; Levels of COMP and aggrecan were elevated in comparison with the reference group. Neither acute nor chronic concentrations of the four biomarkers were associated with the development of radiographic knee OA at the 16 year follow-up	3
C2C, KS, C4S, C6S	SF	Preradiographic high-grade focal cartilage damage	62 ACL injury patients; Progression of high-grade cartilage damage, observed arthroscopically, was significantly correlated with baseline levels of Δ di-C6S (OR = 0.23), Δ di-C4S (OR = 0.08), KS (OR = 0.11), and Δ di-C6S/ Δ di-C4S ratio (OR = 0.06)	4
IL1 α , IL1 β , IL2, IL4, IL6, IL8, IL10, IL12p70, IL13, IL1ra, SF TNF α , INF γ , CTXII, COMP, sGAG, MMP1, MMP3, MMP9, CPII, NTX	SF	Articular cartilage degeneration in MRI	26 ACL injury patients; The high sGAG cluster showed higher relaxation times compared with inflammation cluster	6
CTX-I, CTX-II, CTX-Ia, CTX-Ib, NTX-I	S, U	Change in MRI bone features	RKOA patients (KL1 = 75, KL2 = 306, KL3 = 219); Biomarkers were not predictive of changes in BMLs or osteophytes. uCTX-II was associated with BMLs, large osteophytes, bone area and shape at baseline and changes in bone shape over 24 months	7
MMP-3, HA, Coll2-1NO2	S	Change in MRI features of synovitis	RKOA patients (KL1 = 75, KL2 = 306, KL3 = 219); At baseline, sHA and sMMP-3 were associated with moderate to large effusion synovitis but there was no significant association between biomarkers (baseline and 12 and 24 month) and changes in MRI markers of synovitis	8
hsCRP, GSP	S	RKOA	54 Subjects classified in 3 groups: (i) incident accelerated KOA (ii) incident typical KOA and (iii) No KOA; GSP and CRP were not significantly associated with incident accelerated RKOA while lower and higher GSP levels were associated with incident typical KOA compared with adults with concentrations (log) closer to 5.7	10
TRAcP5b, cath-K	S	Knee pain	129 RKOA (97 with 3-year follow-up); Serum TRAcP5b activity was associated with baseline WOMAC pain (β = 1.28) and pain change measured by NHANES I (β = 0.67) during a 3-year follow up.	11
C1M, C2M, C3M, CRPM, IL6	S	Pain and function, radiographic progression	429 KOA (KL = 2-3) patients randomized to either an 18 month exercise control group (E), weight loss diet (D), or D & E. No marker was associated with change in WOMAC pain or radiographic progression. C3M and CRPM were positively associated with change in WOMAC function. Change in IL6 was positively associated with change in C1M, C3M, and CRPM.	14
Coll2-1, Fib-3-2	S	Symptomatic improvement	192 obese KOA, The clinical improvement after a substantial weight loss and weight maintenance in KOA patients was not associated with decrease in markers of cartilage breakdown Coll2-1 or Fib3-2, even with indications of a slightly negative effect.	15

Odd ratio (OR), , chondroitin-6-sulfate (C-6S), cross-linked C-terminal telopeptide of type 2 collagen (CTX-II), , C-terminal crosslinked telopeptide of type I collagen (CTX-I), type I collagen degraded by MMP (C1M), type II collagen degraded by MMP (C2M).

of synovial inflammation, MMP-3 and hyaluronic acid (HA), were associated with moderate to large effusion MRI-detected synovitis; but there was no association between the markers and changes in MRI features of synovitis (Hoffa and effusion synovitis)⁸. In contrast with the result of a recent meta-analysis⁹, serum high-sensitivity C-reactive protein (hsCRP) was not predictive of incident accelerated (>1 knee developed advance-stage KOA (KL Grade 3 or 4) within 48 months) or typical (>1 knee increased in radiographic scoring within 48 months (excluding those with accelerated KOA)) RKOAs¹⁰.

Two osteoclast biomarkers (TRAcP5b and cath-K) were measured in serum samples of KOA patients from the Prediction of Osteoarthritis Progression cohort. Baseline TRAcP5b was associated with OA pain and pain change during a 3-year follow up as evaluated by the National Health and Nutrition Examination Survey (NHANES) I pain questionnaire¹¹.

Generating OA prediction models

Combining biochemical markers with other markers (i.e., imaging, genetic, and clinical markers) and bioinformatics may facilitate earlier detection of OA. Using data of the prevention of knee osteoarthritis in overweight females study, a randomized controlled trial of middle-aged women with high BMI, authors generated 5 predictive models for KOA incidence including clinical variables, food and pain questionnaire data, as well as biochemical markers and imaging based information¹². Several biomarkers of extracellular matrix tissue turnover provided information on the risk of KOA incidence. Serum type I collagen degraded by MMP

(C1M) and type II collagen degraded by MMP (C2M) seemed to be negatively associated with the incidence of KOA. In a similar way, the urine nitrated form of peptide Coll2 (Coll2-1NO2) exhibited a negative relationship with incidence of KOA. A prognostic model for 10-year incident radiologic hip OA (HOA) was developed utilizing 2327 individuals of Rotterdam study¹³. A basic model including the demographic, questionnaire, and clinical examination variables or a model containing genetic markers or uCTX-II levels alone were not good predictors of incident HOA. By contrast, a model including the basic model with imaging features reached a fair predictive value (AUC = 0.78) and might be applicable in clinical practice when validated in other studies¹³. These findings suggest that biomarkers together with other predictors may provide valuable information on OA development and prediction of disease incidence.

Association with clinical scores and changes

Using longitudinal data from an 18-month clinical trial of exercise and weight loss for KOA, serum biomarkers of type I and type III collagen degradation were found to be decreased in response to weight loss or exercise plus weight loss intervention when compared to an exercise only group. The decrease in these markers over the course of the study and two systemic markers of inflammation, MMP-derived inflammation (CRPM) and interleukin-6 (IL-6), were strongly associated with weight loss indicating that overweight and obese adults with KOA who lose weight experienced reduced inflammation at a systemic as well as tissue level¹⁴.

(Table I). In contrast, among overweight KOA patients who followed a 16-week weight loss intervention and 52-week weight maintenance, the clinical improvement after weight loss and weight maintenance was not associated with change in serum markers of Coll2-1 and fibulin3-2¹⁵ (Table I).

Type II collagen is a major structural protein of the cartilage and together with other collagen types and non-collagenous proteins, such as COMP, provides a tensile meshwork for cartilage². Several studies showed that serum and SF Levels of COMP and fibulin-3, marker of extracellular matrix, and uCTX-II were related to KOA severity^{16–19}. Serum N terminal propeptide of collagen II α (PIIINP), representing collagen synthesis, was lower in individuals with a greater burden of HOA and KOA²⁰ (Table II).

Low-grade inflammation occurs in OA and correlates with disease severity and progression as well as pain and disability. In a post-hoc analysis of baseline data from a randomized clinical trial (RCT), local inflammation represented by SF cytokines was associated with self-reported knee pain. Pro-inflammatory biomarker profiles associated with movement and rest pain were different, i.e., IL-6, IL-8, and tumor necrosis factor alpha (TNF- α) playing a role in pain upon movement, and TNF- α in pain at rest²¹. Serum IL-16 levels significantly correlated with the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score and its

subscores in KOA patients²² while serum C1M and SF IL-6 were associated with synovitis and neuropathic pain features among end stage KOA²³. Moreover, hsCRP was associated with knee and hand OA severity²⁴, greater painful joint count among women, but not men²⁵, and BML and knee effusion²⁶. Serum levels of IL-1 and TNF- α in postmenopausal women were associated with severity of KOA and the lack of estradiol was associated with the pathogenesis of KOA in postmenopausal women²⁷.

Ghrelin has been proven to inhibit inflammation and promote cartilage growth. SF levels of ghrelin were negatively related to TNF- α , IL-6 levels, and disease severity in patients with primary KOA²⁸. Another study showed that ghrelin were significantly associated with increased knee symptom, infrapatellar fat pad (IPFP) (local adipose tissue situated in the knee under the patella) signal intensity alteration²⁹. They reported that serum levels of ghrelin were positively associated with serum levels of MMP-3, MMP-13, N-telopeptide of type I collagen (NTX-I), and N-terminal procollagen III propeptide (PIIINP), suggesting that ghrelin may be associated with increased cartilage breakdown and bone resorption in patients with KOA²⁹.

