Osteoarthritis and Cartilage 24 (2016) 9–20

Osteoarthritis and Cartilage

Review

Osteoarthritis year in review 2015: soluble biomarkers and the BIPED criteria

A.C. Bay-Jensen † *, D. Reker †, C.F. Kjelgaard-Petersen †, A. Mobasheri ‡ § ||, M.A. Karsdal †, C. Ladel ¶, Y. Henrotin #, C.S. Thudium †

† *Rheumatology, Biomarkers and Research, Nordic Bioscience, Herlev, Denmark*
‡ *Faculty of Health and Medical Sciences, University of Surrey, United Kingdom*
§ *Arthritis Research UK Centre for Sport, Exercise and Osteoarthritis, Arthritis Research UK Pain Centre, Medical Research Council and Arthritis Research UK Centre for Musculoskeletal Ageing Research, University of Nottingham, United Kingdom*
|| *Center of Excellence in Genomic Medicine Research (CEGMR), King Fahd Medical Research Center (KFMRC), King AbdulAziz University, Jeddah, Saudi Arabia*
¶ *OA Research & Early Clinical Development, Merck KGaA, Darmstadt, Germany*
# *Bone and Cartilage Research Unit, Arthropole Liège, University of Liège, Institute of Pathology, Liège, Belgium*

ARTICLE INFO

Article history:
Received 1 July 2015
Accepted 21 October 2015

Keywords:
Biomarkers
Osteoarthritis (OA)
BIPED
Year in a review

SUMMARY

Objective: To review and summarize biomarker data published from April 2014 to May 2015 to provide insight to the ongoing work in the field of osteoarthritis (OA). Furthermore, to summarize the BIPED criteria and set it in context of the medical needs of 2015.
Methods: PubMed was used as searching machine: Time period 2014/04/01–2015/05/01, MeSH term [Biomarker] AND [Osteoarthritis], Language; English, Full text available. Reviews were excluded. Only papers describing protein based biomarkers measured in human body fluids from OA patients were included.
Results: Biomarkers of joint tissue turnover, cytokines, chemokines and peptide arrays were measured in different cohorts and studies. Amongst those were previously tested biomarkers such as osteocalcin, Carboxy-terminal cross-linked fragment of type II collagen (CTX-II) and cartilage oligomeric matrix protein (COMP). A majority of the biomarker were classified as I, B or B biomarkers according to the BIPED criteria.
Work is continuing on testing biomarkers in OA. There is still a huge, unmet medical need to identify, test, validate and qualify novel and well-known biomarkers. A pre-requisite for this is better characterization and classification of biomarkers to their needs, which may not be reached before higher understanding of OA phenotypes has been gained. In addition, we provide some references to some recent guidelines from Food and Drug Administration (FDA) and European Medicines Agency (EMA) on qualification and usage of biomarkers for drug development and personalized medicine, which may provide value to the field.

Introduction

There is still a need for biomarkers in osteoarthritis (OA) for diagnosis, prognosis and monitoring of disease activity in clinical practice, as well as biomarkers for patient selection and study design optimization in clinical interventional trials[1]. To date limited biomarkers have been approved by the regulatory authorities for theses uses in OA; however multiple biomarkers have been developed and tested over the last decades. Also, in 2014, new biomarkers were investigated, alongside evaluation of well-known biomarkers.

The US Food and Drug Administration (FDA) and European Medicines Agency (EMA), have recently published different guidelines recommending a higher level of integration of biomarkers in the development and testing of new drugs to advance decision-making on dosing, time and treatment effect, trial design,

* Address correspondence and reprint requests to: A.C. Bay-Jensen, Rheumatology, Biomarker and Research, Nordic Bioscience, Herlev, Denmark. Tel: 45-44-54-77-30.
E-mail address: acbj@nordicbioscience.com (A.C. Bay-Jensen).

http://dx.doi.org/10.1016/j.joca.2015.10.014

and risk/benefit analysis and personalized medicine (please see references at the end of the article) It is believed that by implementing biomarkers for screening of drug candidates in early clinical development (e.g., *in vitro* and/or pre-clinically), potential safety issues can be addressed in advance, and hence increase the drug development efficiency, and possibly reduce the overall cost of running and OA clinical trial[2]. The primary objective of using biomarkers in drug development is to identify a sub-population of responsive subjects that will provide best evidence for rejecting the null hypothesis of no treatment effect, and thereby demonstrate the efficacy and the safety of a drug candidate. Since biomarkers are considered as objective measures of biological, pathological or pharmacological events, the probability of false positives and false negatives selection becomes reduced compared to patient recruitment based on objective measures such as Visual analog score (VAS) pain. Consequently, the power calculation for future clinical trials will result in a smaller sample size.

Biomarkers can be used not only in the process of drug development, but also in assessment of individual patient's response to treatment. By evaluating the biomarker result, clinicians will be able to conclude whether the treatment has the desired effect or not. The BIPED classification categorizes biomarkers according to key parameters for evaluation and qualification of the utility of biomarkers. Those are: Burden of disease (B), Investigative (I), Prognostic (P), Efficacy of intervention (E) and Diagnostic (D)[3].

In this review, we provide a systematic review of soluble biochemical markers (biomarkers) recently tested in clinical studies and cohorts. Furthermore, we highlight recent studies applying biomarkers in OA. In conclusion, we provide interpretation on how to classify biomarkers according to the BIPED criteria by differentiating each of the categories as of 2015.

Methods

The article consists of three parts. The first part summarizes peer reviewed papers on biomarkers published between April 1st 2014 and May 1st 2015. PubMed was used to search the time range, in combination with the MeSH terms BIOMARKER AND OSTEOARTHRITIS. From the initial search 115 articles were listed. Next, the list was further processed by only including publications written in English, reducing the list to 113; all were available as full text papers. Fourteen review articles were excluded from the list, leaving 99 original research articles for inclusion in the review process.

Each article was carefully studied and only articles including measurement of protein or peptide-based soluble biomarkers in serum, plasma, synovial fluid (SF) and urine of either OA patients or preclinical OA-associated models were included. Six articles describing the usage of biomarkers tested in animal models (Table S1)[92–96] and 41 describing the testing in clinical studies (Table I) were reviewed and tabulated in this review article. Fifty two articles were excluded because they were either: (1) not assessed in OA associated models/samples, (2) not soluble protein-derived biomarkers, or (3) not utilizing biomarkers in the study/publication although.

The second part of the article investigates the total number of publications publically available on each of the biomarkers described in the first part which reflects joint tissue turnover. The number of publications was investigated by searching on PubMed, using the biomarker name together with the MeSH term OSTEOARTHRITIS. Review articles were excluded. The limitation of the search was spelling mistakes and uncommon abbreviations, which may have led to unidentified publications. The numbers given in Table II are therefore an estimate, which should only be used as an information pointing towards the number of investigations published on individual biomarkers during the last decades.

The third part is a perspective (narrative) view on the usage of the biomarkers and how to use the BIPED classification to understand the utility of the biomarkers.

Biomarkers tested in clinical OA studies and cohorts

Different classes of biomarkers were tested in the 2014/2015 period targeting mainly two classes: (1) biomarkers of joint tissue turnover and (2) biomarkers of (pro-) inflammatory status. Table I summarizes the biomarkers tested in human OA samples. The BIPED classification in which the authors tested the hypothesis is given in the table.

During the past year several inflammatory biomarkers have been described, including cytokines, chemokines, or cell type markers important for OA pathology[4–8]. Another group of the newly identified biomarkers were biomarkers reflecting the turnover of the extracellular matrix (ECM) of the diseased cartilage[9–11], while the remaining biomarkers targeted auto-antibodies, signaling molecules, or growth factors[12–19]. Many of these biomarkers were investigated based on the knowledge of OA pathology: Daghestani[4] worked from the hypothesis that inflammatory OA has a higher degree of activated macrophages, leading to a shedding of the macrophage cell markers: CD163 and CD14. In their study, they found these two markers indeed were increased and correlated to the severity and progression of OA symptoms[4].

The hypothesis of another study was based on fractalkine (CX3CL1) playing a role in inflammation and chronic pain, and this study showed that CX3CL1 levels in serum and SF were correlated with WOMAC pain and WOMAC total scores[5]. Some biomarkers were identified using high-throughput methods like affinity proteomics and mass spectrometry. Affinity proteomics and antigen microarrays were used to detect auto-antibodies in human OA serum. Normally auto-antibodies are known from autoimmune disease like rheumatoid arthritis, but this study found auto-antibodies against potassium channels, carbohydrate sulfotransferase, and interleukin-6 (IL-6) in human OA serum[14].

