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Review

Osteoarthritis Year in Review 2016: biomarkers (biochemical markers)

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SUMMARY

Purpose: The aim of this "Year in Review" article is to summarize and discuss the implications of biochemical marker related articles published between the Osteoarthritis Research Society International (OARSI) 2015 Congress in Seattle and the OARSI 2016 Congress in Amsterdam.
Methods: The PubMed/MEDLINE bibliographic database was searched using the combined keywords: 'biomarker' and 'osteoarthritis'. The PubMed/MEDLINE literature search was conducted using the Advanced Search Builder function (http://www.ncbi.nlm.nih.gov/pubmed/advanced).
Results: Over two hundred new biomarker-related papers were published during the literature search period. Some papers identified new biomarkers whereas others explored the biological properties and clinical utility of existing markers. There were specific references to several adipocytokines including leptin and adiponectin. ADAM Metallopeptidase with Thrombospondin Type 1 motif 4 (ADAMTS-4) and aggrecan ARGS neo-epitope fragment (ARGS) in synovial fluid (SF) and plasma chemokine (CeC motif) ligand 3 (CCL3) were reported as potential new knee biomarkers. New and refined proteomic technologies and novel assays including a fluoro-microbead guiding chip (FMGC) for measuring C-telopeptide of type II collagen (CTX-II) in serum and urine and a novel magnetic nanoparticle-based technology (termed magnetic capture) for collecting and concentrating CTX-II, were described this past year.
Conclusion: There has been steady progress in osteoarthritis (OA) biomarker research in 2016. Several novel biomarkers were identified and new technologies have been developed for measuring existing biomarkers. However, there has been no "quantum leap" this past year and identification of novel early OA biomarkers remains challenging. During the past year, OARSI published a set of recommendations for the use of soluble biomarkers in clinical trials, which is a major step forward in the clinical use of OA biomarkers and bodes well for future OA biomarker development.

Introduction

Osteoarthritis (OA) is a low-grade inflammatory disease of synovial joints and the most common form of arthritis[1]. It is a leading cause of chronic pain and physical disability in older individuals[2]. OA is one of the most costly and disabling forms of joint disease, being far more common than rheumatoid arthritis (RA) and other forms of joint disease[3]. It is characterized by progressive deterioration and loss of articular cartilage[4] with concomitant structural and functional changes in the entire joint, including the synovium, meniscus (in the knee), periarticular ligaments, and subchondral bone[5]. Cohort studies have

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[b] http://www.d-board.eu/dboard/index.aspx.
[c] http://www.approachproject.eu.

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Abbreviations

ADAMTS-4	ADAM Metallopeptidase with Thrombospondin Type 1 motif 4
ARGS	aggrecan ARGS neo-epitope fragment
AUC	area under the curve
C3M	MMP-degraded type III collagen
CCL3	chemokine (C–C motif) ligand 3
CHECK	Cohort Hip and Cohort Knee
Col2-1 NO2	nitrated type II collagen degradation fragment
COMP	cartilage oligomeric matrix protein
COX-2	cyclooxygenase-2
CRP	C-reactive protein
CRPM	MMP-degraded C-reactive protein
CTX-II	C-telopeptide of type II collagen
DMOADs	disease-modifying osteoarthritis drugs
FMGC	fluoro-microbead guiding chip
FNIH	Foundation for the National Institutes of Health
HA	hyaluronan, hyaluronic acid
hsCRP	high-sensitivity C-reactive protein
IL-1β	interleukin-1β
IL-1Ra	interleukin-1 receptor antagonist
IL-4	interleukin-4
IL-6	interleukin-6
IL-36α	interleukin-36α
JSW	joint space width
LC–MS	liquid chromatography–mass spectrometry
MIA	monoiodoacetate
MMPs	matrix metalloproteinases
MMP-1	matrix metalloproteinase 1
MMP-3	matrix metalloproteinase 3
MPO	myeloperoxidase
MRI	magnetic resonance imaging
MS	mass spectrometry
MSD	musculoskeletal disease
NF-κB	nuclear factor κB
NIH	National Institutes of Health
NOS-2	nitric oxide synthase 2
NTX-I	N-terminal telopeptide of type I collagen
OA	osteoarthritis
OAI	Osteoarthritis Initiative
OARSI	Osteoarthritis Research Society International
PIIANP	N-terminal propeptide of collagen IIA
RA	rheumatoid arthritis
s	serum
S100-A6	S100 Calcium Binding Protein A6
SF	synovial fluid
SME	synovial membrane explant
TNF-α	tumor necrosis factor-α
u	urinary
ucMGP	uncarboxylated matrix Gla-protein
VAS	visual analogue scale
VDAC	voltage-dependent anion-selective channel
WAT	white adipose tissue
WOMAC	Western Ontario and McMaster Universities Arthritis Index

demonstrated that age, obesity and metabolic disease are major risk factors for the development of OA[6,7].