Adipokines are pro-inflammatory molecules secreted systemically by adipose tissue but also locally in the synovial tissue. Adiponectin is increased in KOA patients and associated with OA

Table II
Cross-sectional and case–control observational studies on associations with OA features

Biomarker	Sample	Outcome	Design	Study size and results	Ref.
COMP	S, SF	Radiographic severity	CS	124 KOA patients and 105 HCs; 77 samples SF only, 78 samples to serum only, and for 74 individuals, both SF and serum samples; both COMP levels in SF and serum were higher in patients than in controls. SF COMP levels were considerably higher than serum levels for both groups. Severity of disease (KL grade) was positively correlated with SF COMP levels.	16
Fibulin-3	S, SF	Radiographic severity	CS	209 KOA subjects and 165 HCs. Significant positive correlation between serum and SF fibulin-3 concentrations with KL grades.	17
COMP, MMP-3, Coll2-1, CRP	S	Radiographic severity	CS	56 KOA (KL1 = 17, KL2-3 = 39) (isolated KOA = 24, generalized OA = 32) and 31 HCs; higher serum COMP and MMP-3 levels in KOA patients compared to controls; Patients with KL2-3 KOA had greater median COMP levels than KL1 patients. In the generalized OA group (symptomatic in two or more locations: hands, hips, knees, spine, and feet), mean MMP-3 levels were higher than in the isolated KOA group (did not have any signs of OA in any other region).	18
CTX-II	U	Radiographic severity	CS	82 KOA and 20 HCs; CTX-II level was higher in KOA patients and correlated with KL grade.	19
PIIINP	S	Radiographic severity	CS	1235 participants from the Genetics of Generalized Osteoarthritis study; Higher PIIINP concentrations were associated with lesser burden of osteoarthritic features (osteophyte and joint space narrowing) in lower extremity joints (knees and hips).	20
IL1 β , IL6, IL8, TNF- α , CTX-II (SF, U)	SF, U	Radiographic severity, Pain	CS	70 KOA subjects; sf/u CTX-II was associated with radiographic severity, but not with knee pain. sf IL6 and IL-8 were associated with pain on movement. sf IL1b was inversely associated with pain. SF TNF- α was associated with WOMAC total pain and both pain on movement and at rest.	21
COMP, IL16	S	Radiographic and clinical severity	CS	90 KOA (KL2 = 30, KL3 = 27, KL4 = 7) and 30 HCs; no differences in levels of COMP and IL16 between HCs and KOA patients and within the different KL grades in the KOA patients. IL16 levels significantly correlated with the WOMAC score and its subscales, pain, stiffness, and physical function.	22
C1M, IL6	S, SF	Pain, MRI Synovitis score	CS	104 severe KOA; sf-IL6 was associated with synovitis in the parapatellar subregion, and an association between serum C1M and synovitis in the periligamentous subregion. sf-IL6 was significantly associated with pain (WOMAC, NPQ).	23
hsCRP	S	Radiographic severity	CS	2376 participants from ongoing prospective Dong-gu Study; neither osteophytes nor JSN, in hand or knee OA, were associated with the level of hs-CRP; total OA score, erosion and sclerosis in both knee and hand OA and malalignment in hand OA showed positive associations with high hs-CRP levels.	24
CRP, COMP	S	Pain	CS	189 late stage hip/knee OA; a dose–response association between painful joint burden in OA and systemic inflammation, and the association was sex-specific (women).	25
CRP, GSP	S	BML, effusion	CS	343 high risk subjects (KL0-KL1); CRP was related to the presence of BMLs and effusion among normal weight individuals and abnormal GSP was associated with effusion.	26
IL1, IL6, TNF- α , CRP, estradiol	S, SF	Cartilage injury in CC arthroscopy	CS	58 postmenopausal KOA, 58 patients with menstrual disorders without KOA, and 35 HCs. Serum IL1 and TNF- α increased with increasing of cartilage injury while serum E2 was gradually decreased. Serum levels of IL1, IL6, TNF- α , and CRP increased in KOA patients compared with both control groups.	27

(continued on next page)

Table II (continued)

Biomarker	Sample	Outcome	Design	Study size and results	Ref.
Ghrelin, IL6, TNF- α	S, SF	Radiographic and clinical severity	CS	52 KOA and 52 HCs; No difference in serum ghrelin levels between KOA and HCs; sf ghrelin levels were significantly negatively correlated with KL grade and symptomatic severity; more diagnostic value for sf ghrelin than IL-6 and TNF- α for assessing radiographic progression in ROC.	28
Ghrelin, COMP, CTX-I, NTXI, PIIINP, MMP-3, S MMP-10, MMP-13		Radiographic and MRI features, Pain	CS	146 KOA (mild to moderate); Positive association between ghrelin and WOMAC scores, IPFP signal intensity alteration, NTXI, PIIINP, MMP-3, MMP-13	29
Leptin, resistin, adiponectin, C2C, Ghrelin, IL6, CRP	S	Radiographic severity, pain score	CS	50 KOA (mostly severe) and 50 HCs; Total adiponectin was higher in women with OA compared to women from the HS group. Total adiponectin was borderline associated with the KL grade.	30
Adiponectin	S	Radiographic and clinical severity	CS	60 KOA and 25 HCs; There was a positive correlation between adiponectin level and KL grading scores and clinical variables (VAS and WOMAC total scores). SF-36 scores were inversely associated with adiponectin levels.	31
Leptin, adiponectin, resistin, visfatin, hsCRP, SF osteopontin, omentin, chemerin, IL6, TNF- α		Clinical severity	CS	115 symptomatic KOA female; an association was found for adiponectin with pain and for resistin and visfatin with function	32
ox-LDL, PONI, LDL, HDL	S	Radiographic and clinical severity	CS	203 KOA (KL1 = 50, KL2 = 54, KL3 = 49, KL4 = 50) and 194 HCs; ox-LDL and oxidant parameters were significantly higher in patients compared to controls whereas PONI was significantly lower. ox-LDL was positively correlated with radiographic severity, WOMAC score, and oxidant parameters.	33
Glucose, insulin, c-peptide, cholesterol, OxS markers	S	Radiographic severity	CC	55 severe hip/knee OA and 55 HCs; OA patients have increased levels of OxS and decreased antioxidant capacity. OA was associated with impaired lipid metabolism and dysglycemia. Radiographic severity was associated with LDL-cholesterol and oxidized LDL.	34
YKL-40, CRP	S, SF	US findings	CS	50 KOA; a strong relationship between sf YKL-40 and GSUS and feeble with PDUS. YKL-40 correlated with inflammatory activity in knee joints and neovascularization detected by US.	35
IL1 β , IL6, IL18, TNF- α , leptin, Nox, Uric acid	S, SF	Radiographic severity	CS	134 post-traumatic KOA (KL1-2 = 103, KL3-4 = 31) and 37 HCs; KL score was correlated with leptin levels in plasma and SF and with the synovial IL18 level. Early OA was associated with serum IL1 β .	36
DKK1, TNF- α , OPG	S	Radiographic severity	CC	148 KOA (KL2 = 35, KL3 = 65, KL4 = 48) and 101 HCs; Lower level of DKK-1 and higher levels of OPG and TNF- α in KOA compared to the controls; DKK1 correlated with the progression of KOA. The serum levels of TNF- α , OPG, and DKK-1 correlated with risk of KOA.	37
FGF-23	S	Radiographic/ symptomatic severity	CS	50 KOA and 20 HCs; FGF-23 level was higher in KOA patients and in patients who had effusion or bilateral involvement. FGF-23 level correlated with WOMAC, KL, age, disease duration	38
Eotaxin 1, MMP-3, IL6	S, SF	Radiographic and clinical severity	CS	143 KOA (KL2 = 50, KL3 = 52, KL4 = 41) and 135 HCs; Elevated plasma eotaxin-1 levels in KOA patients; sf Eotaxin-1 associated with KL grading criteria, WOMAC, MMP-3 and IL6	39
TGF- β 1, PDGF-BB, CTX-1	S	Radiographic severity	CC	160 KOA (KL1 = 28, KL2 = 35, KL3 = 55, KL4 = 42) and 80 HCs; higher TGF- β 1 in cases and positively associated with KL grades.	40
MMP-13, VEGF, IL10, IL8, IL6, IL1, TNF- α , collagenase 2	SF	Radiographic and clinical severity	CS	51 KOA (KL1 = 18, KL2 = 17, KL3 = 16) and 40 meniscus injury (MI). No associations between KL scores and biomarker levels in KOA. In MI patients, TNF- α was associated with MRI score. Higher IL6 in KOA than MI.	41
TIMP-1, Bcl-2	SF	Radiographic severity	CS	70 KOA and 30 HCs; higher expression levels of TIMP-1 and Bcl-2 in KOA patients; positive correlation between severity of KOA and the expression level of TIMP-1 and Bcl-2	42

Cross sectional (CS), case-control (CC), ultrasonography (US), visual analog scale (VAS), neuropathic pain questionnaire (NPOQ), chondroitin-6-sulfate (C-6S), cross-linked C-terminal telopeptide of type 2 collagen (CTX-II), C-terminal crosslinked telopeptide of type I collagen (CTX-I), type I collagen degraded by MMP (C1M), type II collagen degraded by MMP (C2M), osteoprotegerin (OPG), Dickkopf-1 (DKK1), gray-scale ultrasonography (GSUS), power Doppler ultrasonography (PDUS), paraoxonase (PONI), N-telopeptide of type I collagen (NTXI), N-terminal pro-peptide of type III procollagen (PIIINP), platelet-derived growth factor BB (PDGF-BB).

severity^{30,31}. Studies suggest a different pattern of association between adipokines and components of KOA severity, pointing to an association of adiponectin with pain and a link between resistin and visfatin with disability³². Oxidized low density lipoprotein (ox-LDL), induced by oxidative stress, contributes to inflammation and plays a role in the pathogenesis of articular cartilage degeneration³³. ox-LDL and oxidative stress parameters were reported to be higher in KOA patients and correlated with KOA severity³³. End-stage HOA/KOA patients have increased levels of oxidative stress markers, decreased antioxidant capacity, impaired lipid metabolism, and dysglycemia³⁴. Moreover, glycoprotein YKL-40, a potential marker for active inflammatory process, is involved in the pathogenesis of KOA synovitis³⁵. Patients with early- and late-stage posttraumatic KOA have increased levels of circulating pro-inflammatory NOx and IL-6 compared with healthy controls (HCs) and KOA severity was correlated with leptin levels in both plasma (P) and SF³⁶. Dickkopf-1, a key factor of bone metabolism in

normal joint bone, and osteoprotegerin together with TNF- α , were predictors of KOA severity³⁷.