Mass spectrometry was used to investigate tryptic digested cartilage slides from young, aging, and OA horses to identify ECM proteins and ECM fragments related to OA cartilage and non-aging cartilage. This study found disease-specific fibromodulin and biglycan peptides that were not linked to aging, and that additionally identified a number of fibronectin fragments with increased abundance in OA cartilage as tentative biomarkers[9].

Selected reaction monitoring mass spectrometry, which enable measurements of several proteins in one sample, was used to identify clusterin and lubricin in human OA plasma as predictors of OA progression, with a prediction level equal to age[17].

In general the biomarkers identified were either identified as diagnostic biomarkers or burden of disease markers in serum, plasma, or SF. However, many of them were found in small sample sizes, and it is therefore important to further investigate these biomarkers in larger sample sizes. Additionally biomarkers measured in human SF were only measured in OA SF, as it is an invasive and unethical procedure to obtain SF from healthy control individuals. Biomarkers measured in SF were only correlated to serum or plasma levels and used to look at progression.

More established biomarkers that have previously been suggested as biomarkers that may correlate with features of OA, were tested in larger sample sizes and populations to further characterize the potential use of these biomarkers. Arendt-Nielsen and colleagues showed that the degradation fragment of CRP, CRPM, correlated with the degree of sensitization in a group of symptomatic OA patients ($n = 281$)[20], indicating a connection between pain sensitization and chronic inflammation. Carboxy-terminal cross-linked fragment of type II collagen (CTX-II) was measured in a

Table I
Clinical biomarker overview. The biomarkers are listed in alphabetic order

Biomarker	Sample type	Results (short description)	Ref ID	BIPED classification
Alpha-CTX	Urine	SKOA and RKOA ($n = 149$). α-CTX was strongly correlated with subchondral bone turnover, JSN and osteophyte progression.	37	Prognostic
ADAMTS-4	Serum	Early OA ($n = 44$), intermediate and late OA ($n = 26$), healthy control ($n = 30$). ADAMTS-4 was significantly increased in patients with early OA compared to intermediate and advanced OA groups as well as controls.	38	Burden of disease
ADAMTS-5	Serum	Early OA ($n = 44$), intermediate and late OA ($n = 26$), healthy control ($n = 30$). ADAMTS-5 was significantly higher in intermediate and advanced OA compared to early OA and controls.	38	Burden of disease
ARGS	Serum, Urine, Plasma	Healthy subjects ($n = 20$), Non-surgical OA ($n = 20$), OA subjects undergoing TKR ($n = 20$). Serum and urine ARGS associated with OA. SF ARGS was positively correlated with WOMAC stiffness but not total WOMAC score.	39	Burden of disease
AGRS	Synovial fluid	Acute knee injury ($n = 98$). No association with osteochondral fracture in the knee.	22	Burden of disease
Autotaxin	Plasma, Synovial fluid	Control ($n = 20$), KOA ($n = 70$). Plasma and SF autotaxin were associated with severity of RKOA and positively correlated with WOMAC scores.	40	Burden of disease
BDNF	Plasma, Synovial fluid	Healthy subjects ($n = 19$), RKOA ($n = 27$), Increased plasma levels in RKOA patients compared to controls and positively correlated with WOMAC score. No correlation found with knee OA severity.	19	Diagnostic
C1M	Serum	Non-clinical OA to clinical OA ($n = 281$). C1M showed no association with intensity or duration of pain.	20	Burden of disease
C2C	Serum, Synovial fluid	Subjects 0 days-7 years after knee injury (SF $n = 235$, serum $n = 71$), controls ($n = 8$). Increased levels of C2C in injured knees 1 day-7 years after injury compared to controls. Shortly after injury 1–33 days, C2C concentrations were correlated with CTX-II, ARGS, osteocalcin, osteopontin and IL-8, but not structural joint injury by MRI.	41	Investigative
C2C	Synovial fluid	Traumatic OA ($n = 8$). Average cartilage strain at maximal flexion of the knee in patients with meniscal tear was not associated with C2C.	42	Investigative
C2M	Serum	Non-clinical OA to clinical OA ($n = 281$) − C2M showed no association with intensity or duration of pain.	20	Burden of disease
C2M	Serum	OA KL 0–4 ($n = 271$). Positive association with increased levels of C2M.	10	Burden of disease
C3M	Serum	Non-clinical OA to clinical OA ($n = 281$). C3M showed no association with intensity or duration of pain.	20	Burden of disease
C-Col10	Serum	OA KL 0–4 ($n = 271$). C-Col10 was elevated in patients with KL = 2 compared to KL = 0, and was elevated in patients with above normal hsCRP.	10	Burden of disease
CCL2	Serum, Synovial fluid	Control ($n = 138$), OA ($n = 161$). CCL2 in SF but not in serum associated with symptomatic severity of OA.	43	Burden of disease
CCL3	Plasma	Non-radiographic KOA ($n = 47$), RKOA ($n = 50$) Control ($n = 75$). CCL3 associated with severity of KOA.	8	Burden of disease
CCL4	Plasma	Non-radiographic KOA ($n = 47$), RKOA ($n = 50$) Control ($n = 75$). CCL4 associated with RKOA severity.	8	Burden of disease
CD14	Synovial fluid Plasma	RKOA ($n = 184$). SF CD14 was positively associated with JSN and osteophytes. CD14 in both plasma and SF was positively associated with self reported knee pain in a subgroup. SF CD14 was positively associated with osteophyte progression.	4	Burden of disease Prognostic
CD163	Synovial fluid, plasma	RKOA ($n = 184$). SF CD163 was positively associated with osteophyte progression.	4	Prognostic
CGRP	Serum, Synovial fluid	KOA ($n = 65$), control ($n = 21$). CGRP was positively correlated with KL score, total WOMAC as well as each WOMAC subscale.	13	Burden of disease
Coll-2-1	Serum	KOA ($n = 22$) Curcumin treatment reduced Coll-2-1 serum levels.	44	Efficacy of intervention
COLL-2-1NO_2	Urine	Overweight and obese middleaged women ($n = 254$). COLL-2-1NO_2 at baseline is negatively associated with incidence of knee OA.	45	Prognostic
Coll-2-1NO_2		KOA ($n = 22$). No association observed between Coll-2-1NO_2 and Curcumin treatment.	44	Efficacy of intervention
COMP	Serum	RHOA ($n = 638$). Symptoms of hand OA and higher AUSCAN scores were associated with higher levels of COMP. No association was found with radiographic hand OA.	21	Burden of disease
COMP	Synovial fluid	Traumatic OA ($n = 8$). Average cartilage strain at maximal flexion of the knee in patients with meniscal tear was not associated with COMP levels.	42	Investigative

(*continued on next page*)

Table I (*continued*)

