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Review

Osteoarthritis Year in Review 2014: we need more biochemical biomarkers in qualification phase

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SUMMARY

The current diagnosis of osteoarthritis (OA) relies on the description of pain symptoms, affected joint stiffness, and radiography used as the reference technique for determining the grade of joint destruction. Limitations of the presently available diagnostic tests have provided an impetus for the substantial increase in interest in finding new specific biological markers for cartilage degradation to facilitate the early diagnosis of joint destruction, evaluate disease progression and improve disease prognosis. Biomarkers for OA are also useful for drug development, treatment monitoring, and as a basis for personalized evidence-based action plans. This review summarizes 29 manuscripts published during 2013 with a focus on soluble biochemical biomarkers, primarily those utilizing proteomic, metabolomics, lipidomic and imaging mass spectrometry technologies.

Introduction

An important objective for osteoarthritis (OA) research is the conceptualization and development of early diagnostic strategies. OA is clinically silent in most individuals during its initial stages, therefore extensive deterioration of cartilage already exists at the time of diagnosis. The current diagnosis of OA relies on the subjective description of pain symptoms by patients, affected joint stiffness, and radiography used as the reference technique for determining the grade of joint destruction. Limitations of the presently available diagnostic tests have provided an impetus for the increased interest in finding new specific biological markers for cartilage degradation to facilitate the early diagnosis of joint destruction, evaluate disease progression and improve disease prognosis.

A biomarker has been defined 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.” Biomarkers for OA are also useful for drug development, treatment monitoring, and as a basis for personalized evidence-based action plans. This “Year in Review” manuscript will focus on soluble biochemical biomarkers, primarily those studies utilizing proteomic and metabolomics technologies.

Methodology

Relevant articles and abstracts were identified through a PubMed/MEDLINE and EMBASE search of English language articles published between April 1, 2013 and April 1, 2014. The initial search strategy included the terms: osteoarthritis, biomarker, biomarkers, biological marker, proteomics, lipidomics, and metabolomics. The initial search yielded 153 articles. Human studies were then given preference over animal studies and biomarkers other than biochemical biomarkers were eliminated from consideration. Finally, 29 relevant articles were selected by the author according to their quality. In this review, the descriptions of and comments on the selected papers follow the phases of biomarker development shown in Fig. 1.

Phase I: discovery phase

Biomarker research involves a series of steps moving from discovery to the launch of a commercial biomarker product (Fig. 1). Proteomics and metabolomics have generated great expectations for discovery of biomarkers to improve the diagnosis of a wide range of diseases. There are two general approaches for proteomic biomarker discovery: global/nondirected and target-specific. Because global/nondirected approaches are unbiased and high-

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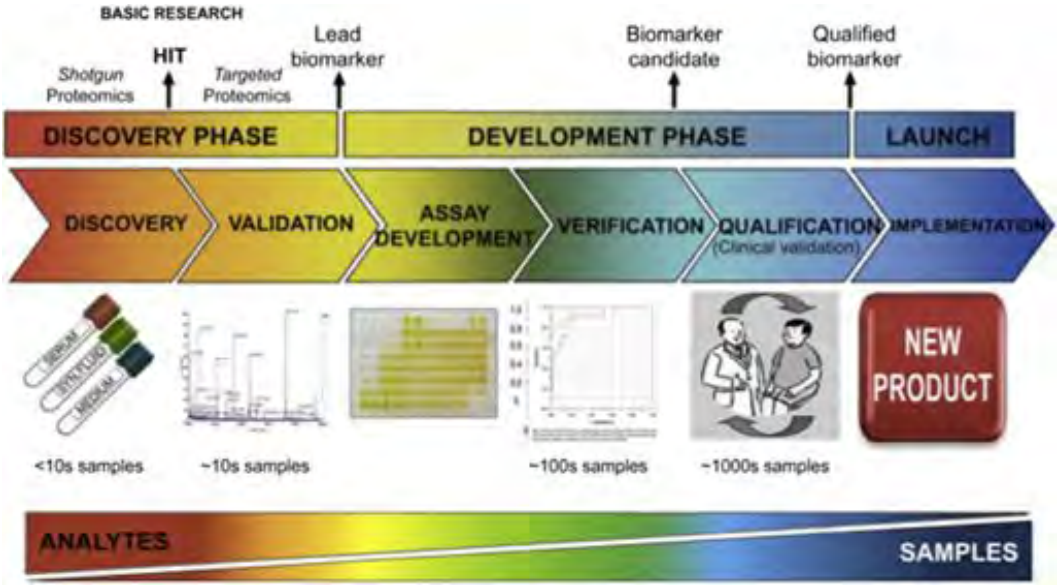