Plasma levels of fibroblast growth factor 23 (FGF-23)³⁸ and eotaxin-1 levels in SF of KOA patients were significantly associated with severity of KOA³⁹. Moreover, SF eotaxin-1 was associated with MMP-3 and IL-6 levels in SF, suggesting possible induction of breakdown of cartilage matrix. FGF-23 has been shown to be highly expressed in osteoarthritic chondrocytes and have low expression levels in normal chondrocytes³⁸. Transforming Growth Factor beta-1 (TGF- β 1) was also reported to be correlated with KOA severity⁴⁰, while another study did not show any association between knee Kellgren Lawrence (KL) grade and SF levels of growth factor marker, vascular endothelial GF⁴¹.

The relative expression of TIMP-1 and B-cell lymphoma/leukemia-2, an anti-apoptotic protein that promotes cell survival by inhibiting apoptosis, were significantly increased in the SF of KOA patients compared with the control group⁴². Moreover, the

expression levels of both markers in the synovial tissues of KOA patients were positively correlated with KOA severity.

Early and late assessment of intervention efficacy and understanding of mode of action

Biochemical markers may be used to facilitate drug development. They can be used for different purposes; from playing a role as a pharmacodynamic marker to companion diagnostic data utilized in the clinic. Table III shows the results of the papers that evaluated biomarkers associated with an intervention. IL-1 β is a

key pathogenic factor in OA. Animal studies suggested that inhibiting IL-1 α / β may reduce pain and slow structural progression in OA. In a phase 1 trial, KOA patients were randomized to get an anti-IL1 α / β drug (ABT-981) in 3 different doses or a placebo control⁴³. Treatment of patients with ABT-981 reduced serum biomarkers of inflammation including CRP, C1M, C3M, and CRPM. Decreased levels of the markers occurred within 4 days of the first dose and, once a nadir was reached, remained suppressed throughout the treatment duration. The final observations took place 70 days after the last dose for all groups, when ABT-981 serum concentrations were 5% of maximum levels, yet many of the biomarkers among

Table III
Associations with treatment response in interventional studies

Biomarker	Sample type	Sample size and results	Ref.
C1M, C2M, C3M, CRPM, IL6	S	RKOA ($n = 429$); C1M and C3M reduced with diet and diet & exercise	14
Coll2-1, Fib-3-2	S	Obese KOA ($n = 192$); No association observed between the markers and weight loss	15
IL1 α , IL1 β , IL1RA, C1M, C2M, C3M, CRPM, COMP, CTX-I, CRP, MMP-9, Creatinine, CTX-II, TIINE-5OH, VEGF, VICM, AGN \times 1 COMP, hyaluronan, sCD14, aggrecanase-1 (ADAMTS-4)	S, U	RKOA ($n = 36$); IL1 α , IL1 β , C1M, C3M, CRPM, and CRP levels reduced with treatment of anti-interleukin-1 α / β dual variable domain immunoglobulin	43
COMP, hyaluronan, sCD14, aggrecanase-1 (ADAMTS-4)	SF, P	Knee cartilage defects ($n = 54$); presence of ADAMTS-4 activity in SF decreased the response to autologous chondrocyte implantation	44
HA, CRP, adiponectin, leptin, PIIANP, CTX-I, MMP-1, MMP-3	S	KOA ($n = 119$); greater response to chondroitin sulfate treatment on cartilage volume loss in patients with low level of inflammation (HA, leptin, adiponectin) and/or greater level of cartilage catabolism (PIIANP, CTX-I, MMP-1, MMP-3)	45
Coll2-1	S	RKOA ($n = 60$); Coll2-1 level decreased by intra-articular injection of an autologous plasma-rich-platelet; improvements in WOMAC by intra-articular injection	47
C1M, C2M, C3M, COMP, CS846, MMP3, PIINAP, CTX-I, CTX-II	S, U	RKOA ($n = 163$); serum CTX-I and urine CTX-II levels decreased with intra-articular TissueGene-C treatment; TG-C was associated with significant improvements in functions and pain	48
COMP	S	Erosive radiographic hand OA ($n = 143$); decreased COMP level in treatment with intramuscular clodronate; significant reduction in the consumption of anti-inflammatory or analgesic drugs and increasing the functionality of the hands by treatment	66
C-6S, CTX-II, TOS, TAC, MMP-3, CRP	S, SF	Symptomatic RKOA ($n = 20$); intra-articular N-acetyl cysteine reduced C-6S and CTX-II levels; significant reduction in WOMAC score after treatment with NAC	67
CTX-II, COMP	S, U	Symptomatic RKOA ($n = 166$); treatment with choline-stabilized orthosilicic acid (ch-OSA) reduced the level of both markers in men only; reducing symptoms of KOA by treatment only among men	68
MMP-9, TIMP-1, COMP, IL6, CRP, ESR, CTX-II, fructosamine	S, U	Symptomatic RKOA (KLO-2) ($n = 87$); <i>Litsea japonica</i> fruit extract reduced the MMP-9 levels and reduced joint pain and stiffness and improved joint function	69
CTX-II, C2C, PIICP, MMP-13	S, U	Symptomatic RKOA (KLO-2) ($n = 44$); No association observed between the markers and <i>Ajuga decumbens</i> extract supplement treatment; treatment improved joint function	70
TNF- α , CTX-II	S, U	Symptomatic RKOA ($n = 135$); a decrease observed between urinary level of CTX-II for extract of <i>Morus alba</i> and <i>Acacia catechu</i> treatment; improvements in pain and function that were comparable with placebo and glucosamine/chondroitin	71
C2C, PIICP, C2C/PIICP	S	Healthy subjects ($n = 120$); C2C levels and C2C/PIICP ratio decreased with N-acetyl-glucosamine in subjects with BMI<25 and KL = 0	72
C1,2C, COMP, CS846, PIICP	S	Knee joint discomfort ($n = 55$); C1,2C decreased with proteoglycan treatment among subjects with high levels of knee pain and physical dysfunction	73
Coll2-1	S	Symptomatic RKOA ($n = 81$); Coll2-1 decreased with intraarticular injection of hyaluronic acid; No differences on the changes in function or pain between treatment and placebo groups	74
PINP, β -CTX, COMP	S	RKOA ($n = 61$); reduced COMP level in treatment with higher dose of Wnt pathway inhibitor; reducing pain and improving function with the treatment	75
hsCRP, TNF- α , COMP	S	Healthy overweight ($n = 105$); no association with treatment with <i>Terminalia chebula</i> fruit; improvement in function and pain by treatment	76
MMP-3, TNF- α	S	Symptomatic KOA ($n = 72$); markers level reduced in response to treatment with L-carnitine supplement; decrease on severity of disease by treatment compared with placebo	77
IL6, IL1 β , MMP-3, MMP-8, CRP	S	Obese RKOA ($n = 17$); IL-6, IL-1 β , MMP-3 and pain significantly decreased with strawberry supplementation	78
hs-CRP, IL1 β , TNF- α	S	RKOA ($n = 90$); level of all markers doped significantly with treatment with Moxibustion plus <i>Duhuo Jisheng</i> decoction	79
IL6, uCTX-II	S, U	Knee pain ($n = 16$); IL-6 level decreased in the treatment with a supplement containing glucosamine, chondroitin sulfate, and five bioactive ingredients; pain and joint stiffness decreased with treatment	80
COMP	S	Symptomatic KOA ($n = 20$); no association observed between COMP and joint stabilization exercises; decreasing pain, improving ROM and muscle strength with stabilization exercise	81
MMP-2, TIMP-2	SF	Symptomatic KOA ($n = 27$); A significant reduction in MMP-2 observed with intra-articular HA treatment	82
IL1 β , TNF- α , IL8, IL6, IL10, TGF- β , cortisol, eHsp72	S	Symptomatic KOA ($n = 21$); levels of IL-1 β , TNF- α , IL-8, IL-6, TGF- β , eHsp72 were markedly decreased with pelotherapy; improved OA-related pain, stiffness and physical function with pelotherapy	83

(continued on next page)

Table III (continued)