Biomarker	Sample type	Results (short description)	Ref ID	BIPED classification
COMP	Synovial fluid	Acute knee injury ($n = 98$). No association with osteochondral fracture in the knee.	22	Burden of disease
COMP	Serum Synovial fluid	KOA with joint effusion ($n = 34$). No correlation between COMP and radiographic or ultrasonographic findings were observed.	23	Burden of disease
CRP	Serum	Non-clinical OA to clinical OA ($n = 281$). CRP showed no association with intensity or duration of pain.	20	Burden of disease
CRP	Serum	KOA ($n = 22$) treated with Curcumin. No association observed between CRP and Curcumin treatment.	44	Efficacy of intervention
CRPM	Serum	Non-clinical OA to clinical OA ($n = 281$). CRPM correlated with degree of centralized sensitization.	20	Burden of disease
CTX-II	Urine	SKOA and RKOA ($n = 149$). CTX-II was strongly associated with cartilage/bone interface bone turnover, JSN, ostephyte severity and OA progression based on ostephyte score.	37	Burden of disease Prognostic
CTX-II	Urine	KOA ($n = 22$) treated with Curcumin. No association observed between CTX-II and Curcumin treatment.	44	Efficacy of intervention
CTX-II	Urine	Population ($n = 1040$). Correlation between CTX-II and KL score in women above 60 with OA.	46	Burden of disease
CX3CL1	Serum Synovial fluid	OA ($n = 193$), Healthy controls ($n = 182$). CX3C1 levels in serum and SF were positively associated with self reported pain and disability measured by WOMAC score.	5	Burden of disease
FAC	Synovial fluid	OA ($n = 17$), non-OA ($n = 17$) Negative correlation with ROA at hip, but positively with microdamage surgery in non-OA patients.	11	Burden of disease
FGF21	Serum, Synovial fluid	OA ($n = 186$), control ($n = 108$) Positive correlation between increased levels of FGF21 and OA and Ahlbäck grade (radiographic bone loss).	15	Burden of disease
Fib3-1	Serum	KOA ($n = 22$) treated with Curcumin. No association observed between Fib3-1 and Curcumin treatment.	44	Efficacy of intervention
Fib3-2	Serum	KOA ($n = 22$) treated with Curcumin No association observed between Fib3-2 and Curcumin treatment.	44	Efficacy of intervention
Fib3-2	Serum	In the PROOF study ($n = 242$), baseline Fibulin 3 epitopes (Fib3-1, Fib3-2 and Fib3-3) concentrations are highly associated to the incidence of clinical knee OA among middle-aged overweight and obese women.	45	Diagnostic
HIF-1a	Serum, Synovial fluid	KOA ($n = 278$), healthy control ($n = 203$) Association between higher levels of HIF-1a and increased radiographic severity of KOA.	12	Burden of disease
hmwAPN	Serum	RHOA ($n = 227$). hmwAPN showed no association with progression of RHOA.	47	Prognostic
IL-1β	Synovial fluid	Acute knee injury ($n = 98$). No association with osteochondral fracture in the knee.	22	Burden of disease
IL-6	Synovial fluid	Acute knee injury ($n = 98$). No association with osteochondral fracture in the knee.	22	Burden of disease
IL-6	Plasma	Non-radiographic KOA ($n = 47$), RKOA ($n = 50$) Control ($n = 75$). Associated with RKOA severity.	8	Burden of disease
IL-8	Synovial fluid	Acute knee injury ($n = 98$). Osteochondral fractures with disrupted cortical bone had increased IL-8 compared to non-osteochondral fractures	22	Burden of disease
IL-8	Plasma	Non-radiographic KOA ($n = 47$), RKOA ($n = 50$) Control ($n = 75$). Associated with RKOA severity.	8	Burden of disease
IL-10	Serum	EHOA + Fenofibrate ($n = 14$). Fenofibrate treatment of EHOA was associated with a reduction in IL-10 levels.	48	Efficacy of intervention
ihh	Synovial fluid	Normal ($n = 25$), early OA ($n = 50$), late OA ($n = 47$). Increased ihh concentration in SF in early but not late stage OA compared to controls.	18	Burden of disease
Leptin	Serum	Elderly Boston Study population ($n = 653$). Increased levels of leptin is associated with increased risk of OA, suggesting that part of the BMI risk factor is mediated through increased leptin concentrations.	49	Prognostic
Leptin	Serum Synovial fluid	KOA with joint effusion ($n = 34$). Serum leptin correlated with HAQ and length of medial ostephytes.	23	Burden of disease

Table I (*continued*)

Biomarker	Sample type	Results (short description)	Ref ID	BIPED classification
MMP-1	Serum	Early OA ($n = 44$), intermediate and late OA ($n = 26$), healthy control ($n = 30$). MMP-1 was significantly higher in intermediate and advanced OA compared to early OA and controls.	38	Burden of disease
MMP-3	Serum	Early OA ($n = 44$), intermediate and late OA ($n = 26$), healthy control ($n = 30$). MMP-3 was significantly higher in intermediate and advanced OA compared to early OA and controls.	38	Burden of disease
MMP13	Serum Synovial fluid	KOA with joint effusion ($n = 34$). No correlation between MMP13 and radiographic or ultrasonographic findings were observed.	23	Burden of disease
MPO		KOA ($n = 22$) treated with Curcumin. No association observed between MPO and Curcumin treatment.	44	Efficacy of intervention
Neuropeptide Y (NPY)	Synovial fluid	KOA ($n = 100$), healthy control ($n = 20$). NPY was correlated with increased pain on the Hideo Watanabe pain score and Tomihisa Koshino pain score.	50	Burden of disease
NTX-I	Urine	Population ($n = 1040$). No association was found between NTX-I levels and the different KL scores.	46	Burden of disease
Osteocalcin	Synovial fluid	Acute knee injury ($n = 98$). No association with osteochondral fracture in the knee.	22	Burden of disease
Osteopontin	Synovial fluid	Acute knee injury ($n = 98$). No association with osteochondral fracture in the knee.	22	Burden of disease
Prostaglandin E2	Synovial fluid	Traumatic OA ($n = 8$). Average cartilage strain at maximal flexion of the knee in patients with meniscal tear was not associated with, Prostaglandin E2.	42	Investigative
Resistin	Serum Synovial fluid	KOA with joint effusion ($n = 34$). SF resistin correlated with effusion.	23	Burden of disease
Resistin	Plasma	Non-radiographic KOA ($n = 47$), RKOA ($n = 50$) Control ($n = 75$). Resistin associated with RKOA severity.	8	Burden of disease
S100A8/A9	Serum	OA ($n = 162$) No correlation with clinical features of OA. Negative correlation with sum score of osteophytes. Small positive association with ESR.	51	Burden of disease
Sclerostin	Plasma, Synovial fluid	KOA ($n = 95$), healthy control ($n = 95$). Inverse correlation between plasma and SF levels of Sclerostin and RKOA.	16	Burden of disease
sCreatinine	Serum	CHECK population ($n = 738$). Change in serum creatinine does not explain the increase observed in uCTX-II at menopause in the CHECK cohort.	52	Investigative
sGAG	Synovial fluid	Traumatic OA ($n = 8$). Average cartilage strain at maximal flexion of the knee in patients with meniscal tear was not associated with sGAG.	42	Investigative
sGAG	Synovial fluid	Acute knee injury ($n = 98$). No association with osteochondral fracture in the knee.	22	Burden of disease
sHA	Serum	RHOA ($n = 638$) Symptoms of hand OA and higher AUSCAN scores were associated with higher levels of sHA. Hand radiographic OA associated with sHA.	21	Burden of disease
SPARC	Synovial fluid	Acute knee injury ($n = 98$). Osteochondral fractures with disrupted cortical bone had increased SPARC compared to non-osteochondral fractures.	22	Burden of disease
TNF-α	Synovial fluid	Acute knee injury ($n = 98$). Increased TNF-α associated with osteochondral fracture in the knee. Osteochondral fractures with disrupted cortical bone additionally had increased SPARC, IL-8, and TNF-α compared to non-osteochondral fractures.	22	Burden of disease
totAPN	Serum	RHOA ($n = 227$). Inversely associated with progression of RHOA. hmwAPN showed no association with progression of RHOA.	47	Prognostic
VEGF	Serum Synovial fluid	KOA with joint effusion ($n = 34$). VEGF correlated with degree of KL score in OA patients and length of medial osteophytes.	23,53	Burden of disease
Proteomic and Multiplex approaches				
Multiplex	Synovial fluid	Unicompartmental KOA ($n = 30$), Bicompartmental KOA ($n = 29$) Increased cytokine levels in bicompartmental OA compared to unicompartmental.	54	Investigative

(*continued on next page*)

Table I (*continued*)

Biomarker	Sample type	Results (short description)	Ref ID	BIPED classification
Multiplex	Plasma Synovial fluid	KOA ($n = 31$), Healthy control ($n = 15$). PECAM-1,HGF, VEGF, angiopoietin-2, follistatin, G-CSF, and IL-8 were increased in OA compared to controls. Plasma angiopoietin differentiated advanced OA from early. SF VEGF was positively correlated with severity. Plasma follistatin was negatively correlated with severity.	7	Burden of disease
Multiplex	Serum	Normal ($n = 100$), OA ($n = 100$), RA ($n = 100$). Systems biology approach to distinguish OA patients from RA and controls.	55	Diagnostic
Mass spectrometry	Synovial fluid	OA ($n = 80$). OA can be divided into distinct metabolic subgroups.	56	Investigative
Mass spectrometry	Plasma Synovial fluid	OA ($n = 69$) Modest correlation between metabolite concentrations in synovial fluid and plasma.	57	Investigative
Mass spectrometry	Serum Synovial fluid	Late stage OA ($n = 13$), Cohort ($n = 253$). Peptides from clusterin, lumican, lubricin were associated with joint space narrowing. Peptides from clusterin and lubricin were as predictive of OA progression as age.	17	Investigative
Mass Spectrometry	Cartilage explants	An iTRAQ-based quantitative proteomic analysis of secretomes from healthy human articular cartilage explants, comparing their protein profile to those from unwounded (early disease) and wounded (advanced disease) zones of osteoarthritic tissue.	58	
Antigen microarray (NAPPA)	Serum	OA ($n = 21$), RA ($n = 20$), control ($n = 21$) Auto-antibodies identified, distinguishing OA, RA and control subjects. Proteomic approach to identify a panel of auto-antibodies for diagnosis and prognosis of OA.	14	Investigative
Protein microarray	Synovial fluid Serum	Long-term TMJ OA ($n = 28$), Early TMJ OA ($n = 12$, Healthy control ($n = 12$). Protein microarray of 50 biomarkers. Synovial fluid levels ANG, GDF15, TIMP-1, CXCL16, MMP-3 and MMP-7 correlated with bone apposition in the tempomandibular joint. Serum levels of ENA-78, MMP-3, PAI-1, VE-Cad-herin, VEGF, GM-CSF, TGFb1, IFNg, TNFa, IL-1a, and IL-6 correlated with bone resorption at this site.	59	Investigative
Affinity chromatography	Synovial fluid	Acute trauma ($n = 19$), Knee pain ($n = 16$), OA ($n = 20$), RA ($n = 20$). Identification of 12 novel COMP fragments from RA, OA or trauma patients.	60	Investigative
IL-6, KC/GRO, IL-8, (MCP-1), (MIP-3a), IL-1b, TNF and L+-lactate	Serum Synovial fluid	Clinical OA ($n = 6$), Healthy controls ($n = 6$). When comparing the cytokine profile between synovial fluid and serum from OA patients only MIP-3α correlated significantly.	61	Investigative