Fig. 1. Phases of proteomics biomarkers development. The Discovery phase encompasses discovery and analytical validation sub-phases. The aim of the Discovery phase is to find prospective biomarkers using a small number of samples. The Development phase is composed of assay development, verification and qualification (clinical validation) sub-phases. The aim of the Development phase is to define biomarker candidates and qualify/verify biomarkers using clinical application.

throughput screens, they possess an important potential for biomarker discovery. There are also two strategies for nondirected approaches: those that profile unidentified proteins and those that generate patterns of identified proteins. Profiling of unidentified proteins often, but not always, utilizes matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Overall, the main advantage of nondirected approaches is speed in processing many samples, making them highly advantageous for clinical screening. However, target-specific approaches frequently use antibodies to screen specific proteins by utilizing western blot analysis, enzyme-linked immunosorbent assay (ELISA), or antibody arrays, making them useful for validation in the discovery phase (Fig. 1).

Blood (plasma and/or serum) and other body fluids are excellent sources of protein biomarkers for proteomic analyses because of their contact with most tissues. Through this contact, body fluids pick up proteins secreted or shed by tissues. A major advantage of using plasma and/or serum is ready availability. However, the proteins secreted or released from a specific tissue or cell type that hold the highest potential as biomarkers are often so diluted in blood as to make them undetectable by current methods. This has generated great interest on analyses focusing on “proximal” body fluids (i.e., synovial fluid [SF]), those that contact only one or a few tissues; thus less dilution of tissue-derived proteins would be expected.

Biomarkers in discovery phase

Because SF bathes all the intrinsic structures of diarthrodial joints, analyses of its constituents offer a unique opportunity to study the entire diseased OA joint. Three papers, using different approaches, have reported several biomarkers in SF[1–3]. Using two-dimensional differential gel electrophoresis (2D-DIGE) and MS, 66 proteins were identified as differentially present in healthy and OA SF[1]. Among these proteins, three major pathways were identified: the acute phase response, and the complement and coagulation pathways. An analysis focusing on those transcripts corresponding to the proteins found to be differentially present also indicated that synovial and cartilage tissues may both contribute to the OA SF proteome. This study also compared age-matched knee SF samples from control subjects and patients with early- and late-stage OA and found no important differences between the OA stages[1].

High-resolution MS identified 545 proteins not previously reported in OA SF[2]. However, multiple reaction monitoring (MRM) analysis validated only three of these proteins, aminopeptidase N (ANPEP), Dickkopf-related protein 3 (DKK3) and osteoglycin (OGN), in ten OA SF samples. Further evaluations of some of these newly identified proteins may reveal their potential as specific targets or useful biomarkers for OA. The authors suggest that improved knowledge of these proteins could provide insights into the underlying mechanism of OA pathogenesis and lead to better therapeutic strategies[2].

One of the major functions of SF in articular joints is lubrication of the surfaces of cartilage, menisci, tendons, and ligaments. Boundary lubrication by SF lowers the friction between apposed and pressurized articular cartilage surfaces. SF contacts 10% of the total joint area and is necessary to protect and maintain intact cartilage surfaces. Three major components of SF have been proposed to independently or additively mediate boundary lubrication: membrane phospholipids, lubricin, and hyaluronan (HA). Despite the evidence that phospholipids are important boundary lubricants, a complete qualitative and quantitative chemical analysis of all phospholipids in SF has only been possible since the recent development of sophisticated lipidomic methods. This technology has enabled the identification of all known phospholipid classes and many individual species in OA and rheumatoid arthritis (RA) SF. Certain phospholipids may act as boundary lubricants, while others perform functions, such as immune modulation during inflammation, cartilage destruction, cell differentiation, apoptosis, and signaling.

Quantitative differences were observed in 117 phospholipid species in SF obtained from the knees of control subjects and patients with early and late OA and RA[3]. Compared to controls, SF from patients with early and late OA had a higher content of total phospholipids, major phospholipid classes, and phospholipid species. .Furthermore, the concentrations of 66 phospholipid species were significantly altered depending on the stage of OA. These data indicate that disease- and stage-dependent differences exist in the

composition and levels of phospholipid species in human knee SF from patients with early and late OA and RA. These differences could alter the lubrication and reactive oxygen species (ROS)-scavenging functions of SF and, thus, modulate the inflammatory status of joints. These authors suggest that certain phospholipids, including phosphatidylcholine, lysophosphatidylcholine, and plasmalogens, may have an association, to some extent, with the pathogenesis of OA and RA[3].