Biomarker	Sample type	Sample size and results	Ref.
CRP, IgG glycan (Plasma glycan profiles)	SF, P	RKOA ($n = 17$); no association between markers and intra-articular injection of the final microfragmented adipose tissue product; pain reduction with treatment	84
uCTXII	U	healthy postmenopausal women with exercise-induced joint pain in knee ($n = 60$); natural eggshell membrane decreased uCTX-II; reduced pain and stiffness with treatment	85
IL1 β , CXCL13, TNF- α	SF	Symptomatic RKOA ($n = 57$); arthroscopic knee surgery can reduce IL-1 β , CXCL13, and TNF- α levels; markers reduction correlates with clinical improvement	86
uNTX, uCTX-II, COMP, HA, PIIANP, YKL	S, U	Symptomatic KOA ($n = 80$); urinary CTX-II decreased with intra-articular corticosteroid injections; uCTX-II baseline level and change from baseline to 3 weeks post injection correlated with joint space narrowing	87
MMP-1, MMP-3, MMP-13, CTX-II, ADAMTS-4, ADAMTS-5, Helix-II, sGAG	S, SF	Severe RKOA ($n = 33$); ADAMTS-5 in serum decreased after 3 weeks treatment with maritime pine bark extract	88
MMP-3, MMP-9, TIMP-1, COMP, IL-6, MDA, Fructosamid, uCTX-II	S, U	KOA ($n = 50$); no association between markers and Deer bone extract supplementation treatment; no significant difference in pain and function between treatment and placebo	89
IL6, IL8, TNF- α , IL18, CD14, CTX-I, CTX-II, hsCRP	S, SF, U	Symptomatic KOA ($n = 109$); colchicine treatment reduced hs-CRP and SF CTX-I but did not reduce KOA symptoms over a 16-week study period.	90
COMP	S	Symptomatic RKOA ($n = 112$); no association between marker and <i>Channa striatus</i> treatment; improvement in stiffness and function with treatment compared to placebo	91
COMP, CRP, IL6, TNF- α , IL1 β , calprotectin	S	RKOA ($n = 18$); no association between marker, KOOS scores, or muscle strength and creatine monohydrate supplementation	92

Chondroitin-6-sulfate (C-6S), cross-linked C-terminal telopeptide of type 2 collagen (CTX-II), total oxidant status (TOS), total antioxidant concentration (TAC),, type II collagen synthesis (PIICP), C-terminal crosslinked telopeptide of type I collagen (CTX-I), malondialdehyde (MDA),, type I collagen degraded by MMP (C1M), type II collagen degraded by MMP (C2M), vascular endothelial growth factor (VEGF), MMP-degraded and citrullinated vimentin (VICM), aggrecan (AGNx1).

groups had not returned to pre-dose baseline levels. This slow rebound of biomarker levels after termination of treatment suggests that alleviation of pro-inflammatory cytokines may have acute and prolonged beneficial effects on tissue turnover.

Currently, there are no wet biomarker tests available to clinicians that can predict the outcomes of cell therapy. Four markers of COMP, HA, sCD14, and aggrecanase-1 (ADAMTS-4) activity in SF and COMP and HA in plasma samples were measured for 54 patients with cartilage defects in the knee treated with autologous chondrocyte implantation (ACI)⁴⁴. Improved Lysholm score at least 10 points after 1 year was classified as responders. The presence or absence of ADAMTS-4 activity in SF was the most important predictive factor. When ADAMTS-4 activity was detectable in SF, the odds of being a responder were 3 times reduced than when ADAMTS-4 activity was not detectable. Other predictive factors were the baseline Lysholm score, age at ACI, and defect patch type used. Authors suggest that ADAMTS-4 activity levels in the SF of joints with cartilage defects may be used to help identify patients who will have a poorer outcome after conventional ACI treatment.

In a post-hoc study explored in a 2-year RCT of KOA patients, some serum biomarkers were found to be associated with a better response to chondroitin sulfate (CS) in reducing cartilage volume loss. The results suggested a greater response to CS treatment on cartilage volume loss in KOA patients with low level of inflammation (HA, leptin and adiponectin) and/or greater level of cartilage catabolism (PIIANP, CTX-I, MMP-1, MMP-3)⁴⁵. In another study, serum biomarker phenotypes associated with the occurrence of all-cause TJR were retrospectively explored using data from a phase III trial of tanezumab⁴⁶. Patients included in this analysis were reclassified into three groups based on nonsteroidal anti-inflammatory drugs (NSAID) use before and during tanezumab treatment. Biomarkers of synovial inflammation (C3M) were the strongest biomarkers for distinguishing between cases and controls in the non-NSAID group as well as in the NSAID group where high levels appear to be protective of a TJR, suggesting that NSAID use could be suppressing synovial inflammation. Bone metabolism markers of CTX-I, osteocalcin (OC), and Dkk1 were present in the phenotypes of 71% of the cases and controls in the NSAID-user group, but was lower in the non-NSAID and scrNSAID groups (used during screening and for <90 days during treatment). This suggests an important interaction exists between NSAID use and bone metabolism.

Medications which protect or stimulate healing of the cartilage are still under investigation. The effect of one dose injection of intra-articular platelet-rich plasma (PRP) in the knee joint on a serum biomarker of cartilage degeneration, Coll2-1 was evaluated over a short period of 3 months. They reported a reduction in Coll2-1 following injection emphasizing that PRP could be a promising safe and tolerable effective therapeutic option⁴⁷. In another study, clinical efficacy of TissueGene-C (TG-C), a cell and gene therapeutic consisting of non-transformed and retrovirally transduced chondrocytes to overexpress TGF- β 1 (3:1), was evaluated for KOA patients. TG-C was associated with statistically significant improvement in function and pain in KOA patients. Moreover, decreased levels of serum CTX-I and uCTX-II, indicating less subchondral bone and cartilage degradation, respectively, were reported in patients treated with TG-C⁴⁸.

Understanding the biology of joint deterioration

Trauma-induced cytokine response after knee injury has a role in the development of posttraumatic KOA. An exploratory analysis of a RCT showed that surgical ACLR in the acute post-injury phase represents a second trauma to the injured knee resulting in a prolonged elevation of already high SF levels of inflammatory cytokines such as IL-6, IL-8, IL-10, TNF, and IFN- γ even after 5 years⁴⁹. The lowest concentrations of inflammatory biomarkers after injury were seen in the rehabilitation alone group. Interestingly, in the group treated with rehabilitation alone, subjects who underwent non-ACL arthroscopic surgeries during the 5-year period showed no significant differences in levels of any of the biomarkers at the 5-year visit compared with patients without arthroscopic procedures⁴⁹.

Irrespective of how OA is classified, mechanical load - either abnormal load on a normal joint, or normal load on a joint that has lost its mechano-protective mechanisms, is an etiological factor in development of OA⁵⁰. The association between biomechanical outcomes of walking gait 6 months following ACLR and biomarkers of serum type-II collagen turnover, plasma degenerative enzymes, and a pro-inflammatory cytokine were evaluated for 17 patients⁵¹. Individuals with lesser biomechanical loading on the ACLR limb at the 6-month follow-up exam, compared to the contralateral limb, demonstrated greater concentrations of plasma MMP-3 and IL-6, as well as serum type-II collagen turnover, indicating early

interactions between mechanical joint loading and metabolic processes that may influence the future breakdown of cartilage and future KOA onset. In a small intervention study ($n = 16$), changes to serum level of C1,2C and CS846 in response to a 30-min walk in patients with medial KOA was evaluated⁵². Regional cartilage thickness changes were measured from MRI obtained at study entry and at 5-year follow-up. Subjects with increased C1,2C had greater medial tibial cartilage thinning 5 years later than those with decreased C1,2C, suggesting that an increase in biomarker concentration following stimulus reflects progression of medial OA. Cartilage thicknesses in the medial compartment have been associated with individual variations in gait mechanics. Thus, gait differences between patients with medial compartment KOA would preclude finding a proportional relationship between biomarkers and cartilage changes⁵². In another study including asymptomatic subjects with MRI-evidence of cartilage loss and asymptomatic HCs, specific gait mechanics and systemic inflammation differences (increased serum TNF- α) were suggested to precede the onset of symptoms of KOA⁵³. The study finding that asymptomatic subjects with cartilage loss had gait and inflammatory characteristics similar to those previously reported in symptomatic KOA patients^{54,55} supports the idea that there are specific mechanical and biological factors that precede the onset of knee pain in the pathogenesis of KOA⁵³.

In a small cross-sectional study, DKK1 and FRZB (antagonists of the WNT-signaling pathway), and GREM1 (antagonist of the BMP-signaling pathway) in SF samples from subjects with knee injury or KOA were evaluated⁵⁶. All three antagonists decreased with increasing time after injury as well as with increasing age, but the temporal change after injury was less accentuated for FRZB compared to that of DKK1 and GREM1. They found positive correlations between FRZB concentrations and levels of sulfated glycosaminoglycan (sGAG), ARGS-aggregcan, COMP, and type II collagen degradation (epitope C2C). On the other hand, levels of DKK1 and GREM1 inversely correlated with concentrations of C2C, and DKK1 negatively correlated with ARGS-aggregcan. This suggests that directly after knee injuries DKK1 and GREM1 work co-operatively to block and/or balance the catabolic signaling, and this process is partly separated from the action of FRZB⁵⁶.

An aberrant immune response has been implicated in the pathogenesis of OA. CD4+T cells, particularly T follicular helper (TFH) cells, are known to regulate B-cell activation and functional differentiation. In a case-control study, expression of IL-21 + TFH cells in KOA patients demonstrated a positive correlation with KOA disease activity, serum CRP levels, and WOMAC⁵⁷. Furthermore, serum IL-21, IL-17A, and IFN- γ levels in KOA patients were significantly higher than those in HCs.