Abbreviations: FCA, Freund's complete adjuvant; FMCP, Fragmented medial coronoid process; Ihh, Indian Hedgehog; IL-1β, Interleukin-1β; KC/GRO; Keratinocyte chemoattractant/human growth-regulated oncogene; MCP-1, monocyte chemoattractant protein 1; MIP-3α, macrophage inflammatory protein 3α; MMP, Matrix metalloproteinase; MPO, Myeloperoxidase; TNF-α, Tumor necrosis factor-α, WT; Wild-type.

large Japanese cohort of 1040 subjects. Here they found that CTX-II correlated with OA severity in the form of KL score in OA patients above 60 years of age.

Another highly investigated marker is cartilage oligomeric matrix protein (COMP), which has been measured in a number of trials within this last year, with varying outcomes; it was shown to correlate with hand OA symptoms and increased AUSCAN scores, but not radiographic hand OA in cross-sectional study of 663 OA patients[21]. In another set of studies looking at traumatic KOA, no association was found between traumatic KOA severity and concentrations of COMP[22]. Finally, Kim and colleagues looked at the correlation between COMP and OA in patients with synovitis, and found no association with effusion[23].

Probably the best tested biomarkers in OA are those reflecting joint tissue turnover such as urinary CTX-II or serum COMP, which both are believed to measure cartilage degradation[24]. One of the reasons why these may be of higher interest for OA is that these often have shown higher sensitivity and analyte stability, whereas cytokines is generally of more acute nature with short half-life[25].

Table II lists the biomarkers from Table I that measured tissue turnover and which have been tested in human OA studies or cohorts within the last 3 decades. Both CTX-II and COMP have been tested in more than 50 different studies, and may be the best validated OA biomarkers to date. However although they have been thoroughly tested the different datasets provide differential and somewhat contradictory results. This may be due to the type of OA patients tested, the design of the studies in respect to the hypothesis tested, and the statistical analyze conducted. This can make it rather difficult to qualify the biomarkers according to specific BIPED categories; however from a general view point these biomarkers can be classified as burden of disease, diagnostic and prognostic biomarkers.

When studying the list it stands out that most biomarkers measure cartilage degradation or bone resorption. What is lacking on the list are tests of cartilage formation/repair and synovial inflammation, which are also believed to play an important role in the pathogenesis of OA and are therefore imperative for a future diagnostic toolbox for OA. Examples of formation/repair biomarkers which have been developed and tested in human OA studies are CS846[26] and PIIANP[27,28]. For these to be validated more clinical anabolic interventional studies with prospective biomarker strategies are needed.

Table II
Number of publications the biomarkers identified in Table I, which reflect joint tissue turnover. *The number of publications on the individual biomarkers is an estimate provided by pivotal search PubMed

Biomarker	Description	References (Max. 5 recent Publications)	Number of publications*
Alpha CTX-I	Cathepsin K degraded newly formed type I collagen	37	1
ARGS (different biomarker assays)	Aggrecanase mediated degradation of aggrecan	22,39,62–64	22
C1M	MMP-mediated degradation of type I collagen	20,65	2
C2C	MMP-mediated degradation of type II collagen	66–70	24
C2M	MMP-mediated degradation of type II collagen	10,20,65,71,72	5
C3M	MMP-mediated degradation of type III collagen	20,65	2
COMP (several different biomarker assays)	Cartilage oligomeric matrix protein turnover/degradation	60,71,73–75	>50
C-Col10	Release of type X collagen from cartilage	10	1
Coll-2-1	Protease-mediated degradation of type II collagen	44,76–79	5
Coll-2-1-NO_2	Protease-mediated degradation of nitrosylated type II collagen	44,76–79	5
CTX-II	Protease-mediated degradation of type II collagen	52,80–83	>50
Fib3-1	Protease-mediated degradation of Fibulin 3	44,84	2
Fib3-2	Protease-mediated degradation of Fibulin 3	44,84	2
NTX-I	Cathepsin K degraded type I collagen	46,68,74,85,86	26
Osteocalcin (several different biomarkers)	Bone formation	87–91	>50

The BIPED criteria

The BIPED classification categorizes biomarkers according to key parameters for evaluation and qualification of the utility of biomarkers. Those are: Burden of disease (B), Investigative (I), Prognostic (P), Efficacy of intervention (E) and Diagnostic (D)[3]. D-biomarkers are those that will enable identification of those patients with OA in the general population or specific phenotype of OA patients (e.g., metabolic vs trauma), whereas B-biomarkers are those which will aid in describing the disease burden or disease severity at a given point in time (e.g., metabolic status, degree of cartilage degradation). The B-biomarker is a snapshot of current status of the disease. Present diagnostic and burden of disease markers are radiographic images, as well as sign and symptoms recorded by the physician and/or patient.

With respect to prognosis or prediction, FDA clearly distinguishes between these P markers. A *prognostic* biomarker is a baseline characteristic that categorizes patients by degree of risk for disease occurrence or progression of a specific aspect of a disease. A prognostic biomarker informs about the natural history of the disorder in that particular patient in the absence of a therapeutic intervention. It can be used as an *enrichment strategy* to select patients likely to have clinical events of interest or to progress rapidly.

A *predictive* biomarker is a baseline characteristic that categorizes patients by their likelihood of response to a particular treatment relative to no treatment. A predictive biomarker can be used as an enrichment strategy to identify a subpopulation likely to respond to a treatment intervention in a particular way. It may predict a favorable response or an unfavorable response (i.e., adverse event)." As such, The "P" (prognostic) biomarker is a marker which predicts future outcomes, such as the risk (odds) for structural progression, or other outcomes or events (e.g., joint inflammation, bone marrow lesion, and joint failure). An example would be that a P biomarker would identify those in most need of aggressive treatment, which then may be eligible for special care or intervention.

An example of a Prognostic biomarker can be found in the field of rheumatoid arthritis (RA), where the biomarker C1M was prognostic of structural progression[29], while a combination of biomarkers were predictive of response to tocilizumab, an anti-interleukin 6 receptor treatment[29,30]. A very important point for a clear differentiation between a prognostic and predictive biomarker is the needed level of qualification of the individual biomarkers.

It is current BIPED description the predictive biomarker is an integrated part of the E category. An "E" biomarker is marker for measuring whether the drug is efficacious and can be used in Phase II dose-finding studies and, optimally, even prior to Phase II, for faster evaluation of potential efficacy. An example would be that an early change in a biomarker will predict whether a drug will be efficacious; that is meeting its clinical endpoint. This is in contrast to the predictive biomarker that will predict who will respond to a given treatment at baseline. In OA, it would be desirable to have available such biomarkers due to the long and vastly populated clinical trials, as it would allow for earlier decision-making.

The predictive E biomarker can used to determine which patients are specially eligible for a given treatment, such as herceptin is approved for the treatment of early-stage breast cancer that is **H**uman **E**pidermal growth factor **R**eceptor **2**-positive (HER2+) and has spread into the lymph nodes, or is HER2+ and has not spread into the lymph nodes. If it has not spread into the lymph nodes, the cancer needs to be estrogen receptor/progesterone receptor (ER/PR)-negative or have one high risk feature. This way, the diagnostic tool is linked to subsequent use of a treatment, in case of a positive test. Hence, a predictive E biomarker would most optimally be used to determine which kind of treatment strategy a patient would benefit from, for example an anti-inflammatory drug (e.g., IL-1 anti-IL1), an anti-resorptive drug[31] (e.g., Zoledronate), or a cartilage anabolic drug (e.g., FGF18)[32].

