Osteoarthritis and Cartilage 20 (2012) 1451–1464

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Review

Osteoarthritis year 2012 in review: biomarkers

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ARTICLE INFO

Article history:
Received 13 May 2012
Accepted 17 July 2012

Keywords:
Osteoarthritis
Articular cartilage
Synovium
Inflammation
Biomarker
Biochemical marker
Proteomics
Complement
Chemotactic proteins
Adipokines

SUMMARY

Purpose: Biomarkers provide useful diagnostic information by detecting cartilage degradation in osteoarthritis (OA), reflecting disease-relevant biological activity and predicting the course of disease progression. They also serve as surrogate endpoints in the drug discovery process. The aim of this narrative review was to focus on OA biomarker-related papers published between the osteoarthritis research society international (OARSI) 2011 meeting in San Diego and the OARSI 2012 meeting in Barcelona.
Methods: The PubMed/MEDLINE and SciVerse Scopus bibliographic databases were searched using the keywords: 'biomarker' and 'osteoarthritis' and/or 'biomarker' and 'proteomics'.
Results: Ninety-eight papers were found with the keywords 'biomarker' and 'osteoarthritis'. Fifteen papers were found with the keywords 'biomarker' and 'proteomics'. Review articles were also included. The most relevant published studies focused on extracellular matrix (ECM) molecules in body fluids. Enrichment of the deamidated epitope of cartilage oligomeric matrix protein (D-COMP) suggests that OA disease progression is associated with post-translational modifications that may show specificity for particular joint sites. Fibulin-3 peptides (Fib3-1 and Fib3-2) have been proposed as potential biomarkers of OA along with follistatin-like protein 1 (FSTL1), a new serum biomarker with the capacity to reflect the severity of joint damage. The 'membrane attack complex' (MAC) component of complement has also been implicated in OA.
Conclusion: Novel OA biomarkers are needed for sub-clinical disease diagnosis. Proteomic techniques are beginning to yield useful data and deliver new OA biomarkers in serum and urine. Combining biochemical markers with tissue and cell imaging techniques and bioinformatics (i.e., machine learning, clustering, data visualization) may facilitate the development of biomarker combinations enabling earlier detection of OA.

Introduction

Osteoarthritis (OA) is the most common form of degenerative joint disease and a leading cause of pain and chronic physical disability in older individuals[1]. OA has a multifactorial etiology, and can be considered the product of interactions between systemic, mechanical and local factors within the joint[2]. Although OA is primarily associated with aging, there are other key contributing factors, including obesity (which increases mechanical stress and systemic levels of inflammatory mediators such as adipokines[3]), a history of joint trauma, repetitive use or injury, genetics, heritable metabolic disorders, muscle weakness, underlying anatomical and orthopedic disorders (i.e., congenital hip dislocation), joint infection, crystal deposition, previous rheumatoid arthritis (RA) and various disorders of bone turnover and blood clotting. The metabolic alterations that occur in obesity along with biomechanical factors and pro-inflammatory mediators produced by white adipose tissue in the chronically overweight are thought to be major factors in the progression of the disease[4]. Stepwise approaches to the management of OA have been proposed by a number of leading investigators. Thus far the evidence suggests that with the exception of joint replacement, the currently available treatments are, at best, modestly efficacious, and are frequently associated with substantial side-effects or costs, or both[5]. This highlights, and reinforces, the need for new treatments and therapeutics.

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". However, radiographic evidence is seen only after significant cartilage degradation has

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1063-4584/$ – see front matter
http://dx.doi.org/10.1016/j.joca.2012.07.009

already taken place. The early stages of the disease may remain latent and asymptomatic for many years. Therefore, the "gold standard" is inadequate and there is an acute need for reliable new biomarkers and diagnostic tests that can facilitate earlier diagnosis of OA, and inform the prognosis, monitoring and therapeutic strategies for chronic and disabling forms of the disease. Most of the studies carried out to date have focused on late stages of the disease in humans or animal models. Studies on early stages of OA are lacking and there are no biomarkers for early diagnosis of the disease.

The Biomarkers Definitions Working Group[a] defines 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[6]. Biomarkers can be anatomic, physiologic, biochemical, or molecular parameters associated with the presence and severity of specific diseases and are detectable by a variety of methods, including physical examination, laboratory assays, and imaging. Biomarkers are also used as indicators of pharmacologic responses to therapeutic interventions. In OA biomarkers can be classified as "dry" biomarkers or "wet" soluble biomarkers. Dry biomarkers include imaging parameters (i.e., from radiographs, magnetic resonance imaging (MRI), and ultrasound), questionnaires and data from visual analog scales. Soluble biomarkers correspond to genetic (RNA, DNA) and biochemical (carbohydrates, proteins, protein fragments, peptides, metabolites) molecules[7]. Biochemical markers can be measured in blood, serum, urine and synovial fluid.

There is an important relationship between the biomarker and pharmaceutical pipelines. Biomarker and drug discovery are protracted, risky and costly. Currently there are many challenges facing the pharmaceutical sector. The drug pipeline is convoluted, "leaky" and highly susceptible to recessions and financial crises. The pharmaceutical industry has not managed to bring effective and safe disease-modifying osteoarthritic drugs (DMOADs) to patients suffering from this debilitating disease[8]. The heterogeneity and slow progressive nature of OA in the human population combined with prolonged periods of asymptomatic, degenerative changes have hampered the development of new drugs[9]. The paucity of biomarkers has been a key contributor to the lack of progress in this area because of the mutual interdependency of the drug and biomarker pipelines.

Currently there are no reliable, quantifiable and easily measured biomarkers that provide an earlier diagnosis of OA, inform on the prognostic of OA disease and monitor responses to therapeutic modalities. Linking a biomarker to a clinical endpoint facilitates the drug discovery process. The process of "qualification" in the biomarker pipeline (Fig. 1) may support the use of suitable biomarkers as surrogate endpoints in drug discovery and development, post approval and regulatory decision-making. Applying the biomarkers toolbox in drug discovery and development provides the opportunity to establish quantifiable decision points in the drug pipeline. Biomarkers of joint tissue turnover enable a more rational and personalized approach to healthcare management because they have the capacity to reflect disease-relevant biological activity and provide useful diagnostic and therapeutic information. However, new biomarkers are needed to discriminate between catabolic and maintenance events since many existing biomarkers reflect normal cartilage turnover, tissue repair, or extracellular matrix (ECM) remodeling.

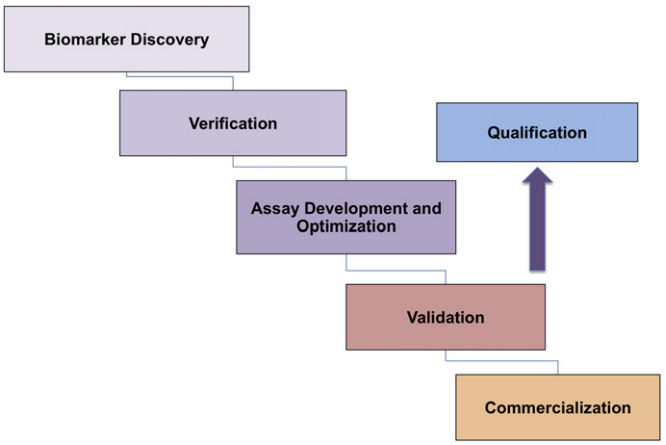

Fig. 1. The biomarker pipeline. The discovery of new biomarkers is followed by independent scientific and analytical verification of the biomarker. This process involves verification of the analytical performance characteristics and clinical correlation of a biomarker with a biological process or clinical outcome. Assay development and optimization are followed by validation, which involves assessing all the technical aspects of the biomarker assay. Qualification allows biomarker to be linked to a clinical endpoint to support its use as a surrogate endpoint in drug discovery, development or post approval and, where appropriate, in regulatory decision-making.