Endotypes/phenotypes and novel OA markers

Although inflammation is associated with the onset and progression of OA, different inflammatory signaling may be present in patients with HOA vs KOA. A large panel of cytokines, chemokines, and GFs were measured in serum and SF samples of patients with KOA, HOA, and controls in a cross-sectional study⁵⁸. The three groups showed distinct serum cytokine profiles. Epidermal GF, FGF2, monocyte chemotactic protein-3, and IL-8 significantly differed between HOA and KOA. Specifically within the HOA subjects, IL-6, monocyte-derived chemokine and interferon gamma-induced protein 10 were associated with pain and were also found to be present in SF and synovial membrane (except IL-6) of patients with HOA⁵⁸. In another study of late stage HOA/KOA patients undergoing total joint arthroplasty, IL-17 and several adipokines and inflammatory markers were measured in SF and serum samples⁵⁹. IL-17 is a pro-inflammatory cytokine that induces

upregulation of inflammatory cytokines and adipocytokines. Of study population 9% had detectable IL-17 in SF. These patients had higher levels of IL-6, leptin, resistin, chemokine (C-C motif) ligand 7, and nerve GF in SF and reduced osteophytes, sclerosis, and minimum joint space width. Increased SF levels of IL-6 were found for both HOA and KOA patients with detectable IL-17. So it seems that the presence of IL-17 in SF could identify a subset of primary end-stage OA patients with a distinct radiographic, inflammatory cytokine, and adipocytokine profile⁵⁹.

Studies have shown that lower serum level of adropin, a newly discovered peptide hormone, induces weight gain and impacts lipid metabolism and glucose homeostasis. Adropin levels inversely correlated to white blood cell, neutrophil to lymphocyte ratio, and TNF- α levels and decreased parallel to the increase in BMI in particular in KOA patients with BMI>30⁶⁰. KOA patients showed lower levels of adropin and higher levels of TNF- α compared with the HCs. There was a significant decrease in adropin levels and increase in TNF- α levels parallel to the increase in the KL grade. The study provides a new link between adropin and diseases with inflammation⁶⁰. Another study suggested that serum measures of impaired glucose homeostasis such as glycated serum protein (GSP) may predict individuals at risk of incident typical KOA but not accelerated KOA¹⁰. A U-shaped relationship between incident typical KOA and GSP indicates that there are two clinically relevant subsets at risk for typical KOA - those with low GSP concentrations and others with high GSP concentrations. The study findings support the notion that accelerated KOA may be a distinct disorder from typical KOA. Among the 78 different investigated proteins, three proteins, C3, ITIH1 and S100A6, significantly differed between KOA patients and HCs, with the potential to be of additive value for the diagnosis and monitoring of KOA. Serum levels of two of these proteins, C3 and ITIH1, differed also among KOA and RA patients, which suggests that C3 and ITIH1 are proteins specifically increased in KOA⁶¹.

A cross-sectional analysis of the Johnston County Osteoarthritis Project showed that OA of the synovial facet joint was associated with HA while spine OA, affecting intervertebral discs that contain collagen type II, was associated with uCTX-II⁶², consistent with other study that showed lumbar intervertebral disc degeneration patients had higher uCTX-II than HCs⁶³. These findings suggest that biomarkers may reflect structural differences between spine degeneration phenotypes⁶².

The identification of biomarkers capable of discriminating erosive hand osteoarthritis (EHOA) from non-erosive hand OA (NEHOA) could improve the diagnostic accuracy. A cross-sectional study demonstrated a significant increase in myeloperoxidase serum levels in patients with EHOA and NEHOA in comparison to the control group and a significant increase in EHOA compared with NEHOA⁶⁴. Myeloperoxidase levels significantly correlated with the disease duration in the NEHOA group and with the Kallman score in EHOA patients. Moreover, higher serum levels of visfatin in EHOA in comparison to NEHOA was found, speculating its potential usefulness as biomarker to distinguish the EHOA from NEHOA⁶⁵.

Conclusions

This "Year in review" included a low number of articles published about other joints other than the knee. Only nine of the included articles were about other joints including hand ($n = 4$)^{24,64–66}, hip ($n = 3$)^{34,46,59}, and spine ($n = 2$)^{62,63}. A large number of these studies focused on the systemic and tissue inflammatory markers as well as cartilage markers.

The number of investigative biomarkers has increased rapidly with expansion of proteomics. Metabolic changes of joint tissues start long before the onset of structural alterations. Therefore,

identification of early OA biomarkers will help for diagnosis or developing new therapeutic alternatives⁹³. To date, there are an extensive list of OA biomarkers and efforts are now under way to use some of these markers to identify sub-clinical phenotypes given the heterogeneous nature of the disease. Moreover, a consensus has been reached that the assessment of multiple biomarkers associated with different joint tissue types like cartilage, bone, and synovium, products of pathological pathways and even genetic factors, is required for considering a personalized medication protocol for the treatment of OA in the near future. However, currently, lack of clinically validated biomarkers, in particular early OA biomarkers, hinders therapeutic advancements necessary to develop disease-modifying drugs⁹⁴. The areas and questions that still require further attention in the identification of biochemical markers for OA are described below:

- Research is needed to explore whether there are specific biomarkers for specific joints
- Can current analytical platforms be refined to improve sensitivity and specificity?
- Biomarker development needs to be much more bound to drug development
- Need for studies combining biochemical markers with other types of markers like MRI- and ultrasound-based markers
- Collaboration between different experts in the OA field and large well designed consortiums are probably needed to overcome the above issues

International standardization of future investigations are warranted for further advances in OA biomarker qualification and progress towards the clinical utility of biochemical markers.

Author contributions

FSH was the main author who searched, reviewed, and wrote the first draft of the manuscript. ABJ and SBZ contributed to the selection and justification process, and were involved in revising the article critically for important intellectual content and expert view.

Declaration of interest

The authors do not have any commercial relationships that could be construed as biased or inappropriate. Anne-Christine Bay-Jensen works for Nordic Bioscience, a company involved in biomarker identification, validation and development. Dr. Bierma-Zeinstra reports grants from Dutch Arthritis Foundation, the Netherlands Organization for Health Research and Development, EU Horizon 2020, Stichting Coolsingel, Nuts-Ohra, and EU Fp7, other from Regeneron, Infir first healthcare, personal fees from Osteoarthritis & Cartilage, personal fees from OARSI, EULAR, Regeneron, and Infir first healthcare, outside the submitted work.

Appendix

Search term

Embase.com

(osteoarthritis/exp/mj OR (osteoarthritis* OR ((hand OR hip OR knee) NEAR/3 oa) OR (osteo NEXT/1 arthritis*) OR (degenerat* NEAR/3 (arthritis* OR 'joint disease' OR 'joint diseases')) OR arthropath*):ab,ti) AND (marker/exp OR 'disease marker'/exp OR 'C reactive protein'/exp OR 'cartilage oligomeric matrix protein'/exp OR (marker* OR biomarker* OR (Crosslink* NEAR/3 telopeptide*)) OR 'uctx ii' OR 'uctx 2' OR crp OR hsCRP OR 'ctx ii' OR 'ctx 2' OR 'ntx ii' OR 'ntx 2' OR comp OR sCOMP OR 'C1,2C' OR C2C OR Col2CTx):ab,ti'

OR 'ntx 2' OR comp OR sCOMP OR 'Col2:3/4Cshort' OR 'C1,2C' OR 'Col2:3/4Clongmono' OR 'COL2-3/4Clong' OR C2C OR Col2CTx-x):ab,ti) NOT ([animals]/lim NOT [humans]/lim) AND [english]/lim NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Conference Paper]/lim OR [Editorial]/lim) AND [2017-2018]/py.

Medline (OvidSP)

(exp osteoarthritis/OR (osteoarthritis* OR ((hand OR hip OR knee) ADJ3 oa) OR (osteo ADJ arthritis*) OR (degenerat* ADJ3 (arthritis* OR joint disease OR joint diseases)) OR arthropath*):ab,ti.) AND (exp Biological Markers/OR C-Reactive Protein/OR cartilage oligomeric matrix protein/OR (marker* OR biomarker* OR (Crosslink* ADJ3 telopeptide*)) OR uctx ii OR 'uctx 2' OR crp OR hsCRP OR ctx ii OR "ctx 2" OR ntx ii OR "ntx 2" OR comp OR sCOMP OR "Col2 3/4Cshort" OR "C1,2C" OR "Col2 3/4Clongmono" OR "COL2-3/4Clong" OR C2C OR Col2CTx):ab,ti.) NOT (exp animals/NOT humans/) AND english.la. AND (2017 OR 2018).yr.

Cochrane central

((osteoarthritis* OR ((hand OR hip OR knee) NEAR/3 oa) OR (osteo NEXT/1 arthritis*) OR (degenerat* NEAR/3 (arthritis* OR 'joint disease' OR 'joint diseases')) OR arthropath*):ab,ti) AND ((marker* OR biomarker* OR (Crosslink* NEAR/3 telopeptide*)) OR 'uctx ii' OR 'uctx 2' OR crp OR hsCRP OR 'ctx ii' OR 'ctx 2' OR 'ntx ii' OR 'ntx 2' OR comp OR sCOMP OR 'C1,2C' OR C2C OR Col2CTx):ab,ti) AND ('follow up' OR longitudin* OR cohort* OR prospecti* OR trial* OR ((clinical* OR control*) NEAR/3 stud*)):ab,ti).