It is important to note that FDA (and EMA) uses a different nomenclature when defining prediction and prognosis. According to the FDA, the main separating factor is that a prognostic biomarker provides you with an odds for progression, whereas a predictive biomarker would be used to decide the exact treatment regimen for individual patients, and would therefore have a significant impact on the patient's life. A predictive biomarker will often become a companion diagnostic[33]. Please find references to important guidelines and documents at the end of the article.

In 2011, Kraus *et al.* modified the BIPED characterization by adding a "S" for safety[34]. The purpose of the "S" biomarker was ideally to monitor the health status of the joint tissue or general cytotoxic status in response to treatment. One could imagine that a relative ratio between an "E" and "S" biomarker could provide a risk/benefit ratio for a drug in a trial.

Finally, an "I" biomarker is a marker of which not sufficient information is available yet to allow its inclusion in one of the other categories. As no biomarkers have been qualified as biomarkers for OA, this category covers the majority of biomarkers tested in OA.

It is important to test and validate a biomarker to a hypothesis that covers just one of the classifications or test the classification individually. Although we in this paper and other in other papers try to categorize the biomarkers according to the BIPED there is still

some question marks regarding the categorization, as biomarker most cohorts are tested retrospectively and no prospectively for exactly validating a BIPED hypothesis. Also predictiveness toward treatment efficacy still needs to be tested, as there are only trials for DMOARDs and even fewer (if any) testing biomarkers as outcome measure. A good biomarker would only has to be validated for one of the classifications other than "I" to qualify for the clinical usage.

Ongoing initiatives for validating and qualifying biomarkers

The issues in understanding OA patho-physiology and subsequent translational application of *in vitro* diagnostics of such understanding in drug development are large and complex, Independent efforts have led to slow and insufficient clinical progress, as outlined above. Also the use of drug development tools (DDT) is of particular interest, and biomarkers for e.g., progression would be an advantage for patient selection in interventional trials. Some of these challenges can be best addressed and the obstacles be overcome by Public-Private-Partnerships (PPP) of engaged, knowledgeable, and complimentary industrial, academic, patient and governmental experts who can provide innovative and viable solutions. Considering the scale of the problem and societal impact, there remains a major unmet need as current treatments are predominantly restricted to symptomatic relief or costly and invasive surgical intervention.

Multiple reasons have been identified as underlying causes of past clinical failures:

- Limited understanding of OA pathogenesis. Emerging data suggest OA is a heterogeneous disease with a variety of pathophysiologic drivers, some of which are amenable to pharmacologic intervention, and some of which are expected to be less so.
- Variable disease course. The majority of an unselected OA population do not progress radiographically or clinically in a given 2 year window; companies have not had the tools or knowledge base to prospectively identify patients at risk of progression who stand to benefit the most from effective therapies.
- Absence of personalized medicine mindset. Clinical development plans have frequently used a 'one size fits all' approach rather than matching mechanism of action to specific OA patient subpopulations and using specific DDTs.
- Reliance on relatively insensitive endpoints. X-ray-based joint space narrowing (the current standard endpoint to demonstrate disease modification) is insensitive, tends to be slowly evolving and does not allow visualization of the tissue most associated with the disease (cartilage).
- Lack of a qualification strategy for the applied biomarkers and DDTs in a clinical setting and for diagnosis.

In PPP consortia, these points are addressed in close collaboration of different academic and industry partners. The FNIH/OARSI initiative on OA biomarkers and imaging[35] has just presented first comprehensive data on imaging and biochemical markers out of the analysis (http://oarsi.org/sites/default/files/docs/2015/fnih_2015_pre_congress_workshop_final.pdf) and also reference intervals for healthy subjects for the selected biomarkers[36]. A phase 2 of the initiative will further substantiate the findings and characterize and validate biomarkers in different clinical cohorts. In Europe the Innovative Medicine Initiative (IMI) has started to bring further knowledge in the field. In both initiatives several topics will be addressed:

- Consolidated existing complementary OA data sets (public and private)
- Definition and characterization of OA patient subsets, also using bioinformatics
- Identification of novel biomarkers for OA
- Biochemical marker assay platform validation and development
- Validation of biochemical markers and next generation imaging technologies in a cohort of OA patients
- User-friendly interface where data can be publically accessed and analyzed (e.g., OAI)
- Work with regulators to finalize guidance and initiate regulatory biomarker qualification process

Lastly the D-BOARD consortium, an EU funded PPP initiative, is working on identification and validation of novel biomarkers and pushing those forward toward qualification, by applying the BIPED categories for hypothesis testing (http://www.d-board.eu/dboard/index.aspx).

Conclusion

Work is continuing on testing biomarkers in OA and will need to continue in the future, as present data do not reach and answer the medical need in the field. However much of validation and qualification work is halted by the lack designated biomarker studies and successful drug trials. Although interesting biomarkers have been tested in 2014/2015, little clinical validation or qualification studies have been presented. Thus there is still a huge, unmet medical need to identify, test, validate and qualify novel and well-known biomarkers. A pre-requite for this is better characterization and classification of biomarkers to their needs, which may not be reached before understanding of OA phenotypes has been much improved. However this needs to be substantiated and supported in a broader context including a reflection on the guidelines provided by the regulatory authorities, which extent outside of this review. The running and upcoming PPP initiatives are expected to select and push the best biomarkers forward towards qualification.

Documents of interest from the regulators

Following links provide some of the guidelines on qualification and approval routes published by the regulators, which may be of great value for biomarker development for use drug development and personalized medicine and health care.

- Presentation given by Dr. Shashi Amur (OTS, CDER, FDA) at the M-CERSI symposium, C-Path (August 2015) providing encouragement and considerations for biomarker development and qualification. The talk was titled "FDA's efforts to encourage biomarker development and qualification": http://c-path.org/wp-content/uploads/2015/08/EvConsid-Symposium-20150821-I-01-SAmur-FINAL.pdf
- Guidance for qualification of drug development tools (DDT such as biomarkers): http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM230597.pdf
- Report titled "Report on the use of omic technologies in the development of personalized medicines (March 2013): http://ec.europa.eu/health/files/committee/70meeting/pharm616.pdf". The report highlights the potentials and issues in research and development of personalized medicine
- List of letters which include high-level summary of the novel methodology, context of use, available data, and on-going and future investigations: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000319.jsp&mid=WC0b01ac0580022bb0

- Guidance for industry on how to technically validated biomarkers (May 2001). http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf
- A document titled: "Paving the way for personalized medicine: FDA's role in a new Era of medical product development". Descripting the rational for personalized medicine and providing examples of the biomarkers in use (October 2013): http://www.fda.gov/downloads/scienceresearch/specialtopics/personalizedmedicine/ucm372421.pdf)

Contribution

ACBJ and CST were the main drivers and authors of the manuscript. DRE and CFKP made the first round of database search and review of those findings. CL and YH provided specific paragraphs and expert review of the paper. MK and AM reviewed and corrected the last version of the manuscript.

Conflict of interest

ACBJ, MAK and CST are full-time employees of Nordic Bioscience. MAK and ACBJ hold stock in Nordic Bioscience. CFKP and DRE are PhD students partly financed by the Danish research foundation and by the D-BOARD consortium. CL is a full-time employee of Merck KGaA. YH is founder of Artialis SA. AM declare no conflict of interest.

Acknowledgments

We acknowledge funding through the D-BOARD Consortium funded by European Commission Framework 7 programme (EU FP7; HEALTH.2012.2.4.5-2, project number 305815, Novel Diagnostics and Biomarkers for Early Identification of Chronic Inflammatory Joint Diseases). AM is a member of the Arthritis Research UK Centre for Sport, Exercise, and Osteoarthritis, funded by Arthritis Research UK (Grant Reference: 20194). AM has also received funding from the Deanship of Scientific Research (DSR), King AbdulAziz University of Saudi Arabia (grant no. 1-141/1434 HiCi).

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2015.10.014.