Although OA has been viewed as a "wear and tear" disease for many decades, it is now generally accepted to be an inflammatory and biomechanical whole-organ disease[1,8–10] associated with systemic co-morbidities and death[11]. The pathogenesis and progression of OA is influenced by a number of factors including bone shape and joint dysplasia[12], obesity[13], synovitis[14–16], complement proteins[17], inflammatory mediators[1,18], inflammaging[19,20], innate immunity[21], low-grade inflammation[8] induced by metabolic syndrome[1,22] and diabetes mellitus[23].

The Osteoarthritis Research Society International (OARSI)[d] has recently endorsed a new definition of OA and launched an initiative to critically evaluate and standardize pre-existing definitions of OA. "*Osteoarthritis is a disorder involving movable joints characterized by cell stress and extracellular matrix degradation initiated by micro- and macro-injury that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity. The disease manifests first as a molecular derangement (abnormal joint tissue metabolism) followed by anatomic, and/or physiologic derangements (characterized by cartilage degradation, bone remodeling, osteophyte formation, joint inflammation and loss of normal joint function), that can culminate in illness*"[24] (Fig. 1). OARSI anticipates that this updated definition of the disease process will be subject to further critique and refinement as new scientific and clinical information emerges and our knowledge of OA pathogenesis and progression expands.

It is clear that OA is a heterogeneous disease with a variety of pathophysiologic drivers leading to multiple phenotypes, many of which may overlap in patients[25] (Fig. 2). Each OA phenotype may potentially be treated and targeted differently, paving the way for the development of stratified medicines for OA[26]. Some of these phenotypes will be amenable to pharmacologic intervention but others are less likely to respond to drugs[27]. The key aim of OARSI and the OA research community is to define and characterize OA phenotypes, develop novel, specific and sensitive disease endpoints, improve the design of OA clinical trials, identify patients that respond to treatment and thus alleviate roadblocks to development and clinical evaluation of disease-modifying osteoarthritis drugs (DMOADs)[28–30].

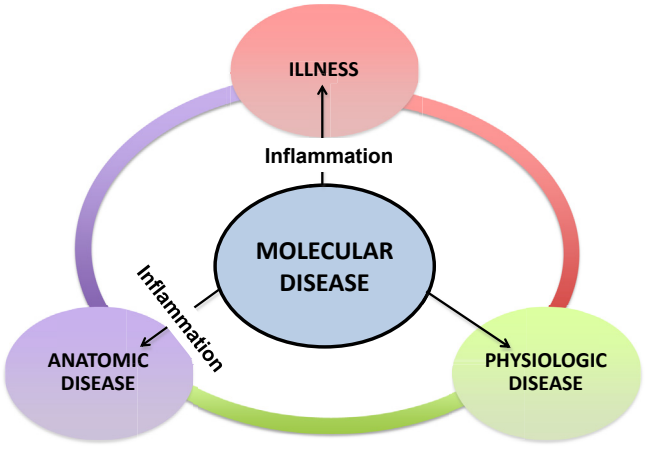

Fig. 1. New definition of OA, as endorsed by OARSI. This figure highlights the relationships between the disease and illness components of OA. The molecular component represents the silent and asymptomatic early stage of OA. Molecular changes precede the anatomic and physiologic aspects by years or even decades. This creates a challenge for detecting the disease early and an opportunity for finding new early marker of disease. Figure adapted from[24] with the kind permission of Dr Virginia Kraus.

[d] https://www.oarsi.org.

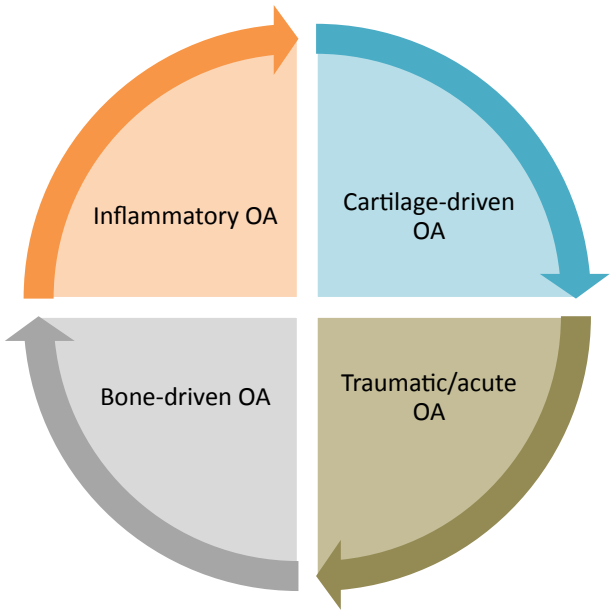

Fig. 2. OA phenotypes. OA is a heterogeneous disease with a variety of pathophysiologic drivers leading to multiple phenotypes, many of which may overlap in patients. Each phenotype may be treated and targeted differently, paving the way for patient "stratification" and the development of "precision" medicines for OA. Some of these will be amenable to pharmacologic intervention but others may be less likely to respond to drugs.