The recent proliferation of post-genomic technologies has resulted in rapid growth and progress in biomarker research. Omic technologies (i.e., genomics, transcriptomics, proteomics, metabolomics) are increasingly applied in biomarker discovery, especially in the area of cancer biomarkers. Combinations of omic technologies, bioinformatics, advanced imaging and basic information such as family history and genetic background may hold special promise for the discovery of novel "combination biomarkers" that will form the foundation for new diagnostic tests. These technologies are likely to dominate the biomarker research arena and play increasingly important roles in the identification of new biomarkers and their validation. The full complement of ECM proteins has been described as the "core matrisome" comprising ~300 proteins in addition to ECM modifying enzymes, ECM-binding growth factors, and other ECM-associated proteins[10,11]. The cartilage ECM contains a limited number of molecules (Fig. 2). Although this limits the total number of ECM proteins that may end up in serum as biomarkers of joint disease and inflammation, the processes of biomarker verification, validation and qualification remain challenging, costly and time-consuming.

The "Year in Review" articles are becoming a well-established tradition in Osteoarthritis and Cartilage. They provide a unique opportunity to build on the "Year in Review" papers from the previous years. This paper is a narrative review of the biomarker papers published between the osteoarthritis research society international (OARSI) 2011 Congress held from 15 to 18 September 2011 in San Diego, California and the OARSI 2012 meeting, held from 26 to 29 April 2012 in Barcelona, Spain. It summarizes the progress in the field by reviewing the key published papers related to OA biomarkers and continues a theme established by the "Year in Review" papers from the 2010 and 2011 OARSI meetings[12,13]. The methodology involved searching the PubMed/MEDLINE and SciVerse Scopus bibliographic databases 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

[a] Downing GJ, National Institutes of Health (US), United States, Food and Drug Administration. *Biomarkers and Surrogate Endpoints: Clinical Research and Applications*. In: *Proceedings of the NIH-FDA Conference Held on 15–16 April 1999 in Bethesda, Maryland, USA*. Downing GJ, Ed. Office of Science Policy National Institutes of Health Bethesda, Maryland, USA 2000 ELSEVIER Amsterdam.

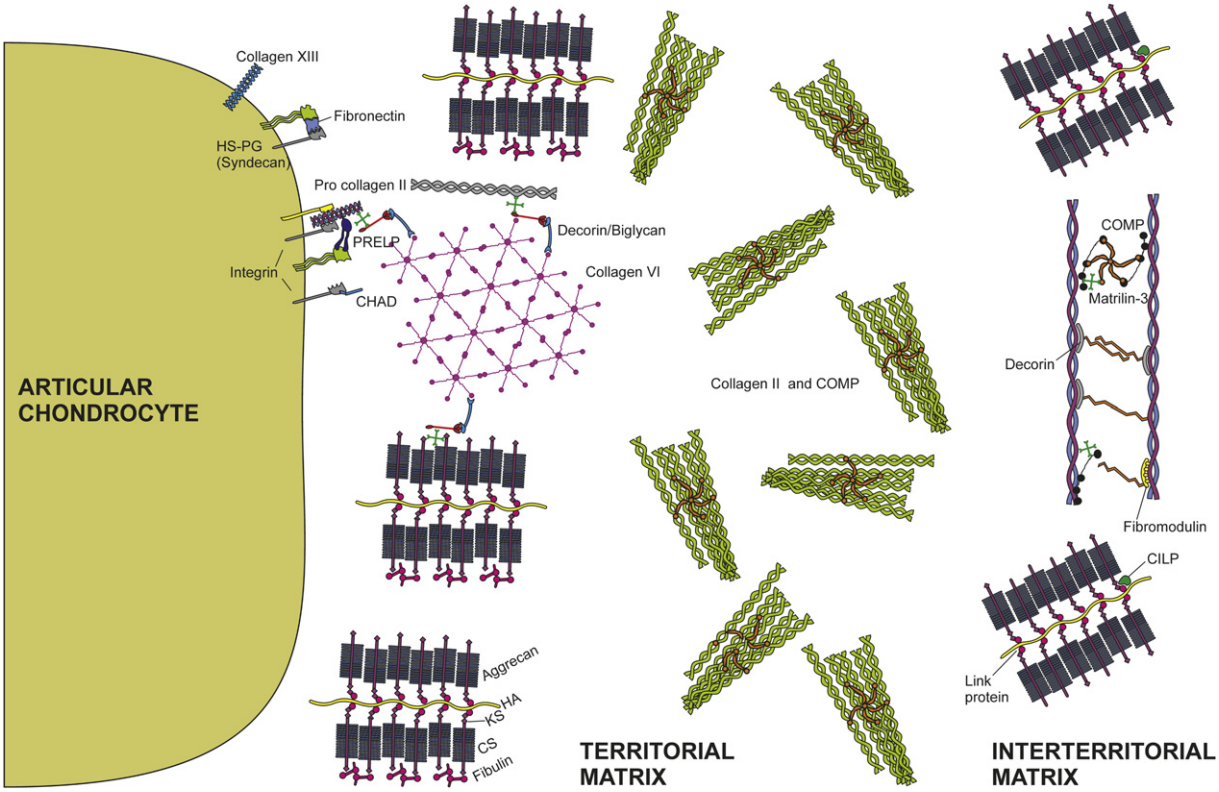

Fig. 2. Schematic illustration of the cartilage matrisome. This figure illustrates the major molecular constituents of the ECM of articular cartilage and the physical and functional association between its constituents. The molecules are arranged into large multi-molecular assemblies in the territorial and interterritorial ECM of chondrocytes. Adapted from Heinegård and Saxne, 2011[75].

the 8 months between the 2011 and 2012 meetings. Ninety-eight papers were found with the keywords 'biomarker' and 'osteoarthritis' (Table I) and 15 papers included the keywords 'biomarker' and 'proteomics' (Table II). Review articles published in the same period including the keywords 'biomarker' and 'osteoarthritis' are summarized in Table III.

Selected OA biomarker studies published between 2011 and 2012

Table I summarizes the key papers published between September 2011 and April 2012. Due to space and article length limitations, it was not possible to review all these papers in great detail. Therefore, only a selected number of papers from the last 8 months are discussed in the following sections.

The importance of large cohorts of human patients in OA biomarker research is highlighted in a clinical study by van Spil and co-workers[14]. In this study they quantitatively assessed a wide spectrum of biomarkers in Cohort Hip and Cohort Knee (CHECK), a large 10-year prospective cohort of 1,002 individuals with (very) early symptomatic knee and/or hip OA. The authors examined 14 biomarkers by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) and used principal component analysis (PCA) to analyze the data. This approach helped to five "clusters", consecutively designated as 'bone-CTX-II', 'inflammation', 'synovium', 'C1, 2C-adipokines', and 'cartilage synthesis' clusters. Based on their analysis they propose that urinary C-terminal telopeptide II (uCTX-II) may be a marker of bone degradation as well as cartilage degradation. They also suggest that cartilage oligomeric matrix protein (COMP) in serum cartilage oligomeric matrix protein (sCOMP) may reflect synovial changes rather than cartilage metabolism. Interestingly, the authors of this study propose that adipokines have no major involvement in joint metabolism. Although the results of this study will require validation by complementary approaches, they do highlight the utility of PCA as a quantitative tool for identifying "clusters" or "groups" of biomarkers.

Animal models continue to have an impact on OA biomarker research. Willett *et al.*,[15] used the Hartley guinea pig (HGP) model to demonstrate that increased advanced glycation endproducts (AGEs) accelerate the progression of spontaneous OA. They measured cartilage pentosidine, an established biomarker for AGEs to show that enhanced levels of non-enzymatic glycation and pentosidine cross-linking are involved in spontaneous OA progression in this model.