References

1. Karsdal MA, Christiansen C, Ladel C, Henriksen K, Kraus VB, Bay-Jensen AC. Osteoarthritis – a case for personalized health care? Osteoarthritis Cartilage 2014;22:7–16.
2. Karsdal MA, Henriksen K, Leeming DJ, Mitchell P, Duffin K, Barascuk N, *et al.* Biochemical markers and the FDA critical path: how biomarkers may contribute to the understanding of pathophysiology and provide unique and necessary tools for drug development. Biomarkers 2009;14:181–202.
3. Bauer DC, Hunter DJ, Abramson SB, Attur M, Corr M, Felson D, *et al.* Classification of osteoarthritis biomarkers: a proposed approach. Osteoarthritis Cartilage 2006;14:723–7.
4. Daghestani HN, Pieper CF, Kraus VB. Soluble macrophage biomarkers indicate inflammatory phenotypes in patients with knee osteoarthritis. Arthritis Rheumatol (Hoboken, NJ) 2015;67:956–65.
5. Huo LW, Ye YL, Wang GW, Ye YG. Fractalkine (CX3CL1): a biomarker reflecting symptomatic severity in patients with knee osteoarthritis. J Investig Med 2015;63:626–31.
6. Gao F, Tian J, Pan H, Gao J, Yao M. Association of CCL13 levels in serum and synovial fluid with the radiographic severity of knee osteoarthritis. J Investig Med 2015;63:545–7.
7. Mabey T, Honsawek S, Saetan N, Poovorawan Y, Tanavalee A, Yuktanandana P. Angiogenic cytokine expression profiles in plasma and synovial fluid of primary knee osteoarthritis. Int Orthop 2014;38:1885–92.
8. Zhao XY, Yang ZB, Zhang ZJ, Zhang ZQ, Kang Y, Huang GX, *et al.* CCL3 serves as a potential plasma biomarker in knee degeneration (osteoarthritis). Osteoarthritis Cartilage 2015;23(8):1405–11, http://dx.doi.org/10.1016/j.joca.2015.04.002.
9. Peffers MJ, Cillero-Pastor B, Eijkel GB, Clegg PD, Heeren RMA. Matrix assisted laser desorption ionization mass spectrometry imaging identifies markers of ageing and osteoarthritic cartilage. Arthritis Res Ther 2014;16:R110.
10. He Y, Siebuhr AS, Brandt-Hansen NU, Wang J, Su D, Zheng Q, *et al.* Type X collagen levels are elevated in serum from human osteoarthritis patients and associated with biomarkers of cartilage degradation and inflammation. BMC Musculoskelet Disord 2014;15:309.
11. Abrams GD, Safran MR, Shapiro LM, Maloney WJ, Goodman SB, Huddleston JI, *et al.* Fibronectin-aggrecan complex as a marker for cartilage degradation in non-arthritic hips. Knee Surg Sports Traumatol Arthrosc 2014;22:768–73.
12. Chu H, Xu ZM, Yu H, Zhu KJ, Huang H. Association between hypoxia-inducible factor-1a levels in serum and synovial fluid with the radiographic severity of knee osteoarthritis. Genet Mol Res 2014;13:10529–36.
13. Dong T, Chang H, Zhang F, Chen W, Zhu Y, Wu T, *et al.* Calcitonin gene-related peptide can be selected as a predictive biomarker on progression and prognosis of knee osteoarthritis. Int Orthop 2015;39:1237–43.
14. Henjes F, Lourido L, Ruiz-Romero C, Fernández-Tajes J, Schwenk JM, Gonzalez-Gonzalez M, *et al.* Analysis of autoantibody profiles in osteoarthritis using comprehensive protein array concepts. J Proteome Res 2014;13:5218–29.
15. Li ZC, Xiao J, Wang G, Li MQ, Hu KZ, Ma T, *et al.* Fibroblast growth factor-21 concentration in serum and synovial fluid is associated with radiographic bone loss of knee osteoarthritis. Scand J Clin Lab Invest 2015;75:121–5.
16. Mabey T, Honsawek S, Tanavalee A, Wilairatana V, Yuktanandana P, Saetan N, *et al.* Plasma and synovial fluid sclerostin are inversely associated with radiographic severity of knee osteoarthritis. Clin Biochem 2014;47:,547–551.
17. Ritter SY, Collins J, Krastins B, Sarracino D, Lopez M, Losina E, *et al.* Mass spectrometry assays of plasma biomarkers to predict radiographic progression of knee osteoarthritis. Arthritis Res Ther 2014;16:456.
18. Zhang C, Wei X, Chen C, Cao K, Li Y, Jiao Q, *et al.* Indian hedgehog in synovial fluid is a novel marker for early cartilage lesions in human knee joint. Int J Mol Sci 2014;15:7250–65.
19. Simão AP, Mendonça VA, de Oliveira Almeida TM, Santos SA, Gomes WF, Coimbra CC, *et al.* Involvement of BDNF in knee osteoarthritis: the relationship with inflammation and clinical parameters. Rheumatol Int 2014;34:1153–7.
20. Arendt-Nielsen L, Eskehave TN, Egsgaard LL, Petersen KK, Graven-Nielsen T, Hoeck HC, *et al.* Association between experimental pain biomarkers and serologic markers in patients with different degrees of painful knee osteoarthritis. Arthritis Rheumatol (Hoboken, NJ) 2014;66:3317–26.
21. Aslam I, Perjar I, Shi XA, Renner JB, Kraus VB, Golightly YM, *et al.* Associations between biomarkers of joint metabolism,