The major challenge faced by OA researchers is early disease identification and selection of fast progressors for treatment[31]. Articular cartilage loss or damage in OA is detected by radiography and measuring decreases in joint space width (JSW) on the radiograph, the so-called "gold standard". In clinical practice, OA is diagnosed radiographically when clinical signs of pain and loss of mobility have already appeared. However, by this stage the joint is actively responding to the injury. Studies have shown that radiographic changes over time are small, and occur in only a subset of patients (progressors), but quantitative JSW measurements have been shown to correlate with 4-year clinical outcomes[32], findings that suggest early treatment with DMOADs are necessary to influence outcomes.

The majority of patients enrolled in OA clinical studies do not progress radiographically (or clinically) during trials that typically last 2–3 years (even changes that may occur over 5 years can be difficult to quantify). Although magnetic resonance imaging (MRI) is increasingly used to evaluate disease progression[33], radiography still remains the required tool for OA diagnosis and efficacy assessment in OA clinical trials. Therefore, we need to develop sensitive and predictive biochemical marker tests and assays to optimize these trials (e.g., patient stratification). Moreover, such biomarkers could ultimately be an alternative for the current radiographic approach and prompt earlier, more targeted and personalized treatments[34,35].

In 2001 the Biomarkers Definitions Working Group defined a biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention[36]. Biomarkers have the capacity to detect early joint degradation in degenerative diseases such as OA. They can provide useful diagnostic and prognostic information by reflecting disease relevant biological activity in the joint and predict the course of disease progression. In addition, they can serve as surrogate endpoints in the discovery and development process for DMOADs[37].

The National Institutes of Health (NIH)-funded OA Biomarkers Network has proposed the "BIPEDs" biomarker classification (which stands for **B**urden of Disease, **I**nvestigative, **P**rognostic, **E**fficacy of Intervention, **D**iagnostic and **s**afety), and offered suggestions on optimal study design and analytic methods for use in OA research and subsequent papers have used this classification to describe the potential usage of selected biomarkers[37–39]. This "Year in Review" article is a narrative review of the biochemical marker papers published between the OARSI 2015 Congress held from 30 April to 3 May 2015 in Seattle, Washington and the OARSI 2016 meeting, held from 31 March to 3 April 2016 in Amsterdam. It summarizes progress in the biochemical marker field by reviewing the key published papers related to OA biomarkers and continues a theme established by the "Year in Review" papers published on OA biomarkers over the last 5 years[35,40–44]. The methodology involved searching the PubMed/MEDLINE bibliographic database using the keywords 'biomarker' and 'osteoarthritis'. In addition, the bibliographic databases were searched using the keywords 'biomarker' and 'proteomics'. The PubMed/MEDLINE literature search was conducted using the Advanced Search Builder function (http://www.ncbi.nlm.nih.gov/pubmed/advanced) and specifically focused on the period between the 2015 and 2016 meetings.

Clinical validation of existing OA biomarkers

During the past year, OARSI has published a set of recommendations for the use of soluble biomarkers in clinical trials. The OARSI-endorsed publications summarize the key steps necessary for the qualification of a biomarker as a drug development tool and the various contexts for which OA biomarkers may be used[45,46]. In an effort to qualify more biomarkers as drug development tools, the Foundation for the National Institutes of Health/Osteoarthritis Initiative (FNIH/OAI) biomarkers consortium[ef] released the results of their soluble (urinary, abbreviated with a prefix u and serum, abbreviated with a prefix s) biomarker analysis[45,46]. Among the important findings, the FNIH/OAI investigators found that time integrated concentrations, over a 24-month period, of urinary C-telopeptide of type II collagen (uCTX-II), serum hyaluronan (sHA) and serum N-terminal telopeptide of type I collagen (sNTX-I) were associated with subject cases that had both progressive pain and radiographic progression of knee OA over a 4-year period (area under the curve (AUC) 0.63). Baseline levels of uCTX-II and sNTX-I predicted pain progression and radiographic progression (AUC 0.59). Plans are underway to qualify these biomarkers using samples and data from already completed DMOAD trials.