STR/ort mice were used in a study by Braza-Boils *et al.*, in which serum levels of matrix metalloproteinase-3 (MMP-3) but not those of tumor necrosis factor-alpha (TNF-α), interleukin 17 (IL-17) or prostaglandin E(2) (PGE_2) were found to correlate with histopathological changes in knee joints[16]. This study adds to the considerable body of evidence implicating MMP-3 in early degenerative changes in OA.

Another recent animal study highlights the utility of the dog as an animal model of OA. Alam *et al.*,[17] measured the levels of tartrate resistant acid phosphatase (TRAP), matrix metalloproteinase-2 (MMP-2), and tissue inhibitor of MMP (TIMP)-2 in synovial fluids and sera from dogs with OA. This study suggests that TRAP, MMP-2, and TIMP-2 in synovial fluid and serum may help to identify early phases of canine OA.

Garner and co-investigators[18] measured the cytokine, chemokine, and matrix metalloproteinase (MMP) profiles of synovial fluid, serum, and urine from dogs with surgically induced and naturally occurring OA were compared with dogs without OA using xMAP technology. Biomarkers that exhibited significant differences

Table I
Summary of selected OA biomarker studies (2011–2012)

Reference	Study type and objectives	Biomarker identified/proteins involved	Species, tissue types, assays and techniques used	Main outcome of the study
van Spil *et al.*, 2012[14]	Clinical study assessing a wide spectrum of biomarkers in a large cohort of individuals with (very) early symptomatic knee and/or hip OA	Fourteen biomarkers were assessed by ELISA or RIA including uCTX-II, uCTX-I, urinary N-telopeptide of type I collagen (uNTX-I), sCOMP, N-terminal propeptide of type IIA procollagen (sPIIANP), sCS846, sC1, 2C, sOC, serum C-terminal telopeptide of type I collagen (sPINP), sHA, N-Terminal propeptide of type III collagen (sPIIINP), pLeptin, pAdiponectin, pResistin	Human; CHECK, a 10-year prospective cohort of 1,002 individuals with early symptomatic knee and/or hip OA	PCA enabled identification of five clusters, consecutively designated as 'bone-CTX-II', 'inflammation', 'synovium', 'C1, 2C-adipokines', and 'cartilage synthesis' cluster uCTX-II may not only be a marker of cartilage but also bone degradation sCOMP may reflect synovial rather than cartilage metabolism No major involvement of adipokines in joint metabolism was identified
Willett *et al.*, 2012[15]	Basic study testing the hypothesis that increased AGEs accelerate the progression of spontaneous OA	Cartilage pentosidine, an established biomarker for AGEs	HGP model; see the primary article for full details of assays and techniques used	Enhanced levels of non-enzymatic glycation and pentosidine cross-linking are involved in spontaneous OA progression AGEs accumulation due to intraarticular ribose-containing injections in the HGP model of spontaneous knee OA did not enhance disease progression
Braza-Boils *et al.*, 2012[16]	Basic study of the early phases of idiopathic OA in STR/ort mice	Serum levels of MMP-3 but not those of TNF-α, IL-17 or PGE_2 correlated with histopathological changes in knees of STR/ort mice	STR/ort mice; see the primary article for full details of assays and techniques used	MMP-3 is a sensitive biomarker for the detection of early OA alterations
Garner *et al.*, 2011[18]	Clinical study in which the cytokine, chemokine, and MMP profiles of synovial fluid, serum, and urine from dogs with surgically induced and naturally occurring OA were compared with dogs without OA	MCP-1, IL-8, KC, MMP-2 and MMP-3 were biomarkers that exhibited significant differences between groups and their sensitivities and specificities were calculated to determine their diagnostic utility	Canine; multiplex bioassays (xMAP technology – bead-based multiplexing of up to 500 analytes per well)	This study used canine samples to provide information relating to pro-inflammatory cytokines, chemokines, and MMPs in OA, and identified potential diagnostic biomarker candidates
Weng *et al.*, 2012[57]	Clinical study aimed at screening serum for biomarkers of knee OA using a phage random peptide library	A phage clone with inserted peptide TGLESGHGPGDS (named KOA1) was identified	Human; a phage random peptide library of random peptide 12-mers was screened with purified immunoglobulin G (IgG) from sera of knee OA patients	The novel knee OA mimic peptide KOA1 could be a potential serum biomarker for knee OA
Xu *et al.*, 2012[26]	Clinical study aimed at demonstrating omentin-1 levels in serum and synovial fluid of patients with knee OA and to investigate their correlation with radiographic disease severity according to the KL grading system	Omentin-1 (also known as intelectin 1)	Human; 197 patients with OA and 65 sex- and age-matched healthy controls	Omentin-1 in synovial fluid might serve as a potential biomarker for reflecting the degenerative process in primary knee OA
González-Álvaro *et al.*, 2011[20]	The objective of this clinical study was to determine whether sIL-15 levels could serve as a biomarkers of disease severity in patients with early OA	sIL-15	Human; see the primary article for full details of assays and techniques used	IL-15 levels in serum may predict a severe disease course in patients with early OA
McCoy *et al.*, 2011	The aim of this animal study was to investigate whether variations in the level of Xylosyltransferase 1 (Xylt1) present in serum can be used to predict OA disease progression	Xylt1	Mouse; see the primary article for full details of assays and techniques used	Serum Xylt1 level increase during early posttraumatic OA
Cattano *et al.*, 2011[21]	The aim of this clinical study was to determine if biochemical differences exist between OA knees with and without effusion	MMP-3, TIMP-1, TIMP-2, and IL-10 were significantly higher in the knees with effusion than in the knees without effusion	Human; cross-sectional study consisting of 22 volunteers (11 with knee effusion, 11 without knee effusion) with confirmed late-stage radiographic knee OA (KL score > 3). Synovial fluid samples	Biochemical differences were observed between knees with and without effusion suggesting the existence of OA subsets characterized by distinct biochemical

			were collected and analyzed using custom multiplex ELISAs	characteristics and clinical findings (e.g., joint effusion)
Fernandez-Moreno *et al.*, 2011[58]	The aim of this clinical study was to assess the incidence of the mtDNA haplogroups on serum levels of two of the main antioxidant enzymes, Manganese Superoxide Dismutase (Mn-SOD or SOD2) and catalase, and to test the potential of these proteins as OA biomarkers	Mn-SOD or SOD2 and catalase	Human; 73 OA patients and 77 healthy controls carrying the haplogroups J, U and H, by ELISA assays	Increased levels of SOD2 were seen in OA patients Mitochondrial haplogroups significantly correlated with serum levels of catalase
Conrozier *et al.*, 2012[22]	The aim of the clinical study was to investigate the effect of HA intra-articular injections (IA) on OA biomarkers in patients with knee OA	uCTX-II and S-HA	Human; see the primary article for full details of patients and assays used	Ninety days after HA intra-articular injections uCTX II levels significantly decreased compared to baseline suggesting a slowdown of type II collagen degradation
Song *et al.*, 2012[27]	The aim of this clinical study was to develop a method to detect COMP as an OA biomarker	COMP	Human; FMGC-based immunoassay and commercial ELISAs for COMP	FMGC-based immunoassay offers a new approach for detecting COMP and other clinically relevant OA biomarkers in blood and synovial fluid
Alam *et al.*, 2011[17]	The aim of this study was to measure the levels of three biomarkers in synovial fluids and sera from dogs with OA	TRAP, MMP-2, and TIMP-2	Canine; western blotting and ELISAs	Assays for TRAP, MMP-2, and TIMP-2 in synovial fluid and serum, may identify early phases of OA
Saetan *et al.*, 2011[59]	The objective of this clinical study was to investigate interferon-γ inducible protein-10 (IP-10) concentrations in plasma and synovial fluid of patients with knee OA and to analyze their relationship with disease severity	Interferon-γ, IP-10	Human; 40 OA patients and 15 healthy controls OA grading was performed according to the KL criteria IP-10 levels in plasma and synovial fluid were assessed by ELISA	Plasma and synovial fluid interferon-γ IP-10 correlates with radiographic severity in knee OA
Duan *et al.*, 2011[24]	The aim of this clinical study was to investigate visfatin levels in synovial fluids and plasma of patients with primary knee OA and establish its relationship with biomarkers of cartilage degradation in synovial fluid	Visfatin, type II collagen and aggrecan	Human; 30 OA patients, 12 SF control, and 12 plasma control subjects Visfatin levels in synovial fluid and plasma were measured by ELISA Radiographic grading of OA in the knee was performed by the KL criteria	Visfatin positively correlated with type II collagen and aggrecan degradation Visfatin may be involved in cartilage matrix degradation
Streich *et al.*, 2011[19]	The aim of this study was to determine the value of urinary biomarkers in the diagnosis of chondral defects after ACL rupture	Cross-linked uCTX-I, uCTX-II and sCOMP	Human; 38 patients with previous ACL rupture were included	uCTX-I, uCTX-II and sCOMP could be used to identify patients with focal cartilage lesions from an early stage of OA
Hao *et al.*, 2011[25]	The aim of this clinical study was to investigate adiponectin levels in plasma and synovial fluids of female patients with knee OA and to analyze the correlation between adiponectin and degradation markers of cartilage matrix in synovial fluid	Adiponectin	Human; see the primary article for full details of patients and assays used	Adiponectin may be involved in the regulation of the degradation of cartilage matrix in OA