Web-of-science

TS=((osteoarthritis* OR ((hand OR hip OR knee) NEAR/3 oa) OR (osteo NEAR/1 arthritis*) OR (degenerat* NEAR/3 (arthritis* OR 'joint disease' OR 'joint diseases')) OR arthropath*)): AND ((marker* OR biomarker* OR (Crosslink* NEAR/3 telopeptide*)) OR "uctx ii" OR "uctx 2" OR crp OR hsCRP OR "ctx ii" OR "ctx 2" OR "ntx ii" OR "ntx 2" OR comp OR sCOMP OR "C1,2C" OR C2C OR Col2CTx)) NOT ((animal* OR rat OR rats OR mouse OR mice OR murine OR pig OR pigs OR piglet OR dog OR dogs OR ovine OR bovine OR equin* OR horse* OR rabbit* OR chick* OR rodent* OR monkey*) NOT (human* OR patient*))) AND LA=(english) AND DT=(article).

References

1. Saberi Hosnijeh F, Runhaar J, van Meurs JBJ, Bierma-Zeinstra SM. Biomarkers for osteoarthritis: can they be used for risk assessment? A systematic review. *Maturitas* 2015;82: 36–49.
2. Glyn-Jones S, Palmer AJR, Agricola R, Price AJ, Vincent TL, Weinans H, et al. Osteoarthritis. *Lancet* 2015;386:376–87.
3. Neuman P, Dahlberg LE, Englund M, Struglics A. Concentrations of synovial fluid biomarkers and the prediction of knee osteoarthritis 16 years after anterior cruciate ligament injury. *Osteoarthritis Cartilage* 2017;25:492–8.
4. Sobue Y, Kojima T, Kurokouchi K, Takahashi S, Yoshida H, Poole R, et al. Prediction of progression of damage to articular cartilage 2 years after anterior cruciate ligament reconstruction: use of aggrecan and type II collagen biomarkers in a retrospective observational study. *Arthritis Res Ther* 2017;19: 265.
5. Yamaguchi R, Yamamoto T, Motomura G, Ikemura S, Iwasaki K, Zhao G, et al. Bone and cartilage metabolism markers in synovial fluid of the hip joint with secondary osteoarthritis. *Rheumatology* 2014;53:2191–5.
6. Amano K, Huebner JL, Stabler TV, Tanaka M, McCulloch CE, Lobach I, et al. Synovial fluid profile at the time of anterior cruciate ligament reconstruction and its association with

- cartilage matrix composition 3 years after surgery. *Am J Sports Med* 2018;46:890–9.
7. Deveza LA, Kraus VB, Collins JE, Guermazi A, Roemer FW, Bowes M, et al. Association between biochemical markers of bone turnover and bone changes on imaging: data from the osteoarthritis initiative. *Arthritis Care Res* 2017;69:1179–91.
 8. Deveza L, Kraus V, Collins J, Guermazi A, Roemer F, Nevitt M, et al. Is synovitis detected on non-contrast-enhanced magnetic resonance imaging associated with serum biomarkers and clinical signs of effusion? Data from the Osteoarthritis Initiative. *Scand J Rheumatol* 2018;47:235–42.
 9. Zhang J. Meta-analysis of serum C-reactive protein and cartilage oligomeric matrix protein levels as biomarkers for clinical knee osteoarthritis. *BMC Musculoskelet Disord* 2018;19:22.
 10. Driban JB, Eaton CB, Amin M, Stout AC, Price LL, Lu B, et al. Glucose homeostasis influences the risk of incident knee osteoarthritis: data from the osteoarthritis initiative. *J Orthop Res* 2017;35:2282–7.
 11. Nwosu LN, Allen M, Wyatt L, Huebner JL, Chapman V, Walsh DA, et al. Pain prediction by serum biomarkers of bone turnover in people with knee osteoarthritis: an observational study of TRAcP5b and cathepsin K in OA. *Osteoarthritis Cartilage* 2017;25:858–65.
 12. Lazzarini N, Runhaar J, Bay-Jensen AC, Thudium CS, Bierma-Zeinstra SMA, Henrotin Y, et al. A machine learning approach for the identification of new biomarkers for knee osteoarthritis development in overweight and obese women. *Osteoarthritis Cartilage* 2017;25:2014–21.
 13. Saberi Hosnijeh F, Kavousi M, Boer CG, Uitterlinden AG, Hofman A, Reijman M, et al. Development of a prediction model for future risk of radiographic hip osteoarthritis. *Osteoarthritis Cartilage* 2018;26:540–6.
 14. Loeser RF, Beavers DP, Bay-Jensen AC, Karsdal MA, Nicklas BJ, Guermazi A, et al. Effects of dietary weight loss with and without exercise on interstitial matrix turnover and tissue inflammation biomarkers in adults with knee osteoarthritis: the Intensive Diet and Exercise for Arthritis trial (IDEA). *Osteoarthritis Cartilage* 2017;25:1822–8.
 15. Bartels EM, Henrotin Y, Bliddal H, Centonze P, Henriksen M. Relationship between weight loss in obese knee osteoarthritis patients and serum biomarkers of cartilage breakdown: secondary analyses of a randomised trial. *Osteoarthritis Cartilage* 2017;25:1641–6.
 16. Arellano RD, Aguilar LS, Argüello R, Hernandez F, Gonzalez FF, Moran J. Cartilage oligomeric matrix protein levels in synovial fluid in patients with primary knee osteoarthritis and healthy controls: a preliminary comparative analysis with serum cartilage oligomeric matrix protein. *Arch Rheumatol* 2017;32:189–96.
 17. Wu Q, Sun XC, Du L. Association of fibulin-3 concentrations with the presence and severity of knee osteoarthritis: a cross-sectional study. *Knee* 2017;24:1369–73.
 18. Georgiev T, Ivanova M, Kopchev A, Velikova T, Miloshev A, Kurteva E, et al. Cartilage oligomeric protein, matrix metalloproteinase-3, and Coll2-1 as serum biomarkers in knee osteoarthritis: a cross-sectional study. *Rheumatol Int* 2017;1–10.
 19. Xin L, Wu Z, Qu Q, Wang R, Tang J, Chen L. Comparative study of CTX-II, Zn2+, and Ca2+ from the urine for knee osteoarthritis patients and healthy individuals. *Medicine (Baltimore)* 2017;96:e7593.
 20. Daghshani HN, Jordan JM, Renner JB, Doherty M, Gerry Wilson A, Kraus VB. Serum N-propeptide of collagen IIA (PIANP) as a marker of radiographic osteoarthritis burden. *PLoS One* 2017;12(12):e0190251.
 21. Leung YY, Huebner JL, Haaland B, Wong SBS, Kraus VB. Synovial fluid pro-inflammatory profile differs according to the characteristics of knee pain. *Osteoarthritis Cartilage* 2017;25:1420–7.
 22. Das Gupta E, Ng WR, Wong SF, Bhurhanudeen AK, Yeap SS. Correlation of serum cartilage oligomeric matrix protein (COMP) and interleukin-16 (IL-16) levels with disease severity in primary knee osteoarthritis: a pilot study in a Malaysian population. *PLoS One* 2017;12(9):e0190542.
 23. Radojčić MR, Thudium CS, Henriksen K, Tan K, Karlsten R, Dudley A, et al. Biomarker of extracellular matrix remodelling C1M and proinflammatory cytokine interleukin 6 are related to synovitis and pain in end-stage knee osteoarthritis patients. *Pain* 2017;158:1254–63.
 24. Wen L, Shin MH, Kang JH, Yim YR, Kim JE, Lee JW, et al. The value of high-sensitivity C-reactive protein in hand and knee radiographic osteoarthritis: data from the Dong-gu study. *Clin Rheumatol* 2017;37:1099–106.
 25. Perruccio AV, Chandran V, Power JD, Kapoor M, Mahomed NN, Gandhi R. Systemic inflammation and painful joint burden in osteoarthritis: a matter of sex? *Osteoarthritis Cartilage* 2017;25:53–9.
 26. Stout AC, Barbe MF, Eaton CB, Amin M, Al-Eid F, Price LL, et al. Inflammation and glucose homeostasis are associated with specific structural features among adults without knee osteoarthritis: a cross-sectional study from the osteoarthritis initiative. *BMC Musculoskelet Disord* 2018;19:1.
 27. Liu YP, Li J, Xin SB, Xu J. Study the relevance between inflammatory factors and estradiol and their association with knee osteoarthritis in postmenopausal women. *Eur Rev Med Pharmacol Sci* 2018;22:472–8.
 28. Zou YC, Deng HY, Mao Z, Zhao C, Huang J, Liu G. Decreased synovial fluid ghrelin levels are linked with disease severity in primary knee osteoarthritis patients and are increased following laser therapy. *Clin Chim Acta* 2017;470:64–9.
 29. Wu J, Wang K, Xu J, Ruan G, Zhu Q, Cai J, et al. Associations between serum ghrelin and knee symptoms, joint structures and cartilage or bone biomarkers in patients with knee osteoarthritis. *Osteoarthritis Cartilage* 2017;25:1428–35.
 30. Toussirot E, Michel F, Béreau M, Dehecq B, Gaugler B, Wendling D, et al. Serum adipokines, adipose tissue measurements and metabolic parameters in patients with advanced radiographic knee osteoarthritis. *Clin Rheumatol* 2017;36:2531–9.
 31. Cuzdan Coskun N, Ay S, Evcik FD, Oztuna D. Adiponectin: is it a biomarker for assessing the disease severity in knee osteoarthritis patients? *Int J Rheum Dis* 2017;20:1942–9.
 32. Calvet J, Orellana C, Albinana Gimenez N, Berenguer-Llergo A, Caixas A, Garcia-Manrique M, et al. Differential involvement of synovial adipokines in pain and physical function in female patients with knee osteoarthritis. A cross-sectional study. *Osteoarthritis Cartilage* 2018;26:276–84.
 33. Ertürk C, Altay MA, Bilge A, Çelik H. Is there a relationship between serum ox-LDL, oxidative stress, and PON1 in knee osteoarthritis? *Clin Rheumatol* 2017;36:2775–80.
 34. Tootsi K, Martson A, Kals J, Paapstel K, Zilmer M. Metabolic factors and oxidative stress in osteoarthritis: a case-control study. *Scand J Clin Lab Invest* 2017;77:520–6.
 35. Karalilova R, Kazakova M, Batalov A, Sarafian V. Correlation between protein YKL-40 and ultrasonographic findings in active knee osteoarthritis. *Med Ultrasonography* 2018;20:57–63.
 36. Panina SB, Krolevets IV, Milyutina NP, Sagakyants AB, Kornienko IV, Ananyan AA, et al. Circulating levels of proinflammatory mediators as potential biomarkers of post-