Several biomarkers have been tested in samples from patients with varying severity of OA during the last year. However, there were only a limited number of novel biomarkers; most were previously identified molecules such as matrix metalloproteinases (MMPs), interleukins, adipokines and joint related serum biomarkers MMP-degraded C-reactive protein (CRPM), MMP degraded type III collagen (C3M), cartilage oligomeric matrix protein (COMP), HA, N-terminal propeptide of collagen IIA (PIIANP), Col2-3/4 C-terminal cleavage product of types I and II collagen, uCTX-II, matrix metalloproteinase-3 (MMP-3) and urinary nitrated type II collagen degradation fragment (uCol2-1 NO_2)[45,46]. The first analytical data came from the OAI biomarker initiative where 18 biomarkers believed to be associated with OA were tested in 129 blood or urine samples from OA patients[45]. The data showed that three

[e] http://www.fnih.org/what-we-do/current-research-programs/oai.
[f] http://www.fnih.org/what-we-do/current-research-programs/biomarkers-consortium-oa.

commercially available biomarkers were associated with age: sHA (rho = 0.19), PIIANP (rho = 0.27) and C1,2C (rho = 0.20). Likewise gender differences were seen for uCTX-II, MMP-3, uCol2-1 NO_2 and sHA[45]. Cartilage damage and concentrations of sCOMP, sCTX-II, sMMP-3, sPIIINP, and sHA were determined in 79 patients who underwent knee arthroscopy or total knee replacement in a study by Jiao and colleagues[47]. PIIANP, serum CTX-II, HA and COMP were measured in this study, but only HA and COMP concentrations were found to be significantly higher in the knee OA patients with early signs of cartilage damage[47]. These results suggest that sCOMP and HA concentrations can be used to predict early cartilage lesions in the knee. In the C4P study, 58 knee OA subjects and 33 healthy controls were tested for the MMP derived collagen products C1M, C2M, C3M together with CRP[48]. The knee OA patients had elevated levels of C1M, C2M and CRP as compared to controls, whereas C3M was significantly lower[48].

There have been a limited number of studies during the past year that attempted to validate existing OA biomarkers in the context of a clinical DMOAD trial. Karsdal *et al.*[49] studied uCTX-I, uCTX-II and serum osteocalcin in the context of two large phase III knee OA trials of oral salmon calcitonin. The investigators found that in one trial with a positive WOMAC pain response to calcitonin, there was a reduction in all three markers over the 24-month trial. Unfortunately, the pain and biomarker responses were not significant at 24 months in the other calcitonin trial and there was a disparate radiographic response between the two trials, making it difficult to validate the biomarkers as surrogates for either pain or radiographic responses. The authors of the calcitonin clinical trial concluded that a definitively successful DMOAD trial (i.e., a trial with a positive pain and radiographic outcome) will be needed to validate predictive and surrogate biomarkers for OA drug development.

There have been four reports from investigators studying large OA cohorts that have addressed the predictive capability of established OA biomarkers[50–52]. In one of these studies, sHA was associated with JSW over a 5 year period in the Iwaki Health Promotion Project[50]. In the Chingford cohort study, which has 20 years of data on development of radiographic knee OA progression in a cohort of middle-aged women with Kellgren and Lawrence (KL) scores of zero at baseline, sCOMP levels in the highest quartile were significantly associated with the development of radiographic knee OA and painful radiographic knee OA[51]. sCOMP was also shown to be associated with increased 5-year risk of radiographic knee OA when tested in 493 subjects with knee OA[51]. In the third report, 5-year data from the Rotterdam study cohort was used to examine biomarker associations with incidence of OA and KL progression[53]. As noted by other investigators, uCTX-II and sCOMP were significantly associated with the incidence (defined as KL < 2 at baseline and KL $\geq$ 2 at follow-up) and progression (defined as KL $\geq$ 1 increase between baseline and follow-up) of OA[53]. In the final report, investigators analyzing 5-year data from the Cohort Hip and Cohort Knee (CHECK) study, found that a number of biomarkers measured at baseline were associated with the presence of baseline knee OA (defined as KL 1), incidence of knee OA (defined as KL 0 at baseline and KL $\geq$ 1 at follow-up) and progression of knee OA (defined as KL 1 at baseline and KL $\geq$ 2 at follow-up). Interestingly, uCTX-II and sCOMP were the most consistently associated biomarkers with the presence, incidence and progression of knee OA[52]. While uCTX-II and sCOMP were positively associated with the presence and progression with knee OA, both biomarkers were negatively associated with the incidence of knee OA. The authors speculated that low levels of cartilage and subchondral bone turnover in the earliest phases of knee OA may explain this latter finding[52].

The past year also included several publications that explored the mechanisms and utility of established inflammatory biomarkers in the pathogenesis of OA and as predictors of OA progression. Data from the Rotterdam study indicated that akin to uCTX-II and sCOMP, CRP was independently associated with the incidence and progression of OA[53]. CRPM, uCTX-II and COMP were independently and positively associated with OA progression[53].