Table II
Summary of selected OA biomarker studies that specifically used proteomic tools and technologies (2011–2012)

Reference	Study type	Biomarker identified/proteins involved	Species, tissue types, assays and techniques used	Main outcome of the study
Wang *et al.*, 2012	Basic study using genetically engineered mice and three different mouse models of OA	C5, C6, the complement regulatory protein CD59a and the MAC Expression of inflammatory and degradative molecules was lower in chondrocytes from destabilized joints from C5-deficient mice than C5-sufficient mice, and MAC induced production of these molecules in cultured chondrocytes. MAC colocalized with MMP-13 and with activated ERK around chondrocytes in human OA cartilage	Mouse; human; see the primary article for full details	Dysregulation of complement in synovial joints plays a key role in the pathogenesis of OA
Vincourt *et al.*, 2012[60]	Clinical study combined with *in vitro* experiments	Matrilin-3 (MATN3)	Human; synovial fluid from human OA patients	MATN3 switched from anti- to pro-anabolic upon integration to the ECM MATN3 downregulated cartilage ECM synthesis and upregulated catabolism when administered as a soluble protein
Mateos *et al.*, 2012[51]	Clinical study of synovial fluids from OA and RA patients	Pooled RA samples: proteins related to complement activation, inflammation and the immune response (major MMPs and several neutrophil-related proteins) Pooled OA samples: fibronectin, kininogen-1, cartilage acidic protein 1 and COMP	Human; immunodepletion, size fractionation, in-gel digestion, reverse-phase peptide separation, MALDI-TOF/TOF, and spectral analysis	One hundred and thirty-six different proteins were identified in synovial fluid Some of the proteins identified are likely to be involved in the etiopathogenesis of RA and OA as putative disease biomarkers and their presence in synovial fluid may be a prelude to their dilution in serum
Onnerfjord *et al.*, 2012[61]	Basic study describing the compositional analysis of ECM proteins in articular cartilages, meniscus, intervertebral disc, rib and tracheal cartilages by relative quantification Compared the relative abundance of 150 proteins	Matrilin-1 and epiphycan in rib and trachea. Asporin in the meniscus. Lubricin in the nucleus pulposus of the intervertebral disc. Asporin, CILP and COMP were difficult to extract may be cross-linked	Human; tissue extraction, trypsin digestion, 2D LC-separations coupled to tandem mass spectrometry, relative quantification with isobaric labeling, iTRAQ	Distinct differences in protein patterns may relate to different tissue mechanical properties, and to the intriguing tropism in different patterns of joint pathology
Sun *et al.*, 2012[62]	Clinical study of human patients with ankylosing spondylitis (AS), RA and OA	Alpha 1-anti-trypsin (ATA1) expression was increased synovial membranes of AS compared with samples from RA and OA	Human; western blotting; immunohistochemistry; Q-PCR (Taqman method)	The study reports increased expression of ATA1 in the synovial tissues of patients with AS
Chiaradia *et al.*, 2012[63]	Clinical study of equine OA and osteochondrosis (OC) using equine synovial fluids	Proteins involved in inflammation, coagulation, oxidative stress and matrix damage	Equine; differential proteomic analysis	Deregulated proteins in OA and OC included inflammatory components, coagulation pathways, oxidative stress and matrix damage, suggesting pathological alterations in articular homeostasis, plasma-synovial fluid exchange, joint nutritional status and vessel permeability
Katano *et al.*, 2011[64]	Clinical study using synovial fluids from OA and RA patients	S100 calcium-binding protein A8 (S100A8)	Human; ELISA; antibody arrays; real-time PCR	S100A8 was significantly elevated in synovial fluids of patients with RA compared to OA patients and may be involved in the exacerbation of RA
Calamia *et al.*, 2011[64]	Basic study of protein modifications inuced by the pro-inflammatory cytokine IL-1β	Cellular chaperones were upregulated concurrent with a down-regulation of the actin cytoskeleton Upregulated by IL-1β: pro-inflammatory mediators, proteases and proteins involved in the transforming growth factor-beta (TGF-β) pathway Downregulated by IL-1β: aggrecan vitamin K-dependent proteins and thrombospondin	Human; stable isotope labeling with amino acids in cell culture (SILAC) technique	Metabolic labeling of chondrocytes enables the quantitative analysis of changes induced by IL-1β treatment
Pan *et al.*, 2011[65]	Clinical study exploring the use of proteomic methods for the identification of OA biomarkers	No specific proteins identified; two peptide peaks were found as potential diagnostic markers for OA	Human; MALDI-TOF-MS	This study highlights the value of proteomic methods for identification of potential biomarkers of OA
Fernandez-Puente *et al.*, 2011[66]	The aim of this study was to identify novel protein biomarkers of moderate and severe OA in serum	Complement components, lipoproteins, von Willebrand factor, tetranectin, and lumican	Human; sera from 50 moderate OA patients, 50 severe OA patients, and 50 non-symptomatic controls. Serum protein levels were analyzed using isobaric tags for relative and absolute quantitation (iTRAQ) and MALDI-TOF/TOF MS	A number of biomarkers were identified but the specificity and selectivity of these candidates need to be validated before new molecular diagnostic or prognostic tests for OA can be developed

Table III
Review articles published including the keywords 'biomarker' and 'osteoarthritis' (2011–2012)