- traumatic knee osteoarthritis development. *J Orthop Traumatol* 2017;18:349–57.
37. 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;36:2351–8.
 38. Mohammed MA, Rady SAK, Mohammed RA, Fadda SMH. Relation of plasma fibroblast growth factor-23 (FGF-23) to radiographic severity in primary knee osteoarthritis patients. *Egypt Rheumatol* 2018, <https://doi.org/10.1016/j.ejr.2018.01.007>.
 39. Li B, Zhang YL, Yu SY. Synovial fluid eotaxin-1 levels may reflect disease progression in primary knee osteoarthritis among elderly Han Chinese: a cross-sectional study. *Cartilage* 2018, <https://doi.org/10.1177/1947603518764280>.
 40. He J, Cao W, Azeem I, Zhao Q, Shao Z. Transforming Growth Factor Beta1 being considered a novel biomarker in knee osteoarthritis. *Clin Chim Acta* 2017;472:96–101.
 41. Ding J, Niu X, Su Y, Li X. Expression of synovial fluid biomarkers in patients with knee osteoarthritis and meniscus injury. *Exp Ther Med* 2017;14:1609–13.
 42. Li XM, Fu XD, Gao YJ, Li H, Wang WL, Shen Y. Expression of tissue inhibitor of metalloproteinases-1 and B-cell lymphoma-2 in the synovial membrane in patients with knee osteoarthritis. *Exp Ther Med* 2018;15:885–9.
 43. Wang SX, Abramson SB, Attur M, Karsdal MA, Preston RA, Lozada CJ, et al. Safety, tolerability, and pharmacodynamics of an anti-interleukin-1 α / β dual variable domain immunoglobulin in patients with osteoarthritis of the knee: a randomized phase 1 study. *Osteoarthritis Cartilage* 2017;25:1952–61.
 44. Wright KT, Kuiper JH, Richardson JB, Gallacher P, Roberts S. The absence of detectable ADAMTS-4 (Aggrecanase-1) activity in synovial fluid is a predictive indicator of autologous chondrocyte implantation success. *Am J Sports Med* 2017;45:1806–14.
 45. Martel-Pelletier J, Raynauld JP, Mineau F, Abram F, Paiement P, Delorme P, et al. Levels of serum biomarkers from a two-year multicentre trial are associated with treatment response on knee osteoarthritis cartilage loss as assessed by magnetic resonance imaging: an exploratory study. *Arthritis Res Ther* 2017;19(1):169, <https://doi.org/10.1186/s13075-017-1377-y>.
 46. Arends RHGP, Karsdal MA, Verburg KM, West CR, Bay-Jensen AC, Keller DS. Identification of serological biomarker profiles associated with total joint replacement in osteoarthritis patients. *Osteoarthritis Cartilage* 2017;25:866–77.
 47. Fawzy RM, Hashaad NI, Mansour AI. Decrease of serum biomarker of type II Collagen degradation (Coll2-1) by intra-articular injection of an autologous plasma-rich-platelet in patients with unilateral primary knee osteoarthritis. *Eur J Rheumatol* 2017;4:93–7.
 48. Kim MK, Ha CW, In Y, Cho SD, Choi ES, Ha JK, et al. A multicenter, double-blind, phase III clinical trial to evaluate the efficacy and safety of a cell and gene therapy in knee osteoarthritis patients. *Hum Gene Ther Clin Dev* 2018;29:48–59.
 49. Larsson S, Struglics A, Lohmander LS, Frobell R. Surgical reconstruction of ruptured anterior cruciate ligament prolongs trauma-induced increase of inflammatory cytokines in synovial fluid: an exploratory analysis in the KANON trial. *Osteoarthritis Cartilage* 2017;25:1443–51.
 50. Vincent TL, Watt FE. Osteoarthritis. *Medicine* 2014;42:213–9.
 51. Pietrosimone B, Loeser RF, Blackburn JT, Padua DA, Harkey MS, Stanley LE, et al. Biochemical markers of cartilage metabolism are associated with walking biomechanics 6-months following anterior cruciate ligament reconstruction. *J Orthop Res* 2017;35:2288–97.
 52. Chu CR, Sheth S, Erhart-Hledik JC, Do B, Titchenal MR, Andriacchi TP. Mechanically stimulated biomarkers signal cartilage changes over 5 years consistent with disease progression in medial knee osteoarthritis patients. *J Orthop Res* 2018;36:891–7.
 53. Edd SN, Favre J, Blazek K, Omoumi P, Asay JL, Andriacchi TP. Altered gait mechanics and elevated serum pro-inflammatory cytokines in asymptomatic patients with MRI evidence of knee cartilage loss. *Osteoarthritis Cartilage* 2017;25:899–906.
 54. 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. *Osteoarthritis Cartilage* 2010;18:1441–7.
 55. Favre J, Erhart-Hledik JC, Andriacchi TP. Age-related differences in sagittal-plane knee function at heel-strike of walking are increased in osteoarthritic patients. *Osteoarthritis Cartilage* 2014;22:464–71.
 56. Huang X, Post JN, Zhong L, Leijten J, Larsson S, Karperien M, et al. Dickkopf-related protein 1 and gremlin 1 show different response than frizzled-related protein in human synovial fluid following knee injury and in patients with osteoarthritis. *Osteoarthritis Cartilage* 2018;26:834–43.
 57. Shan Y, Qi C, Liu Y, Gao H, Zhao D, Jiang Y. Increased frequency of peripheral blood follicular helper T cells and elevated serum IL-21 levels in patients with knee osteoarthritis. *Mol Med Rep* 2017;15:1095–102.
 58. Ren GM, Lutz I, Railton P, Wiley JP, McAllister J, Powell J, et al. Serum and synovial fluid cytokine profiling in hip osteoarthritis: distinct from knee osteoarthritis and correlated with pain. *BMC Muscoskeletal Disord* 2018;19(1):39, <https://doi.org/10.1186/s12891-018-1955-4>.
 59. Snelling SJ, Bas S, Puskas GJ, Dakin SG, Suva D, Finckh A, et al. Presence of IL-17 in synovial fluid identifies a potential inflammatory osteoarthritic phenotype. *PLoS One* 2017;12: e0175109.
 60. Gundogdu G, Gundogdu K. A novel biomarker in patients with knee osteoarthritis: adropin. *Clin Rheumatol* 2018;37(8):2179–86.
 61. Lourido L, Ayoglu B, Fernandez-Tajes J, Oreiro N, Henjes F, Hellstrom C, et al. Discovery of circulating proteins associated to knee radiographic osteoarthritis. *Sci Rep* 2017;7:137, <https://doi.org/10.1038/s41598-017-00195-8>.
 62. Goode AP, Nelson AE, Kraus VB, Renner JB, Jordan JM. Biomarkers reflect differences in osteoarthritis phenotypes of the lumbar spine: the Johnston County Osteoarthritis Project. *Osteoarthritis Cartilage* 2017;25:1672–9.
 63. Brayda-Bruno M, Vigano M, Cauci S, Vitale JA, de Girolamo L, De Luca P, et al. Plasma vitamin D and osteo-cartilaginous markers in Italian males affected by intervertebral disc degeneration: focus on seasonal and pathological trend of type II collagen degradation. *Clin Chim Acta* 2017;471:87–93.
 64. Fioravanti A, Tenti S, Pulsatelli L, Addimanda O. Could myeloperoxidase represent a useful biomarker for erosive osteoarthritis of the hand? *Scand J Rheumatol* 2018;1–3, <https://doi.org/10.1080/03009742>.
 65. Fioravanti A, Cheleschi S, De Palma A, Addimanda O, Mancarella L, Pignotti E, et al. Can adipokines serum levels be used as biomarkers of hand osteoarthritis? *Biomarkers* 2018;23:265–70.
 66. Saviola G, Abdi-Ali L, Povino MR, Campostrini L, Sacco S, Carbonare LD. Intramuscular clodronate in erosive osteoarthritis of the hand is effective on pain and reduces serum COMP: a randomized pilot trial—the ER.O.D.E. study (ERosive Osteoarthritis and Disodium-clodronate Evaluation). *Clin Rheumatol* 2018;37(7):2019, <https://doi.org/10.1007/s10067-018-4082-6>.