In a meta-analysis of knee, hip and hand OA studies from 1992 to 2012, Jin and colleagues found that OA pain symptoms but not radiographic findings were strongly correlated with high-sensitivity C-reactive protein (hsCRP) levels[54]. As demonstrated by Kraus and colleagues, one potential source of inflammation driving CRP production may be inflammatory macrophages in the joints of knee OA patients[55]. During this past year, this group of investigators demonstrated that soluble markers of inflammatory macrophages (CD14 and CD163) in the synovial fluid (SF) and blood were associated with the abundance of activated macrophages in the knee joint as measured by EC20 SPECT imaging and these soluble markers were associated with the severity of joint space narrowing, osteophytes and knee pain[56]. Other well-known markers of inflammation were also validated in a study by Attur, Abramson and colleagues[57]. Previously, this group had shown that peripheral blood leukocyte transcript levels of the pro-inflammatory mediators interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and cyclooxygenase-2 (COX-2) identified patients at risk for knee OA disease progression[58]. In a 24 month prospective cohort of symptomatic knee OA patients, the authors confirmed that elevated baseline levels of IL-1β, TNF-α and COX-2 peripheral blood transcripts predicted a higher risk of JSW narrowing[57]. In another study that analyzed samples from the same 24-month prospective cohort of symptomatic knee OA patients, the authors found that plasma levels of interleukin-1 receptor antagonist (IL-1Ra) were positively associated with the severity and progression of knee OA[59].

In addition to these findings, decreased serum and SF uncarboxylated matrix Gla-protein (ucMGP) levels have been detected in OA patients. The mean serum ucMGP levels of the knee OA patients was significantly lower than that of healthy controls ($P = 0.045$) and negatively correlated with radiographic severity ($r = -0.48$)[60].

Other previously tested biomarkers were adipokines such as leptin and adiponectin. Data from Gandhi *et al.* showed differences between race[61].

Mabey *et al.* showed that interleukin-4 (IL-4) and interleukin-6 (IL-6) levels were significantly higher in OA patients compared to controls and positively correlated with radiographic severity[62].

In a study of 138 knee OA patients the serum levels of adipsin (complement factor D), leptin, adiponectin, resistin and serpin E1, and cartilage volume (MRI) were determined at baseline and 24 months. Higher levels of adipsin and leptin correlated with increased cartilage volume in the global knee and medial femur. Adiponectin showed an inverse correlation with cartilage volume in the medial compartment and femur. Resistin and serpin E1 were not associated with cartilage volume[63].

Early testing of exploratory biomarkers

There have been some interesting studies published on different proteomics approaches, including metabolomics, in the recent period. Plasma profiles of 15 inflammation-related proteins were significantly different in farmers with musculoskeletal disease (MSD) as compared to farmers without MSD[64]. Leucine-rich alpha-2-glycoprotein, haptoglobin, complement factor B, serotransferrin, one isoform of kininogen, one isoform of alpha-1-antitrypsin, and two isoforms of hemopexin were higher in farmers with MSD than in referents. On the other hand, the levels of alpha-2-HS-glycoprotein, alpha-1B-glycoprotein, vitamin D-binding protein, apolipoprotein A1, antithrombin, one isoform of kininogen, and one isoform of alpha-1-antitrypsin were lower in farmers with MSD

than in referents. Although the study was not specifically on OA, it may provide novel insight into biomarker developments. A metabolomic profile was done on urine samples of 22/22 OA structural progressors/non-progressors (18-month follow-up) from the Intensive Diet and Exercise for Arthritis (IDEA) trial. A profile including the following metabolites was found to be able to discriminate between OA progressors and non-progressors: glycolate, hippurate, and trigonelline were among the important metabolites for distinguishing progressors from non-progressors at baseline whereas alanine, N,N-dimethylglycine, glycolate, hippurate, histidine, and trigonelline distinguished between them at 18 months[65]. In another study, arginine was significantly depleted in refractory knee OA patients ($n = 64$) vs controls ($n = 45$), which is most likely due to the over activity of arginine to ornithine pathway, leading to imbalance between cartilage repair and degradation[66]. These novel findings support a role for metabolic factors in the severity (burden of disease) and progression of knee OA and suggest that measurement of metabolites could be useful to predict progression. Further investigation in a larger sample that would include targeted investigation of specific metabolites is warranted.

Biomarkers in interventional studies and clinical validation of other existing markers

In a placebo controlled study in mild to moderate knee OA ($N = 69$) L-carnitine supplements was tested. Significant changes to IL-1β and MMP-1 were observed after treatment, which seemed to be associated with decreases in visual analogue scale (VAS) pain[67].

The biomarker-related studies published in the last 12 months include some of the usual suspects such as COMP, HA, ADAMTS-4, the aggrecan ARGS neo-epitope and the type II collagen markers CTX-II, C2C and COLL2-1 NO_2.

Adipokines (adipocytokines) presumably are an important component of inflammatory OA[68]. There is evidence for racial differences in OA prevalence and incidence[69], and from general population-based studies[70]. Some East Indian and Asian races consistently demonstrate a unique adipokine/insulin serum concentration profile as compared to Caucasians and recent findings published by Gandhi *et al.*, suggest that race may be an important factor to consider when studying serum biomarker concentrations in OA[61]. This study also highlights the fact that the ancestry of patients and research subjects should be taken into consideration with respect to the design of OA biomarker studies and also in connection with the analysis and presentation of biomarker data.