Reference	Purpose of the review
Rousseau and Garnero, 2012[67]	The aim of this review was to use the BIPED classification that appeared in 2006 for OA biomarkers to describe the potential usage of particular biomarkers
Patra and Sandell, 2011[9]	This review highlights some of the biochemical biomarkers in current use in OA, their applications and limitations The authors highlight the potential of omics technologies such as lipidomics and metabolomics for detecting early phenomena in OA
Henrotin 2012[13]	This review is a summary of selected studies related to soluble biomarkers published between 1 September 2010 and 30 August 2011 identified through a PubMed search using the terms "biomarker" and "osteoarthritis"
Patra and Sandell, 2011[68]	This paper reviews recent advances in OA biomarkers and discusses the application of proteomic technologies that have generated several new, non-conventional biomarkers that could allow better profiling of OA The authors propose that biomarker combinations have the ability to subgroup the heterogenous OA population to allow a better scrutiny of diagnosis and treatment options
van der Kraan and van den Berg 2012[69]	This paper comprehensively and elegantly reviews the literature on the role and regulation of chondrocyte terminal differentiation (hypertrophy-like changes) in OA and integrates this information in a conceptual model of primary OA development
Hoch *et al.*, 2011[70]	The purpose of this systematic review and meta-analysis was to answer the following questions: (1) Is sCOMP elevated in patients with radiographically diagnosed knee OA compared to controls? (2) Are there differences in sCOMP levels when comparing differing radiographic OA severities to controls? The authors conclude that sCOMP is elevated in patients with knee OA and is sensitive to OA disease progression and future research studies with a higher level of evidence should be conducted to investigate the use of this biomarker as an indicator for OA development and progression
Madry *et al.*, 2012[71]	The aim of this systematic review was to highlight the molecular basis and histopathological features of early OA Special emphasis is placed on early changes in subchondral bone and other structures of the joint, such as the menisci, the synovial membrane, the joint capsule, ligaments, muscles and the infrapatellar fat pad
Claessen *et al.*, 2012[72]	In this paper the authors conducted a systematic review of reported associations between circulating insulin-like growth factor-1 (IGF-1) and/or IGF-1 gene polymorphisms and radiographic OA No association was found between serum IGF-1 and the occurrence of radiographic OA (moderate level of evidence), and a positive relationship between IGF-1 gene polymorphisms and radiographic OA (moderate level of evidence); however the confounding effect of BMI was insufficiently addressed The authors propose that future well-designed prospective studies should further elaborate the role of the complex GH/IGF-1 system in primary OA
Gharbi *et al.*, 2011[73]	The aim of this elegant review was to gather most of the available information relating to proteomic techniques and their applications to OA research
Mobasheri 2011[74]	This perspective article discusses the relevance and potential of proteomics for studying age-related musculoskeletal diseases such as OA and reviews the contributions of key investigators in the field

between groups included monocyte chemoattractant protein-1 [MCP-1], interleukin-8 [IL-8], keratinocyte-derived chemoattractant [KC], MMP-2 and MMP-3. The sensitivities and specificities of these markers were also calculated to determine their diagnostic usefulness in a future biomarker panel. Interestingly, synovial fluid IL-8 was the most sensitive of the markers although MCP-1 was also highly sensitive and specific. The authors propose that this "panel" of biomarkers may differentiate between cruciate disease and other types of OA. This is a novel and interesting concept that should be borne in mind in our future studies on OA biomarkers.

A similar study carried out by Streich *et al.*, 2011[19] in humans determined the value of urinary biomarkers in the diagnosis of chondral defects after anterior cruciate ligament (ACL) rupture. The authors measured the levels of cross-linked C-terminal telopeptide I (uCTX-I), uCTX-II and sCOMP in human patients with previous ACL rupture were included. The results suggest that these biomarkers may be used to identify patients with focal cartilage lesions from an early stage of OA.

González-Álvaro *et al.*,[20] determined whether interleukin-15 (IL-15) levels in serum IL-15 (sIL-15) could serve as useful biomarkers of disease severity in patients with early OA. The authors performed multivariate longitudinal analyses to show that sIL-15 may predict a severe disease course in patients with early OA and this group of patients may benefit from receiving intensive treatments. This study also reminds us that there may be temporal and joint specific changes in the expression of certain cytokines in OA.

A study by Cattano *et al.*,[21] examined biochemical differences between OA knees with and without effusion. The authors measured MMP-3, TIMP-1, TIMP-2, and interleukin-10 (IL-10) and found these to be significantly higher in the knees with effusion than in the knees without effusion. The measurements were done using custom multiplex ELISAs on synovial fluid samples. This study highlights the fact that joint effusions are important inflammatory events in subsets of OA patients and may be characterized using distinct biochemicals.

Intraarticular injections are important treatments for patients with knee OA. Recent work by Conrozier *et al.*,[22] investigated the effect of intraarticular injections with hyaluronic acid (HA) on urinary type II collagen C-telopeptide (uCTX-II) and serum hyaluronic acid (S-HA). These two markers significantly decreased compared to baseline in treated patients suggesting that HA may hinder the degradation of type II collagen in knee OA. It is likely that intraarticular injections with HA and corticosteroids can help subsets of patients with knee OA. Therefore biomarkers that can demonstrate the effectiveness of intraarticular injections can help to further develop and improve intraarticular formulations for cohorts of patients who may benefit from such treatments.

Visfatin is a newly discovered adipocyte hormone (adipokine) with a direct relationship between plasma visfatin level and type 2 diabetes mellitus. Visfatin was originally cloned as a putative cytokine shown to enhance the maturation of B cell precursors in the presence of interleukin-7 (IL-7) and stem cell factor, it was therefore named "pre-B cell colony-enhancing factor" (PBEF)[23]. Visfatin is also known as nicotinamide phosphoribosyltransferase – an enzyme that catalyzes the condensation of nicotinamide with 5-phosphoribosyl-1-pyrophosphate to yield nicotinamide mononucleotide, an intermediate in the biosynthesis of nicotinamide adenine dinucleotide (NAD). It is the rate-limiting component in the mammalian NAD biosynthesis pathway. A clinical study by Duan *et al.*,[24] investigated visfatin levels in synovial fluids and plasma of patients with primary knee OA and established its relationship with biomarkers of cartilage degradation in synovial fluid. Visfatin positively correlated with the degradation of type II collagen and

aggrecan. This study suggests that visfatin may be involved in cartilage matrix degradation. Further clinical and basic studies are required in to unravel the role of visfatin in joint inflammation and cartilage degradation.

Another study by Hao *et al.*, 2011[25] also focused on adipokines by investigating adiponectin levels in plasma and synovial fluids of female patients with knee OA. Adiponectin is an important adipokine involved in the control of fat metabolism and insulin sensitivity, with anti-diabetic, anti-atherogenic and anti-inflammatory activities. The aim of this study was to analyze the correlation between adiponectin and markers of cartilage matrix degradation in synovial fluid. Adiponectin may be involved in the regulation of the degradation of cartilage matrix in OA. It is possible that adiponectin may be involved in anti-inflammatory activities in early OA.

Omentin-1 (also known as intelectin 1) is an adipokine that is predominantly expressed in visceral adipose tissue. The levels of omentin-1 mRNA are significantly higher in visceral adipose tissue compared to other types of adipose tissues. Although omentin-1 has been shown to have no effect on basal glucose uptake in adipocytes, it does enhance insulin-stimulated glucose uptake and increases AKT (protein kinase B) phosphorylation. Therefore, this adipokine has the capacity of modulating glucose metabolism and insulin signaling. Xu *et al.*,[26] used ELISAs to measure omentin-1 levels in serum and synovial fluid of patients with knee OA and correlated the values with radiographic disease severity using the Kellgren–Lawrence (KL) grading system. There were no significant differences in serum omentin-1 levels between patients with OA and healthy controls. There were also no significant differences in serum omentin-1 levels among patients with OA with different KL grades. However, synovial fluid omentin-1 levels decreased significantly as the KL grades increased. The negative correlation between synovial fluid levels of omentin-1 levels and radiographic severity of knee OA suggests that synovial omentin-1 is a biomarker reflecting degenerative processes in the knee.

The studies by Duan *et al.*,[24], Hao *et al.*,[25] and Xu *et al.*,[26] suggest that adipokines are involved in various forms of OA whereas the study by van Spil and co-workers[14] suggests that adipokines are not involved in cartilage metabolism. Clearly, this is an active area of research and further studies are required to explain the differing outcomes of these studies.

Technological and methodological advances are important for progress in OA biomarker research. Song *et al.*,[27] developed a method to detect COMP using fluoro-microbead guiding chip (FMGC)-based immunoassays and compared it with commercial ELISAs for COMP. The FMGC-based immunoassay clearly distinguished immunospecific from nonspecific binding and thus offers a new approach for detecting COMP and other clinically relevant OA biomarkers in human blood and synovial fluid.