67. Ozcamdalli M, Misir A, Kizkapan TB, Uzun E, Duygulu F, Yazici C, et al. Comparison of intra-articular injection of hyaluronic acid and N-acetyl cysteine in the treatment of knee osteoarthritis: a pilot study. *Cartilage* 2017;8:384–90.
68. Geusens P, Pavelka K, Rovensky J, Vanhoof J, Demeester N, Calomme M, et al. A 12-week randomized, double-blind, placebo-controlled multicenter study of choline-stabilized orthosilicic acid in patients with symptomatic knee osteoarthritis. *BMC Musculoskelet Disord* 2017;18(1):2, <https://doi.org/10.1186/s12891-016-1370-7>.
69. Ahn Y, Kwon O, Kim EA, Yoon WJ, Kim JH, Kim JY. Randomized double-blind placebo-controlled study of the efficacy of *Litsea japonica* fruit extract in subjects with mild to moderate knee osteoarthritis. *J Funct Foods* 2017;34:304–10.
70. Sawada Y, Sugimoto A, Hananouchi T, Sato N, Nagaoka I. Evaluation of the efficacy of *Ajuga decumbens* extract supplement in individuals with knee discomfort associated with physical activity: a randomized, double-blind, placebo-controlled study. *Exp Ther Med* 2017;14:4561–71.
71. Kalman DS, Hewlings SJ. The effects of *Morus alba* and *Acacia catechu* on quality of life and overall function in adults with osteoarthritis of the knee. *J Nutr Metab* 2017;2017:4893104.
72. Tomonaga A, Fukagawa M, Suzuki A, Kurokawa M, Nagaoka I. Evaluation of the effect of N-acetyl-glucosamine administration on biomarkers for cartilage metabolism in healthy individuals: a randomized double-blind placebo-controlled clinical study. *Funct Foods Health Dis* 2017;7:604–27.
73. Tomonaga A, Takahashi T, Tanaka YT, Tsuboi M, Ito K, Nagaoka I. Evaluation of the effect of salmon nasal proteoglycan on biomarkers for cartilage metabolism in individuals with knee joint discomfort: a randomized double-blind placebo-controlled clinical study. *Exp Ther Med* 2017;14:115–26.
74. Henrotin Y, Berenbaum F, Chevalier X, Marty M, Richette P, Rannou F. Reduction of the serum levels of a specific biomarker of cartilage degradation (Coll2-1) by hyaluronic acid (KARTILAGE CROSS) compared to placebo in painful knee osteoarthritis patients: the EPIKART study, a pilot prospective comparative randomized double blind trial. *BMC Musculoskelet Disord* 2017;18:222, <https://doi.org/10.1186/s12891-017-1585-2>.
75. Yazici Y, McAlindon TE, Fleischmann R, Gibofsky A, Lane NE, Kivitz AJ, et al. A novel Wnt pathway inhibitor, SM04690, for the treatment of moderate to severe osteoarthritis of the knee: results of a 24-week, randomized, controlled, phase 1 study. *Osteoarthritis Cartilage* 2017;25:1598–606.
76. Lopez HL, Habowski SM, Sandrock JE, Raub B, Kedia A, Bruno EJ, et al. Effects of dietary supplementation with a standardized aqueous extract of *Terminalia chebula* fruit (AyuFlex) on joint mobility, comfort, and functional capacity in healthy overweight subjects: a randomized placebo-controlled clinical trial. *BMC Altern Med* 2017;17:475.
77. Mahdavi R, Kolahi S, Attari VE, Mahdavi AM. L-carnitine supplementation ameliorates serum tumor necrosis factor-alpha and matrix metalloproteinase-3 in knee osteoarthritis women. *Bangladesh J Pharm* 2017;12:28–34.
78. Schell J, Scofield RH, Barrett JR, Kurien BT, Betts N, Lyons TJ, et al. Strawberries improve pain and inflammation in obese adults with radiographic evidence of knee osteoarthritis. *Nutrients* 2017;9(9), <https://doi.org/10.3390/nu9090949>. pii: E949.
79. Nong XG, Cheng WP, Zeng LJ. Effect of moxibustion plus Duhuo Jisheng decoction on hs-CRP, IL-1 beta and TNF-alpha levels in middle-aged and elderly patients with knee osteoarthritis. *J Acupunct Tuina Sci* 2017;15:277–80.
80. Katayoshi T, Kageyama M, Kobashi R, Minakuchi J, Suzuki N, Takara T, et al. Efficacy and safety of a compound supplement containing glucosamine, chondroitin, and five bioactive ingredients in volunteers with knee joint pain. *Pers Med Universe* 2017;6:17–21.
81. Apparao P, Sudhakar S, Ch GS, Reddy RS. Influence of stabilization exercises on articular cartilage changes in degenerative tibiofemoral joint disease: a pilot study. *Asian J Pharm Clin Res* 2017;10:440–4.
82. Alunno A, Falcinelli E, Luccioli F, Petito E, Bartoloni E, Momi S, et al. Platelets contribute to the accumulation of matrix metalloproteinase type 2 in synovial fluid in osteoarthritis. *Thromb Haemost* 2017;117:2116–24.
83. Ortega E, Galvez I, Hinchado MD, Guerrero J, Martin-Cordero L, Torres-Piles S. Anti-inflammatory effect as a mechanism of effectiveness underlying the clinical benefits of pelotherapy in osteoarthritis patients: regulation of the altered inflammatory and stress feedback response. *Int J Biometeorol* 2017;61: 1777–85.
84. Hudetz D, Borić I, Rod E, Jeleč Ž, Radić A, Vrdoljak T, et al. The effect of intra-articular injection of autologous micro-fragmented fat tissue on proteoglycan synthesis in patients with knee osteoarthritis. *Genes* 2017;8(10), <https://doi.org/10.3390/genes8100270>.
85. Ruff KJ, Morrison D, Duncan SA, Back M, Aydogan C, Theodosakis J. Beneficial effects of natural eggshell membrane versus placebo in exercise-induced joint pain, stiffness, and cartilage turnover in healthy, postmenopausal women. *Clin Interv Aging* 2018;13:285–95.
86. Li ZM, Li M. Improvement in orthopedic outcome score and reduction in IL-1beta, CXCL13, and TNF-alpha in synovial fluid of osteoarthritis patients following arthroscopic knee surgery. *Genet Mol Res* 2017;16, <https://doi.org/10.4238/gmr16039487>.
87. Klocke R, Levasseur K, Kitas GD, Smith JP, Hirsch G. Cartilage turnover and intra-articular corticosteroid injections in knee osteoarthritis. *Rheumatol Int* 2018;38:455–9.
88. Jessberger S, Högger P, Genest F, Salter DM, Seefried L. Cellular pharmacodynamic effects of Pycnogenol® in patients with severe osteoarthritis: a randomized controlled pilot study. *BMC Complement Altern Med* 2017;17(1):537, <https://doi.org/10.1186/s12906-017-2044-1>.
89. Shin D, Kim EA, Kim YY, Kim MS, Kim JH, Ahn CW, et al. Deer bone extract supplementation for mild-to-moderate knee osteoarthritis symptoms: a randomized, double-blind, placebo-controlled trial. *J Med Food* 2018;21:159–66.
90. Leung YY, Haaland B, Huebner JL, Wong SBS, Tjai M, Wang C, et al. Colchicine lack of effectiveness in symptom and inflammation modification in knee osteoarthritis (COLKOA): a randomized controlled trial. *Osteoarthritis Cartilage* 2018;26: 631–40.
91. Azidah AK, Arifah AK, Roslida AH, Mat Jais AM, Omar J, Sadagatullah AN, et al. A randomized, double-blind study comparing multiple doses of *Channa striatus* supplementation for knee osteoarthritis. *Orient Pharm Exp Med* 2017;17: 345–54.
92. Cornish SM, Peeler JD. No effect of creatine monohydrate supplementation on inflammatory and cartilage degradation biomarkers in individuals with knee osteoarthritis. *Nutr Res* 2018;51:57–66.
93. Lotz M, Martel-Pelletier J, Christiansen C, Brandi ML, Bruyere O, Chapurlat R, et al. Value of biomarkers in osteoarthritis: current status and perspectives. *Ann Rheum Dis* 2013;72:1756–63.
94. Bay-Jensen A-C, Henrotin Y, Karsdal M, Mobasheri A. The need for predictive, prognostic, objective and complementary blood-based biomarkers in osteoarthritis (OA). *EBioMedicine* 2016;7:4–6.