Pascarelli *et al.*[71], developed a randomized controlled trial to study the effect of balneotherapy (mud-bath) therapy on serum biomarkers in patients with knee OA. The authors observed a statistically significant improvement in VAS pain and WOMAC subscores but serum levels of COMP, myeloperoxidase (MPO) and hsCRP did not show any significant changes in either group. Interestingly, a significant increase in serum levels of CTX-II was observed in the mud-bath group after the treatment and the authors associate this increase with cartilage turnover induced by the balneotherapy[71]. It is important to note that this is an unusual and counter-intuitive observation that needs to be validated by other studies.

Proteomics, novel OA biomarkers, new biomarker assays and magnetic capture technologies

One of the more interesting technological advances during the past year was the development of a magnetic nanoparticle-based technology to collect biomarkers from a rodent stifle, a technology termed magnetic capture by the authors[72]. The authors used anti-CTX-II antibodies conjugated to the surface of superparamagnetic iron oxide-containing polymeric particles to capture and concentrate this biomarker directly in the affected joint in a rat monoiodoacetate (MIA) model of knee OA. Importantly, measurements were unaffected by SF viscosity. The development of such technologies is important as it allows magnetic capture platforms to be developed for a range of OA biomarkers. Ongoing work by the same authors is aiming to facilitate optimization of the collection of magnetic particle-biomarker conjugates from high-viscosity biological fluids without the need to remove the fluid from a patient[73].

Proteomic techniques continue to remain a focus in biomarker discovery and exploring the basic biology of articular cartilage and other joint tissues. However, proteomic techniques developed for other tissues need to be refined and adapted for studies on articular cartilage and chondrocytes. Hsueh and co-workers have advanced the area of cartilage proteomics by refining the extraction methods for more in depth proteomic studies on the molecular composition of cartilage[74]. They established and refined a novel extraction method for removing chondrocytes from cartilage sections with minimal extracellular matrix protein loss. By adding surfactant to guanidine-HCl extraction buffer they improved protein solubility and used ultrafiltration to remove interference from polysaccharides and salts. They also introduced *in situ* trypsin digestion to increase the number of collagen peptides detectable from cartilage by mass spectrometry (MS)[74]. The addition of surfactant to guanidine-HCl extraction buffer allowed them to improve protein solubility, which resulted in a four-fold increase in the extraction and MS detection of collagen peptides by the *in situ* trypsin digestion method. As expected, proteoglycans were far more abundant within the guanidine-HCl extract. The methodology developed by the authors will allow investigators to specifically focus on the detection of extracellular matrix proteins from different zones of articular cartilage and from diseased samples. For those investigators interested in applying proteomics to chondrocytes and the membranome of these cells, proteomic methods are being developed to enrich hydrophobic and hydrophilic protein fractions. However, despite their best efforts, the total *in situ* digestion method was not appropriate for identifying low abundance proteins since the peptides from high abundant extracellular proteins generally saturates the analytical performance system and attenuated the ability to identify new proteins. This is a major hurdle for identifying new and low abundance biomarkers with clinical utility. Future studies on sub-compartments and carefully generated fractions should allow investigators to focus on the detection of lower abundance proteins.

Proteomics has also been applied to synovitis using an explant model of the synovium. Kjelgaard-Petersen and colleagues primary treated fibroblast-like synoviocytes and synovial membrane explants (SMEs) with various pro-inflammatory cytokines and growth factors and assessed the biological activity of the proteases in this model using C1M, C3M, and active MMP-3 assays in the conditioned medium from the cells and explants. Their work suggests that C1M, C3M, and active MMP-3 may be used as biomarkers of synovitis in *ex vivo* models and provides a novel translational tool for synovitis[75].

At the present time there is insufficient knowledge about the chondrocyte membranome and its molecular composition. We need to learn more about this proteome sub-compartment because many potential drug targets reside in the plasma membrane of living cells. New research from our own laboratory has developed a Triton X-114 based separation technique using nano liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) combined with shotgun proteomics to identify chondrocyte membrane proteins[76]. Chondrocyte proteins were separated into

hydrophobic and hydrophilic fractions; and trypsin-digested fractions were analyzed by nanoLC-MS/MS. A total of 315 proteins were identified. The phase extraction method yielded a high proportion of membrane proteins (56%) including CD276, S100 Calcium Binding Protein A6 (S100-A6 or calcyclin) and three voltage-dependent anion-selective channel (VDAC) isoforms and several glucose transporters. Our work has confirmed the findings of several previous studies and adds new proteins to the proteomic profile of articular chondrocytes. Some of the identified proteins including the CD276 antigen, S100-A6 or VDACs have not been previously been reported to be components of articular chondrocytes. The development and refinement of proteomics-based techniques for studying the chondrocyte membranome and other cellular subcompartments will enable a better understanding of the function of regulatory proteins and enhance the search for new drug targets for OA[76].