hand osteoarthritis, and hand pain and function: the Johnston County Osteoarthritis Project. J Rheumatol 2014;41:938–44.
22. Sward P, Struglics A, Englund M, Roos HP, Frobell RB. Soft tissue knee injury with concomitant osteochondral fracture is associated with higher degree of acute joint inflammation. Am J Sports Med 2014;42:1096–102.
23. Kim H-R, Lee J-H, Kim K-W, Kim B-M, Lee S-H. The relationship between synovial fluid VEGF and serum leptin with ultrasonographic findings in knee osteoarthritis. Int J Rheum Dis 2014, http://dx.doi.org/10.1111/1756-185X.12486.
24. Qvist P, Bay-Jensen AC, Christiansen C, Sondergaard BC, Karsdal MA. Molecular serum and urine marker repertoire supporting clinical research on joint diseases. Best Pract Res Clin Rheumatol 2011;25:859–72.
25. Karsdal MA, Henriksen K, Leeming DJ, Woodworth T, Vassiliadis E, Bay-Jensen AC, *et al*. Novel combinations of Post-Translational Modification (PTM) neo-epitopes provide tissue-specific biochemical markers-are they the cause or the consequence of the disease? Clin Biochem 2010;43:793–804.
26. Mazzuca SA, Poole AR, Brandt KD, Katz BP, Lane KA, Lobanok T. Associations between joint space narrowing and molecular markers of collagen and proteoglycan turnover in patients with knee osteoarthritis. J Rheumatol 2006;33:1147–51.
27. Sharif M, Kirwan J, Charni N, Sandell LJ, Whittles C, Garnero P, *et al*. A 5-yr longitudinal study of type IIA collagen synthesis and total type II collagen degradation in patients with knee osteoarthritis –association with disease progression. Rheumatology 2007;46:938–43.
28. Bay-Jensen AC, Andersen TL, Charni-Ben Tabassi N, Kristensen PW, Kjaersgaard-Andersen P, Sandell L, *et al*. Biochemical markers of type II collagen breakdown and synthesis are positioned at specific sites in human osteoarthritic knee cartilage. Osteoarthritis Cartilage 2008;16:615–23.
29. Siebuhr AS, Bay-Jensen AC, Leeming DJ, Plat A, Byrjalsen I, Christiansen C, *et al*. Serological identification of fast progressors of structural damage with rheumatoid arthritis. Arthritis Res Ther 2013;15:R86.
30. Bay-Jensen AC, Platt A, Byrjalsen I, Vergnoud P, Christiansen C, Karsdal MA. Effect of tocilizumab combined with methotrexate on circulating biomarkers of synovium, cartilage, and bone in the LITHE study. Semin Arthritis Rheum 2014;43:470–8.
31. Karsdal MA, Bay-Jensen AC, Lories RJ, Abramson S, Spector T, Pastoureau P, *et al*. The coupling of bone and cartilage turnover in osteoarthritis: opportunities for bone antiresorptives and anabolics as potential treatments? Ann Rheum Dis 2014;73:336–48.
32. Lohmander LS, Hellot S, Dreher D, Krantz EF, Kruger DS, Guermazi A, *et al*. Intraarticular sprifermin (recombinant human fibroblast growth factor 18) in knee osteoarthritis: a randomized, double-blind, placebo-controlled trial. Arthritis Rheumatol (Hoboken, NJ) 2014;66:1820–31.
33. FDA. *In Vitro* Companion Diagnostic Devices. Guidance for Industry and Food and Drug Administration Staff 20141–13.
34. Kraus VB, Burnett B, Coindreau J, Cottrell S, Eyre D, Gendreau M, *et al*. Application of biomarkers in the development of drugs intended for the treatment of osteoarthritis. Osteoarthritis Cartilage 2011;19:515–42.
35. Hunter DJ. Osteoarthritis. Best Pract Res Clin Rheumatol 2011;25:801–14.
36. Kraus VB. Establishment of reference intervals for osteoarthritis related biomarkers – the FNIH/OARSI OA biomarkers consortium. Osteoarthritis Cartilage 2014;22:S46, http://dx.doi.org/10.1016/j.joca.2014.02.103.
37. Huebner JL, Bay-Jensen AC, Huffman KM, He Y, Leeming DJ, McDaniel GE, *et al*. Alpha C-telopeptide of type I collagen is associated with subchondral bone turnover and predicts progression of joint space narrowing and osteophytes in osteoarthritis. Arthritis Rheumatol (Hoboken, NJ) 2014;66:2440–9.
38. Li W, Du C, Wang H, Zhang C. Increased serum ADAMTS-4 in knee osteoarthritis: a potential indicator for the diagnosis of osteoarthritis in early stages. Genet Mol Res 2014;13:9642–9.
39. Germaschewski FM, Matheny CJ, Larkin J, Liu F, Thomas LR, Saunders JS, *et al*. Quantitation OF ARGS aggrecan fragments in synovial fluid, serum and urine from osteoarthritis patients. Osteoarthritis Cartilage 2014;22:690–7.
40. Mabey T, Taleongpong P, Udomsinprasert W, Jirathanathornnukul N, Honsawek S. Plasma and synovial fluid autotaxin correlate with severity in knee osteoarthritis. Clin Chim Acta 2015;444:72–7.
41. Kumahashi N, Swärd P, Larsson S, Lohmander LS, Frobell R, Struglics A, *et al*. Type II collagen C2C epitope in human synovial fluid and serum after knee injury – associations with molecular and structural markers of injury. Osteoarthritis Cartilage 2015;23(9):1506–12, http://dx.doi.org/10.1016/j.joca.2015.04.022.
42. Carter TE, Taylor KA, Spritzer CE, Utturkar GM, Taylor DC, Moorman 3rd CT, *et al*. *In vivo* cartilage strain increases following medial meniscal tear and correlates with synovial fluid matrix metalloproteinase activity. J Biomech 2015;48:1461–8.
43. Li L, Jiang B-E. Serum and synovial fluid chemokine ligand 2/monocyte chemoattractant protein 1 concentrations correlates with symptomatic severity in patients with knee osteoarthritis. Ann Clin Biochem 2015;52:276–82.
44. Henrotin Y, Gharbi M, Dierckxsens Y, Priem F, Marty M, Seidel L, *et al*. Decrease of a specific biomarker of collagen degradation in osteoarthritis, Coll2–1, by treatment with highly bioavailable curcumin during an exploratory clinical trial. BMC Complement Altern Med 2014;14:159.
45. Landsmeer ML, Runhaar J, Henrotin YE, Middelkoop van M, Oei EH, Vroegindeweij D, *et al*. Association of urinary biomarker COLL2-1NO with incident clinical and radiographic knee OA in overweight and obese women. Osteoarthritis Cartilage 2015, http://dx.doi.org/10.1016/j.joca.2015.04.011.
46. Tanishi N, Yamagiwa H, Hayami T, Mera H, Koga Y, Omori G, *et al*. Usefulness of urinary CTX-II and NTX-I in evaluating radiological knee osteoarthritis: the Matsudai knee osteoarthritis survey. J Orthop Sci 2014;19:429–36.
47. Klein-Wieringa IR, Andersen SN, Herb-van Toorn L, Kwekkeboom JC, van der Helm-van Mil AH, Meulenbelt I, *et al*. Are baseline high molecular weight adiponectin levels associated with radiographic progression in rheumatoid arthritis and osteoarthritis? J Rheumatol 2014;41:853–7.
48. Shirinsky IV, Shirinsky VS. Treatment of erosive osteoarthritis with peroxisome proliferator-activated receptor alpha agonist fenofibrate: a pilot study. Rheumatol Int 2014;34:613–6.
49. Fowler-Brown A, Kim DH, Shi L, Marcantonio E, Wee CC, Shmerling RH, *et al*. The mediating effect of leptin on the relationship between body weight and knee osteoarthritis in older adults. Arthritis Rheumatol (Hoboken, NJ) 2015;67:169–75.
50. Wang L, Zhang L, Pan H, Peng S, Lv M, Lu WW, *et al*. Levels of neuropeptide Y in synovial fluid relate to pain in patients with knee osteoarthritis. BMC Musculoskelet Disord 2014;15:319.
51. Mahler EA, Zweers MC, van Lent PL, Blom AB, van den Hoogen FH, van den Berg WB, *et al*. Association between serum levels of the proinflammatory protein S100A8/A9 and clinical and structural characteristics of patients with established knee, hip, and hand osteoarthritis. Scand J Rheumatol 2015;44:56–60.
52. de Klerk B, Lafeber FPJG, van Spil WE. Associations of CTX-II with biochemical markers of bone turnover raise questions