Fibulin-3 peptides (Fib3-1 and Fib3-2) – potential biomarkers of OA

One of the most exciting discoveries over the last year has been the identification of fibulin peptides in sera from OA patients. Fibulins are a family of proteins that belong to a multigene family with seven members. They are associated with basement membranes and the ECM[28]. They are secreted glycoproteins that become incorporated into a fibrillar ECM. The known members of the fibulin family share an elongated structure with many calcium-binding sites, owing to the presence of tandem arrays of epidermal growth factor (EGF)-like domains[29]. They have overlapping binding sites for basement-membrane proteins, fibrillin, fibronectin and proteoglycans, and they participate in diverse supramolecular structures. The amino-terminal portion of fibulins contain repeated elements with potential disulfide loop structure resembling that of the complement component anaphylatoxins C3a, C4a, and C5a as well as proteins of the albumin gene family and the remaining portion of the molecules consists of a series of EGF-like repeats[29]. There are reports of animal models of fibulin deficiency and human fibulin gene mutations[28]. However, little is known about the pathological roles of members of this family.

Recent studies by Henrotin and co-workers suggest that fibulin-3 peptides (Fib3-1 and Fib3-2) are potential biomarkers of OA[30]. Fibulin 3 is also known as epidermal growth factor-containing fibulin-like extracellular matrix protein-1 (EFEMP1) and epidermal growth factor (EGF) containing fibulin-like ECM protein. The EFEMP1 gene encodes a protein that contains tandemly repeated EGF-like repeats followed by a C-terminus fibulin-type domain. The protein can bind the EGF-receptor (EGFR), inducing EGFR autophosphorylation and activating downstream signaling pathways. It may also function as a negative regulator of chondrocyte differentiation. The authors set out to identify new OA biomarkers by using proteomics. They focused on differentially expressed proteins and found that Fib3-1 and Fib3-2 are present in cartilage and serum of OA patients. They used immunoassays to study the distribution of Fib3-1 and Fib3-2 in cartilage and developed specific immunoassays to detect and quantify them. Expression of both peptides was significantly elevated in the superficial layer of fibrillated cartilage from OA patients. This study is a good example of how a proteomics-based workflow can be applied in new OA biomarker discovery. The authors compared urine samples from women with OA and healthy age-matched controls to quantify differentially expressed proteins by employing differential in gel electrophoresis (DIGE). They identified two fibulin (Fib) peptides as increased in OA samples. Specific ELISAs were developed against these peptides and validated in a larger population of patients. The data from Henrotin *et al.*, suggest that both Fib3-1 and Fib3-2 can be used to discriminate normal and OA samples population, and thus both peptides are potential biomarkers of OA.

Follistatin-like protein 1 (FSTL1): a novel OA biomarker in serum

Recent work suggests that FSTL1 is a serum OA biomarker with the capacity to reflect the severity of joint damage in OA patients[31]. FSTL1 is a secreted glycoprotein that has been implicated in arthritis. The FSTL1 gene encodes a protein similar to follistatin, an activin-binding protein. FSTL1 contains an FS module, a follistatin-like sequence containing 10 conserved cysteine residues. The FSTL1 protein has a multi-specific binding nature; it can bind heparin and may modulate the action of TGF-β superfamily growth factors on cell proliferation and differentiation[32]. This gene product is thought to be an autoantigen associated with RA[33,34, b]. It is overexpressed in RA synovium, the product of which exerts inhibitory activity on synovial cell growth. FSTL1 is expressed in the synovial tissues of RA, but its polymorphisms are not associated with genetic susceptibility[35]. Although new polymorphic sites have been identified for this gene, they are not specifically associated with susceptibility to RA, suggesting that overexpression of FSTL1 is a secondary consequence of inflammatory changes in the synovial environment of RA. In addition, FSTL1 is upregulated in the early stages of collagen-induced murine arthritis and it can exacerbate the disease when delivered by gene transfer[36]. It is overexpressed in human arthritis and its neutralization inhibits murine collagen-induced arthritis and suppresses interferon-gamma (IFN-gamma) and chemokine 10 (CXCL10) production in arthritic joints[36].

[b] http://www.genecards.org/cgi-bin/carddisp.pl?gene=FSTL1.

The aim of the study by Wang and co-workers was to study FSTL1 expression in OA cartilage and synovium and assess its potential utility as a biomarker of joint damage in OA patients. They detected FSTL1 expression by real-time polymerase chain reaction (PCR), western blot and immunohistochemistry in articular cartilage and synovial tissues from OA patients and control patients without OA (age-matched trauma samples). The concentration of FSTL1 in serum and synovial fluid was measured by ELISA assays in OA and control samples. FSTL1 mRNA and protein levels were elevated in the synovial tissues from OA patients compared with control trauma patients. FSTL1 was strongly expressed in the cytoplasm of synovial cells and capillary endothelial cells, but its expression was weak in chondrocytes from OA cartilage. Serum and synovial FSTL1 concentrations were significantly elevated in OA patients compared to controls. Interestingly, serum and synovial FSTL1 levels were higher in female OA patients. Serum FSTL1 levels in female OA patients statistically correlated with KL grade, joint space narrowing (JSN) and the Western Ontario McMaster and Universities Osteoarthritis (WOMAC) indices. Serum FSTL1 levels also correlated significantly with age and disease progression. FSTL1 is known to be upregulated in RA. This study suggests that this protein may a potentially useful serum biomarker in OA patients as well. The authors suggest that FSTL1 that may reflect the severity of joint damage, and may have potential for monitoring the course of disease progression and the efficacy of new pharmacotherapies in OA patients[31].

FSTL1 plays a central role in arthritic diseases by enhancing IFN-γ signaling pathways and activating cellular and molecular mechanisms that bridge innate and adaptive immune responses[36]. Elevated serum FSTL1 levels is associated with both OA[31] and RA[33]. It reflects not only joint diseases but also inflammation and tissue degradation in systemic autoimmune diseases[33]. Serum FSTL1 levels may thus serve as a serological inflammatory marker of disease activity in OA and RA patients and may form the basis of new biomarker assays.

Complement proteins as membrane biomarkers of OA

The complement system consists of a large array of proteins that interact in a carefully regulated manner to destroy invading microorganisms and prevent the deposition of immune complexes in healthy tissues. Complement can be activated by diverse mechanisms signaling through distinct pathways. However, the key players involved in killing cells are just five proteins: C5b, C6, C7, C8 and C9. Although none of these possess enzymatic, proteolytic or lipolytic activity, they converge on a final common pathway and assemble into a multi-molecular complex known as the membrane attack complex (MAC)[37]. MAC is typically formed as a result of the activation of the alternative pathway of the complement system, as one of the effector systems of the immune system. MAC forms transmembrane channels that disrupt the phospholipid bilayer of target cells, leading to cell lysis and cell death (Fig. 3). A number of complement proteins participate in the assembly of the MAC. The first step involves binding of activated C5b to C6 to form a C5b-6 complex. This complex then binds to C7 forming the C5b-7 complex. The C5b-7 complex then binds to C8 forming the C5b-8 complex. C5b-8 subsequently binds to C9 and acts as a catalyst in the polymerization of C9. The active MAC complex consisting of C5b-C6-C7-C8-C9 then inserts into target cell membranes to form a functional pore, resulting in ion flux and ultimately osmotic cell lysis.