Cathepsins are a family of lysosomal proteases that are thought to contribute to OA pathophysiology due to their elevation and activation in pro-inflammatory conditions. In order to monitor the specific activity of cathepsins Ben-Aderet *et al.*[77], developed a cathepsin activity-based probe (ABP), GB123, for investigation of enzyme activity *in vitro* and *in vivo*. In essence, these probes are recognized by cathepsins and are conjugated on to the enzymes after the reaction. Since the probes are tagged with a fluorophore, they emit fluorescence that can be observed and quantified. The ABPs allowed the investigators to monitor cathepsin activity, which was found to correlate well with OA severity and joint inflammation. The authors are developing biomarker assays for downstream targets of cathepsins, thus allowing them to develop new assays with translational potential for non-invasive detection of early OA in preclinical models.

Identification of novel cytokines and adipokines in the joint

Recent studies suggest that other cytokines may exert pro-inflammatory effects on articular cartilage in synovial joints. Conde *et al.*, analyzed the expression of IL-36α in healthy and OA cartilage and demonstrated that this cytokine acts as a pro-inflammatory mediator in cartilage by increasing the expression of markers of inflammation and cartilage catabolism including MMP-13, nitric oxide synthase 2 (NOS-2) and COX-2[78]. Like other members of the interleukin family, IL-36α acts through the activation of nuclear factor κB (NFκB) and p38 MAPK pathway. Therefore, IL-36α and its co-conspirators in the interleukin family can co-operate with other pro-inflammatory cytokines and chemokines to enhance and perpetuate cartilage destruction[78]. Earlier studies by the same group have shown that other cytokines and adipokines produced by white adipose tissue (WAT) are implicated in OA[79]. This work suggests that novel adipokines may exist in different joint tissues and compartments and how these molecules interact and are differentially expressed in healthy and OA joint tissues is largely unknown. Advanced analytical (omics) techniques are likely to identify novel biomarkers to shed more light on these interactions between WAT-derived cytokines, joint inflammation and cartilage degradation.

What's on the horizon in OA biomarker research?

Sleep and its physiological and pathophysiological attributes are seldom considered to be “biomarkers” but new research indicates that sleep is an important biological and disease marker. There is increasing evidence to suggest that sleep is a pathophysiological “marker” of disease progression. Sleep is a complex biological process that involves cyclic changes of brain activity. Sleep is a sensitive biomarker of brain function and depression. Sleep dysregulation is closely linked to the underlying pathophysiology of depressive disorders and sleep studies are essential for antidepressant drug development. There is evidence that sleep disturbance in OA is linked with pain and depression[80]. Depression plays a strong role in the sleep-pain linkage, particularly where pain is severe and sleep is believed to have a unique and predictive role in progression of disability. Sleep studies are not done in conjunction with OA research but interdisciplinary research involving sleep physiology may reveal novel interventions to prevent OA-related functional decline among persons whose sleep is disrupted by OA pain[81] and this exciting possibility can be explored in ongoing longitudinal cohorts or new cohorts in future studies.

Conclusions

OA biomarker research is active and thriving. Applying the biomarker toolbox in the drug discovery and development pathway allows investigators to link a biomarker to a complementary endpoint, thereby acting as a potential surrogate with predictive power. This facilitates the drug discovery process and allows the drug development industry to make rational economic decisions about the continuity of preclinical studies and clinical trials. Biomarkers can be used at critical decision points to make go/no-go decisions. Biomarkers can be used in translational OA research, bridging the gap between the bench and the bedside. Biomarkers may also be used to identify responders and non-responders and

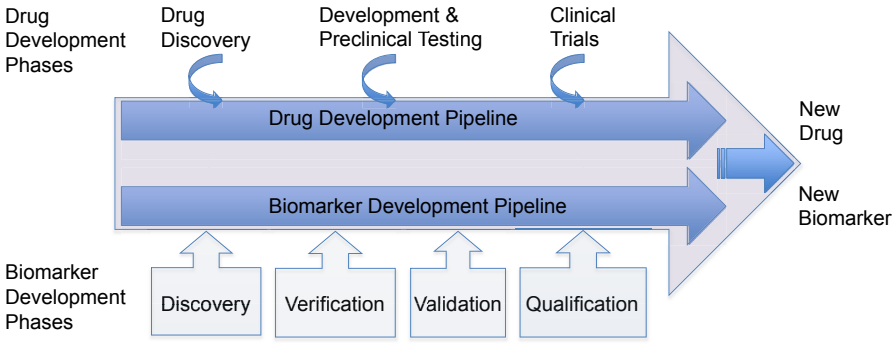

Fig. 3. Application of the biomarker toolbox in OA drug discovery and development. This schematic highlights the mutual interdependency of the biomarker testing and drug development pipelines. By linking an OA biomarker to a complementary endpoint industry can make rational decisions about the continuity of preclinical studies and the scale of OA clinical trials. Biomarkers can be used to identify responders and non-responders and quantify clinical efficacy and patient stratification (i.e., identification of those in need of treatment and selection of patients most likely to respond to treatment).