about its tissue origin: new insights from CHECK. Ann Rheum Dis 2014;73:e39.
53. Saetan N, Honsawek S, Tanavalee A, Yuktanandana P, Meknavin S, Ngarmukos S, *et al*. Relationship of plasma and synovial fluid vascular endothelial growth factor with radiographic severity in primary knee osteoarthritis. Int Orthop 2014;38:1099–104.
54. Moradi B, Rosshirt N, Tripel E, Kirsch J, Barié A, Zeifang F, *et al*. Unicompartmental and bicompartmental knee osteoarthritis show different patterns of mononuclear cell infiltration and cytokine release in the affected joints. Clin Exp Immunol 2015;180:143–54.
55. Heard BJ, Rosvold JM, Fritzler MJ, El-Gabalawy H, Wiley JP, Krawetz RJ. A computational method to differentiate normal individuals, osteoarthritis and rheumatoid arthritis patients using serum biomarkers. J R Soc Interface 2014;11:20140428.
56. Zhang W, Likhodii S, Zhang Y, Aref-Eshghi E, Harper PE, Randell E, *et al*. Classification of osteoarthritis phenotypes by metabolomics analysis. BMJ Open 2014;4:e006286.
57. Zhang W, Likhodii S, Aref-Eshghi E, Zhang Y, Harper PE, Randell E, *et al*. Relationship between blood plasma and synovial fluid metabolite concentrations in patients with osteoarthritis. J Rheumatol 2015;42:859–65.
58. Lourido L, Calamia V, Mateos J, Fernández-Puente P, Fernández-Tajes J, Blanco FJ, *et al*. Quantitative proteomic profiling of human articular cartilage degradation in osteoarthritis. J Proteome Res 2014;13:6096–106.
59. Cevidanes LH, Walker D, Schilling J, Sugai J, Giannobile W, Paniagua B, *et al*. 3D osteoarthritic changes in TMJ condylar morphology correlates with specific systemic and local biomarkers of disease. Osteoarthritis Cartilage 2014;22:1657–67.
60. Åhrman E, Lorenzo P, Holmgren K, Grodzinsky AJ, Dahlberg LE, Saxne T, *et al*. Novel cartilage oligomeric matrix protein (COMP) neoepitopes identified in synovial fluids from patients with joint diseases using affinity chromatography and mass spectrometry. J Biol Chem 2014;289:20908–16.
61. Finn A, Angeby Möller K, Gustafsson C, Abdelmoaty S, Nordahl G, Ferm M, *et al*. Influence of model and matrix on cytokine profile in rat and human. Rheumatology (Oxford) 2014;53:2297–305.
62. Chen P, Zhu S, Wang Y, Mu Q, Wu Y, Xia Q, *et al*. The amelioration of cartilage degeneration by ADAMTS-5 inhibitor delivered in a hyaluronic acid hydrogel. Biomaterials 2014;35:2827–36.
63. Misko TP, Radabaugh MR, Highkin M, Abrams M, Friese O, Gallavan R, *et al*. Characterization of nitrotyrosine as a biomarker for arthritis and joint injury. Osteoarthritis Cartilage 2013;21:151–6.
64. Larsson S, Lohmander LS, Struglics A. An ARGS-aggrecan assay for analysis in blood and synovial fluid. Osteoarthritis Cartilage 2014;22:242–9.
65. Siebuhr AS, Petersen KK, Arendt-Nielsen L, Egsgaard LL, Eskehave T, Christiansen C, *et al*. Identification and characterisation of osteoarthritis patients with inflammation derived tissue turnover. Osteoarthritis Cartilage 2014;22:44–50.
66. Svoboda SJ, Harvey TM, Owens BD, Brechue WF, Tarwater PM, Cameron KL. Changes in serum biomarkers of cartilage turnover after anterior cruciate ligament injury. Am J Sports Med 2013;41:2108–16.
67. Yoshida H, Kojima T, Kurokouchi K, Takahashi S, Hanamura H, Kojima M, *et al*. Relationship between pre-radiographic cartilage damage following anterior cruciate ligament injury and biomarkers of cartilage turnover in clinical practice: a cross-sectional observational study. Osteoarthritis Cartilage 2013;21:831–8.
68. Goode AP, Marshall SW, Kraus VB, Renner JB, Stürmer T, Carey TS, *et al*. Association between serum and urine biomarkers and lumbar spine individual radiographic features: the Johnston County Osteoarthritis Project. Osteoarthritis Cartilage 2012;20:1286–93.
69. Dejica VM, Mort JS, Laverty S, Antoniou J, Zukor DJ, Tanzer M, *et al*. Increased type II collagen cleavage by cathepsin K and collagenase activities with aging and osteoarthritis in human articular cartilage. Arthritis Res Ther 2012;14:R113.
70. Conrozier T, Balblanc JC, Richette P, Mulleman D, Maillet B, *et al*. Early effect of hyaluronic acid intra-articular injections on serum and urine biomarkers in patients with knee osteoarthritis: an open-label observational prospective study. J Orthop Res 2012;30:679–85.
71. Valdes AM, Meulenbelt I, Chassaing E, Arden NK, Bierma-Zeinstra S, Hart D, *et al*. Large scale meta-analysis of urinary C-terminal telopeptide, serum cartilage oligomeric protein and matrix metalloprotease degraded type II collagen and their role in prevalence, incidence and progression of osteoarthritis. Osteoarthritis Cartilage 2014;22:683–9.
72. Bay-Jensen AC, Liu Q, Byrjalsen I, Li Y, Wang J, Pedersen C, *et al*. Enzyme-linked immunosorbent assay (ELISAs) for metalloproteinase derived type II collagen neoepitope, CIIM-Increased serum CIIM in subjects with severe radiographic osteoarthritis. Clin Biochem 2011;44:423–9.
73. Vos LM, Kuijer R, Huddleston Slater JJR, Stegenga B. Alteration of cartilage degeneration and inflammation markers in temporomandibular joint osteoarthritis occurs proportionally. J Oral Maxillofac Surg 2013;71:1659–64.
74. Blumenfeld O, Williams FM, Hart DJ, Spector TD, Arden N, Livshits G. Association between cartilage and bone biomarkers and incidence of radiographic knee osteoarthritis (RKOA) in UK females: a prospective study. Osteoarthritis Cartilage 2013;21:923–9.
75. Bellucci F, Meini S, Cucchi P, Catalani C, Nizzardo A, ARiva. Early decrease of serum biomarkers of type II collagen degradation (Coll2-1) and joint inflammation (Coll2-1 NO_2) by hyaluronic acid intra-articular injections in patients with knee osteoarthritis: a research study part of the Biovisco study. J Orthop Res 2013;31:901–7.
77. Fernández-Moreno M, Soto-Hermida A, Oreiro N, Pértega S, Fenández-López C, Rego-Pérez I, *et al*. Mitochondrial haplogroups define two phenotypes of osteoarthritis. Front Physiol 2012;3:129.
78. Punzi L. Coll2-1, Coll2-1NO2 and myeloperoxidase serum levels in erosive and non-erosive osteoarthritis of the hands. Osteoarthritis Cartilage 2012;20:557–61.
79. Rego-Pérez I, Fernández-Moreno M, Deberg M, Pértega S, Fenández-López C, Oreiro N, *et al*. Mitochondrial DNA haplogroups modulate the serum levels of biomarkers in patients with osteoarthritis. Ann Rheum Dis 2010;69:910–7.
80. Sowa GA, Perera S, Bechara B, Agarwal V, Boardman J, Huang W, *et al*. Associations between serum biomarkers and pain and pain-related function in older adults with low back pain: a pilot study. J Am Geriatr Soc 2014;62:2047–55.
81. Wang B, Pramono HK, Cicuttini FM, Hanna F, Davis SR, Bell RJ, *et al*. Association between urinary C-telopeptide fragments of type II collagen and knee structure in middle-aged women without clinical knee disease. Osteoarthritis Cartilage 2014;22:1136–41.
82. Røtterud J, Reinholt FP, Beckstrøm K, Risberg M, Årøen A. Relationship between CTX-II and patient characteristics, patient-reported outcome, muscle strength, and rehabilitation in patients with a focal cartilage lesion of the knee: a prospective exploratory cohort study of 48 patients. BMC Musculoskelet Disord 2014;15:99.

83. Kwoh CK, Roemer FW, Hannon MJ, Moore CE, Jakicic JM, Guermazi A, *et al*. Effect of oral glucosamine on joint structure in individuals with chronic knee pain: a randomized, placebo-controlled clinical trial. Arthritis Rheumatol (Hoboken, NJ) 2014;66:930–9.
84. Henrotin Y, *et al*. Fibulin 3 peptides Fib3-1 and Fib3-2 are potential biomarkers of osteoarthritis. Arthritis Rheum 2012;64:2260–7.
85. Nishii T, Tamura S, Shiomi T, Yoshikawa H, Sugano N. Alendronate treatment for hip osteoarthritis: prospective randomized 2-year trial. Clin Rheumatol 2013;32:1759–66.
86. He T, Wu W, Huang Y, Zhang X, Tang T, Dai K. Multiple biomarkers analysis for the early detection of prosthetic aseptic loosening of hip arthroplasty. Int Orthop 2013;37:1025–31.
87. Prentice JR, Clark MJ, Hoggard N, Morton AC, Tooth C, Paley MN, *et al*. Metal-on-Metal hip Prostheses and systemic health: a cross-sectional association study 8 Years after Implantation. PLoS One 2013;8.
88. Kumm J, Tamm A, Lintrop M, Tamm A. Diagnostic and prognostic value of bone biomarkers in progressive knee osteoarthritis: a 6-year follow-up study in middle-aged subjects. Osteoarthritis Cartilage 2013;21:815–22.
89. Catterall JB, Stabler TV, Flannery CR, Kraus VB. Changes in serum and synovial fluid biomarkers after acute injury (NCT00332254). Arthritis Res Ther 2010;12:R229.
90. Kenanidis EI, Potoupnis ME, Papavasillioul KA, Sayegh FE, Petsatodis GE, Kapetanos GA. Serum levels of bone turnover markers following total joint arthroplasty. J Orthop Surg (Hong Kong) 2010;18:290–5.
91. Ichchou L, Allali F, Rostom S, Bennani L, Hmamouchi I, Abourazzak FZ, *et al*. Relationship between spine osteoarthritis, bone mineral density and bone turn over markers in post menopausal women. BMC Womens Health 2010;10:25.
92. Hurlbeck C, Einspanier R, Pfeil I, Bondzio A. Evaluation of biomarkers for osteoarthritis caused by fragmented medial coronoid process in dogs. Res Vet Sci 2014;96:429–35.
93. Mickiewicz B, Heard BJ, Chau JK, Chung M, Hart DA, Shrive NG, *et al*. Metabolic profiling of synovial fluid in a unilateral ovine model of anterior cruciate ligament reconstruction of the knee suggests biomarkers for early osteoarthritis. J Orthop Res 2015;33:71–7.
94. Giunta S, Castorina A, Marzagalli R, Szychlinska MA, Pichler K, Mobasheri A, *et al*. Ameliorative effects of PACAP against cartilage degeneration. Morphological, immunohistochemical and biochemical evidence from *in vivo* and *in vitro* models of rat osteoarthritis. Int J Mol Sci 2015;16:5922–44.
95. Cai A, Hutchison E, Hudson J, Kawashima Y, Komori N, Singh A, *et al*. Metabolic enrichment of omega-3 polyunsaturated fatty acids does not reduce the onset of idiopathic knee osteoarthritis in mice. Osteoarthritis Cartilage 2014;22:1301–9.
96. Dymock DC, Brown MP, Merritt KA, Trumble TN. Concentrations of stromal cell-derived factor-1 in serum, plasma, and synovial fluid of horses with osteochondral injury. Am J Vet Res 2014;75:722–30.