Traditionally OA has been viewed as a biomechanical disease resulting from 'wear and tear' and the breakdown of articular cartilage in synovial joints. Recent research has focused on the role of inflammation in OA and evidence for an inflammatory

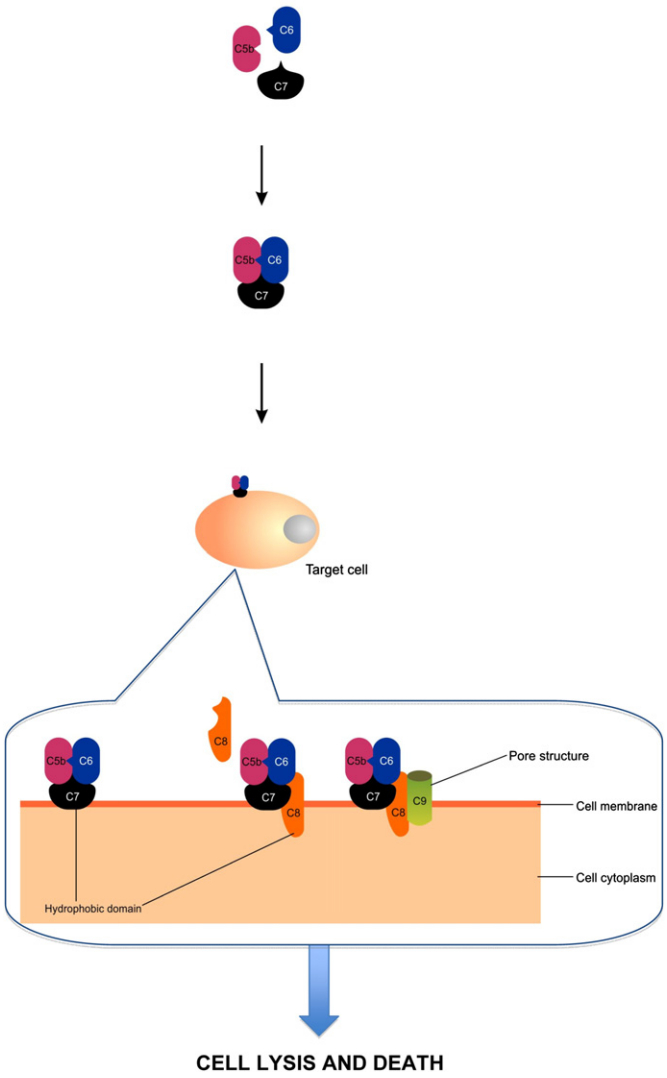

Fig. 3. The MAC of complement. The MAC is a multi-molecular assembly, which involves the following proteins: C5, C6, C7, C8, and C9. These proteins participate directly in the killing of target cells[37]. The formation of this complex is toxic to chondrocytes[39] and has been implicated in OA[38,51] and RA[40–43].

component to OA is increasing despite OA often being regarded as a mechanical and non-inflammatory disease. Recent work by Wang and co-workers suggests that the 'MAC' component of complement is involved in the pathogenesis of OA and complement proteins are aberrantly expressed in OA[38]. The authors attempted to clarify the role of low-grade inflammation in OA by identifying a central role for the complement system in disease pathogenesis. Proteomic and transcriptomic analyses of synovial fluids and membranes from individuals with OA revealed that the expression and activation of complement is abnormally high in human OA joints. The authors used knockout mice genetically deficient in complement component 5 (C5), C6 or the complement regulatory protein CD59a to show that the MAC component of complement is important for disease development in three different murine models of OA. Expression of inflammatory and matrix degrading molecules was lower in chondrocytes from destabilized joints from C5-deficient mice and MAC induced production of these molecules in cultured chondrocytes. The authors used immunohistochemistry and immunofluorescence techniques to colocalize MAC with matrix metalloproteinase-13 (MMP-13) and with activated extracellular

signal-regulated kinase (ERK) around chondrocytes in human OA cartilage.

We have known for more than 20 years that complement is involved in RA and is toxic to chondrocytes[39]. MAC is an initiating mediator inducing the inflammation associated with RA[40]. Complement activation contributes to pathological processes in a number of autoimmune and inflammatory diseases, including RA[41]. Other researchers have previously suspected that complement may be involved in the pathogenesis of arthritis because cartilage from patients with RA and from animals with antigen-induced arthritis is frequently contaminated with complement-containing immune complexes[42]. A possible role for complement in cartilage degradation was modeled by Satsuma and co-workers in 1993[39]. They carried out *in vitro* experiments by exposing chondrocytes to homologous serum, and determining cytotoxicity. Complement activation was found to be highly cytotoxic and the toxicity can be ablated by heat or methylamine treatment but not by ethylene glycol tetraacetic acid (EGTA), suggesting that alternate route of complement is involved in the pathogenesis of cartilage degradation in inflammatory arthritis. However, the international team of Wang and co-workers[38] was the first to shown that inhibiting the complement system could help slow disease progression in OA. Their data provides novel and mechanistic evidence linking the dysregulation of complement in synovial joints and the pathogenesis of OA[38]. Furthermore, the pharmacological modulation of complement that they carried out in wild-type mice confirmed the results obtained with mice genetically deficient in complement proteins.

The accumulating evidence suggests that activation of the complement system is a dynamic calcium dependent process that occurs in both OA and RA. This opens up novel and potentially disease-modifying strategies for developing drugs capable of targeted and controlled inhibition of complement for the treatment of inflammation in OA[c]. The tantalizing possibility of controlled pharmacological manipulation of various components of the complement system is encouraging news for the pharmaceutical industry at a time when economic pressures have forced the closure of OA research divisions in many drug companies and the abandonment of OA as a difficult disease target. The interesting and exciting paper by Wang and colleagues provides a much needed boost and reinvigorates the development of therapeutic strategies for targeting the inflammatory components of complement and may result in drugs capable of controlling joint inflammation and preventing joint destruction[43].

Post-translational modifications in ECM molecules – deamidated COMP

There is increasing interest in post-translational modification of ECM macromolecules. Post-translational modifications increase the complexity of the proteome by increasing the molecular variants of proteins. The modified protein in turn may have altered biochemical, enzymatic and physiological functions and may participate in disease processes. There are numerous types of post-translational modifications and these can be classified into natural and synthetic sub-groups. Natural post-translational modifications include phosphorylation, methionine oxidation, deamidation, glycosylation and ubiquitination. Traditionally phosphorylation of chondrocyte proteins has been studied in the context of cell signaling and it is well-known that the load-bearing properties of many ECM macromolecules are the consequence of sulfation and glycosylation.

Deamidation is a common post-translational modification that results in the conversion of an asparagine residue to a mixture of isoaspartate and aspartate residues. Deamidation is a biologically significant phenomenon in a large percentage of cellular proteins[44]. Deamidation causes time-dependent changes in the charge and conformation of peptides and proteins[44] and has been described as a 'molecular timer' that controls protein turnover[45]. It is well-established that ECM proteins age, as does cartilage. During the aging process ECM molecules accumulate non-enzymatic post-translational modifications that cannot be reversed or repaired. In an elegant recent study Catterall and co-workers hypothesized that post-translational modifications could be used to monitor the loss of ECM macromolecules in OA[46]. To test this hypothesis, they focused on COMP, a well-established OA biomarker and predicted sites of deamidation, which were confirmed, by mass spectroscopy (MS). They found evidence for the presence of deamidated (Asp(64)) and native (Asn(64)) in COMP and calculated the relative contribution of deamidated COMP (D-COMP) to native COMP in cartilage. In addition, they developed a new D-COMP-specific ELISA using the monoclonal antibody 6-1A12An. The ELISA assay was used to study D-COMP levels in the serum of patients undergoing joint replacement surgery. Interestingly, they found that serum D-COMP but not total COMP declined significantly after joint replacement, demonstrating a joint tissue source for D-COMP. The study was further strengthened by analysis of 450 participants from the Johnston County OA Project, a population-based study of knee and hip OA in rural North Carolina[47]. In this study, controlling for age, gender, and race, allowed D-COMP to be associated with radiographic hip but not knee OA severity. In contrast, total COMP was associated with radiographic knee but not hip OA severity. D-COMP levels were higher in extracts from hip cartilage proximal to OA lesions compared with samples from sites remote from the lesions. Although total COMP did not vary by joint site or proximity to the lesion, D-COMP did.