quantify clinical efficacy and patient stratification (i.e., identification of those in need of treatment and selection of patients most likely to respond to treatment) (Fig. 3). In phase II clinical trials biomarkers can be used for dose determination and safety/efficacy studies. They can also help pharmaceutical companies save costs by enabling drug repositioning and determining the cost/benefit ratio for treatment. Biomarkers have the capacity to identify patients who are in the greatest need of treatment, select those who may respond optimally, with the greatest efficacy and lowest safety concerns to a specific treatment, enhancing drug development and targeting strategies for a selected subpopulation of patients thus allowing for more efficient use of healthcare resources. Even in routine clinical practice, biomarkers can be used as important diagnostic and prognostic tools for monitoring disease development and monitoring patient compliance with the recommended therapy. They are also indispensable tools for pharmacovigilance, personalized and precision health care and differentiating compounds from competitors. However, from a clinical practice and trial design perspective, none of the currently available biochemical markers in our toolbox is sufficiently discriminating to aid diagnosis and prognosis of OA, or performs so consistently that it could function as an outcome in clinical trials[42]. Specific biochemical markers and categories of biochemical markers as well as their specificity, origin and metabolism, need further investigation[82]. Therefore, there is an urgent and unmet need to develop new predictive biomarker tests that can provide an early warning of joint alterations, which could prompt earlier, more targeted and personalized treatments.

The aim of this “Year in Review” article is to discuss biomarker related research and review articles published since the 2015 OARSI congress. There has been solid and steady progress in OA biomarker research since OARSI 2015. The OA biomarkers studies published in the last year have included a set of recommendations from OARSI for the use of soluble biomarkers in clinical trials outlining the key steps necessary for the qualification of a biomarker as a drug development tool and the various contexts for which OA biomarkers may be used. This is a major step forward in the clinical use of OA biomarkers. In terms of new biomarkers there have been important but incremental gains in our knowledge in the past year. However, the majority of the published studies have represented “evolutionary” rather than “revolutionary” increments in our knowledge of OA.

We need more effort and faster progress in this area and this is particularly important for OA patients. OARSI has submitted a white paper[g] on December 1, 2016 asking the US Food and Drug Administration (FDA) to consider OA as a serious disease. OARSI is concerned about the growing population of OA patients, many of whom may experience progressive disability and decreased quality of life and the FDA has a unique and influential role in fostering the development of these therapies, which might alter the natural progression of OA. Novel OA biomarkers are needed for subclinical or preclinical disease diagnosis and patient stratification in clinical trials. OA biomarker research remains a challenging area, yet with many exciting opportunities for collaboration between academic, drug development and clinical scientists. Biomarkers can facilitate new research into the underlying mechanisms in OA to validate existing biomarkers and identify new candidates and subsets of OA patients that may have differential responses to therapy. ‘Omics’ techniques and platforms are continuously evolving and increasing in sensitivity, which means that they can be used to identify novel OA subsets. The refinement of proteomic and immunoassay technologies is also likely to contribute to improvements in diagnostic assays for wet biochemical markers in serum, SF and urine. There are differences in biomarkers between joints (knee, hip, hand, spine, etc.) and more research is needed to explore whether there are specific biomarkers for specific joints. The key challenges in OA biomarker research include identification of new and more sensitive/specific biomarkers and improvement of existing biomarker assays and the technologies and platforms used for their detection and measurement. Another important issue is standardization and calibration of biomarkers, enabling comparative studies. The ultimate challenge for OA biomarker researchers is combining biochemical and imaging markers into predictive algorithms and new prognostic tests.

In conclusion, the capabilities of novel and exploratory biomarkers for OA should be regarded as important capabilities for furthering clinical research and patient stratification. Nonetheless, curiosity driven research that drives identification of novel exploratory OA biomarkers is worthy of further pursuit and should remain a high priority. Further advances in OA biomarker qualification and progress towards the clinical utility of biochemical markers will require highly stratified and well-phenotyped cohorts that can be used for answering hypothesis driven research questions that can truly progress the current state-of-the art in OA research.

Declaration of interest

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[g] https://www.oarsi.org/sites/default/files/docs/2016/oarsi_white_paper_oa-serious-disease.pdf.

[h] http://www.d-board.eu/dboard/index.aspx.

[i] http://www.approachproject.eu.

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