Previous studies of participants from the Johnston County OA Project have shown that s-COMP reflects the presence, severity and multiple joint involvement in OA by distinguishing an OA-affected subgroup from an unaffected subgroup[48]. Serum COMP may be useful as a biomarker of pre-radiographic hip joint pathology[49] and its levels also vary by ethnicity and sex[50]. The study by Catterall *et al.*, refines and enhances the previous work in this area by focusing on the utility of D-COMP as an OA biomarker. The authors demonstrate, for the first time, that post-translational modifications in COMP are important and are linked with aging and the disease process. D-COMP appears to be the first biomarker to show specificity for a particular joint site. The presence and enrichment of D-COMP in articular cartilage and the systemic circulation opens up new possibilities for studying post-translational modifications in other cartilage ECM macromolecules, and the development of new assays capable of estimating the ratio between total the amount of protein and the post-translationally modified fraction. The authors propose that this reflects a fundamental underlying difference between hip and knee cartilage turnover and repair responses in OA and the deamidated epitope in hip OA cartilage indicates a lesser repair response of hip OA compared with knee OA cartilage.

This elegant and original article from the Kraus group highlights how it makes sense both 'biologically' and 'systemically' to develop assays for biologically relevant post-translational modifications. D-COMP may turn out to be a longitudinal marker for following disease progression within a human subject or a cohort of patients. The D-COMP assay may also detect occult or pre-radiographic hip disease. The future is looking bright for OA biomarker research especially if more intuitive approaches such as this are employed.

[c] Haas MJ. Big MAC attack in osteoarthritis. *SciBX* 2011;**4**(47). http://dx.doi.org/10.1038/scibx.2011.1311.

Proteomic studies

Quantitative and high-throughput proteomic techniques have made important contributions to the discovery of complement components, lipoproteins and lower-abundance ECM components in body fluids from OA patients. Table II summarizes some of the key papers published between September 2011 and April 2012.

One of the most interesting clinical proteomics papers was published by Mateos *et al.*,[51]. The authors used high performance liquid chromatography (LC) coupled to MS [LC-matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)/TOF] to identify differentially expressed proteins in the synovial fluid of OA and RA patients[51]. The strategy they used involved pooling 20 samples from each disease state into OA and RA groups. According to the authors, this was done to "reduce the contribution of extreme individual values". Samples were immunodepleted and the most abundant plasma proteins were selectively removed to enrich the lower-abundance proteins. The samples were then subjected to protein size fractionation, in-gel digestion and reverse-phase peptide separation in a nano-LC system. Peptide identification was done using a MALDI-TOF/TOF system. This strategy led to the identification of 136 different proteins. Spectral counting revealed a greater abundance of proteins involved in complement activation, inflammation and the immune response. In addition to MMP-1 and several neutrophil-related proteins they identified elevated levels of fibronectin, kininogen-1, cartilage acidic protein 1 and COMP. The authors verified the results obtained for MMP-1, transforming growth factor-beta-induced protein (BGH3), fibronectin and gelsolin by western blotting. Mateos *et al.*, conclude that this clinical proteomics approach may help identify novel proteins and putative disease-relevant biomarkers[51]. The elevated levels of these proteins in synovial fluid may be linked to the etiopathogenesis of RA and OA and a prelude to increased levels of the protein in serum. This study pushes the boundaries and sets new standards for clinical studies of OA patients using proteomics.

Combining proteomics and microscopic imaging

A new technique known as MALDI imaging mass spectrometry (IMS) has been developed for correlating protein expression to specific anatomical areas of joint tissues[52]. This technology has been applied to the analysis of synovial tissues from OA and RA patients. This new technique is powerful and allows morphological data from synovial tissue to be combined and correlated with proteomics. A pathologist initially examines photomicrographs of the stained tissue images and areas of interest are marked digitally. The histology-annotated images are then merged to form a photomicrograph of the section taken before the application of MALDI. Proteins identifications are then linked back to the anatomical locations. MALDI IMS is a powerful tool for the rapid *in situ* detection of proteins in cartilage and synovial tissue sections[52]. Further refinements in this technology and the addition of quantitative capability will make this one of the most powerful technologies in future studies of OA biomarkers.

Conclusions

The incidence of OA is steadily rising throughout the world not only in the aging population, but also in middle-aged individuals. Cartilage degradation and structural changes in subchondral bone result in the production of fragments of ECM molecules. Many of these are well-known biochemical markers or "biomarkers". Biomarkers may be useful markers of disease progression and can be detected in blood, serum, synovial fluid, and urine using traditional biochemical and immunological assays.

Systems biology is increasingly applied in orthopedics and rheumatology to cartilage and synovium. "Omics-based" technologies used in systems biology hold special promise for identifying new biomarkers. These techniques include genomics, transcriptomics, proteomics, metabolomics, glycomics, and bioinformatics and can be applied to the study of cartilage, synovium, synovial fluid, and even blood (serum) or urine from OA patients. Current "omics-based" research aims to develop an "analytical toolbox", which is hoped will contribute to the clinical development process[53,54].

Proteomics involves the application of specialized analytical techniques that allow the evaluation of the protein composition of tissues, cells, and culture supernatants. Proteomics is being increasingly applied in basic cartilage biology and OA research. Proteomic screening techniques are also making significant contributions to the discovery of novel OA biomarkers in serum and urine. Many of the biomarkers identified indicate normal cartilage turnover, tissue repair, or ECM remodeling whereas others reflect catabolic events during the process of cartilage degradation in the later stages of OA. However, there is a need for standardization and consensus guidelines for clinical proteomics. These techniques require further development, standardization, clinical testing and validation. Future progress will require deposition of proteomic datasets in publicly accessible databases and archives. At the present time the databases are too small and contain scant data. Expanding the databases will allow more detailed bioinformatic studies. Combining biochemical markers with tissue and cell imaging techniques and innovative bioinformatic approaches (i.e., machine learning, clustering, data visualization) are likely to increase the predictive power of future 'combination biomarkers'.

Post-translational modifications are biologically significant phenomena in a large percentage of proteins. Future research should focus on post-translational modifications of cartilage ECM proteins with aging and OA. Some post-translational modifications serve as 'molecular timers' that control protein turnover and are likely to have pathophysiological relevance to OA and other joint diseases.

Biomarkers reflect diverse biological activities. It is therefore important to discriminate between catabolic and maintenance events. The definition of 'biomarker' will need to reflect these diverse processes and the validation and qualification processes for novel biomarkers will ultimately depend on the context and specific purpose of their intended applications. New biomarkers require validation according to the Burden of Disease, Investigative, Prognostic, Efficacy of Intervention and Diagnostic and Safety ('BIPEDS') criteria for detecting early, pre-radiographic changes in joints[55]. Combinations of existing and new biomarkers may improve their prognostic accuracy and help identify at-risk patients[56].

Author contributions

Ali Mobasheri drafted the review article and approved the final version to be published.

Study conception and design: Mobasheri.

Acquisition of data: Mobasheri.

Analysis and interpretation of data: Mobasheri.

Role of the funding source

The funding bodies that support my research did not influence any aspect of the research reviewed. They had no role in the design of the study, data collection, analysis and interpretation of the data, the writing of the manuscript or in the decision to submit the manuscript.

Conflict of interest

The author declares that there are no conflicts of interest.

Acknowledgments

I am grateful to Dr Francois Rannou and the program committee of OARSI 2012 for inviting me to present the OA Year in Review 2012 lecture on biomarkers at the 2012 OARSI World Congress in Barcelona.

I wish to acknowledge Dr Stefan Lohmander, the Editor-in-Chief of Osteoarthritis and Cartilage for commissioning this review.

I would like to apologize to all those colleagues whose recent work could not be cited due to space and article length limitations.

My research is supported by grants from the Biotechnology and Biological Sciences Research Council (BBSRC; grants BBSRC/S/M/2006/13141 and BB/G018030/1), the Engineering and Physical Sciences Research Council (EPSRC), the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs; grant number: Mobasheri A. 28102007) and the Wellcome Trust (Grant no. CVRT VS 0901).

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