A complementary lipidomic study of SF quantified the composition of sphingolipids (sphingomyelins, ceramides, and hexosyl- and dihexosylceramides) and minor glycerophospholipid species [(lyso)phosphatidic acid, (lyso)phosphatidylglycerol, and bis(monoacylglycero)phosphate] in knee joint SF from unaffected control subjects and from patients with early and late OA and RA[4]. The results showed that concentrations of phospholipid species increased in the knee joint SF of RA and OA patients compared with those from the controls. A broad spectrum of sphingolipid species, their precursors, and intermediate metabolites was found in human knee SF. Moreover, the concentration of 41 lipids in early OA SF, 48 species in late OA SF, and 50 species in RA SF increased significantly in comparison with control SF. Notably, the levels of 21 lipid species were altered between early OA and late OA SF, indicating that the lipid composition of SF reflects the severity of OA disease. Accordingly, these results may lead to the development of biomarkers able to discriminate healthy joints from those with early OA and early OA joints from those with late OA[4].

Using a liquid chromatography tandem MS (LC-MS/MS) analysis approach, a very interesting study investigated the fatty acids and their oxygenated derivatives (oxylipins) secreted by the infrapatellar fat pad (IPFP) in end-stage OA and normal donors[5]. The IPFP is a special form of adipose tissue located intracapsularly and extrasynovially in the joint, existing in close contact with synovial layers and articular cartilage. The IPFP facilitates SF distribution and absorbs mechanical forces through the knee. Adipose tissue adipocytes and infiltrating immune cells actively secrete numerous cytokines and adipokines, which in turn influence metabolism and inflammatory responses in the body. Thus, these adipose tissue-derived factors may contribute to the development of OA. This metabolomics study found that 29 oxylipins and fatty acids were detectable in fat-conditioned media (FCM)[5]. Statistical analyses revealed an oxylipin/fatty acid profile consisting of 14 mediators associated with end-stage OA (accuracy rate 72%). Among these, the most important contributors to the model were lipoxin A4 (decreased), thromboxane B2 (increased), and arachidonic acid (increased). The statistical model correctly predicted 64% of the mediators found in a second set of OA samples. The significance of this study was the demonstration that the OA IPFP and the normal IPFP generate multiple and different oxylipins[5].

A MS-based metabolic phenotype study identified global metabolic defects associated with OA, as well as metabolic signatures of three other types of arthritis: RA, ankylosing spondylitis and gout, comparing sera from all forms of arthritis with those from healthy controls[6]. The use of a combination of gas chromatography coupled with TOF MS (GC-TOF MS) and ultra performance liquid chromatography quadrupole-TOF MS (UPLC-QTOF MS) identified 196 metabolites in these sera. Metabolic defects common to these forms of arthritis resulting from joint inflammation and lesions are suggested by the identification of a global metabolic profile among all arthritic subjects. Additionally, differentially expressed serum metabolites were identified, providing a unique metabolic signature for each type of arthritis; these metabolic signatures may prove useful as biomarkers for disease diagnosis and patient stratification. The metabolites, 5-oxoproline, tyrosine, citric acid, lysine, acetylornithine, tryptophan, sarcosine, alanine and cis-aconitic acid, represent a metabolic signature that enabled the authors to distinguish OA sera from RA or control sera[6].

Several proteomics studies have been conducted using cartilage or chondrocytes. Because the medial femur condyle is usually more affected than the lateral condyle in OA, a quantitative comparison was made between the secretomes of the medial and lateral femur condyle chondrocytes in the same knee[7]. This same comparison of medial/lateral femur condyle chondrocytes was also made on secretomes from chondrocytes taken from one individual with no clinically apparent joint disease, as well as from OA patients. The authors identified 825 proteins in the secretome from OA chondrocytes; 69 of these proteins were differentially expressed when medial and lateral femoral compartments were compared. Several proteins of interest were identified and relatively quantified, CYTL1 (cytokine-like 1 *protein*), DMD (dystrophin) and STAB1 (signal transducer and activator of transcription 1) for the OA disease mechanism, and TIMP1 (tissue inhibitor of metalloproteinases-1), PPP2CA (protein phosphatase 2A catalytic subunit), and B2M (*beta-2-microglobulin*) as putative early OA disease markers. In this study, the findings of differences in protein abundance between medial and lateral femur condyles in OA patients are in accordance with results of other studies suggesting that there are also differences in protein abundance between cartilage from knee and hip. These findings expand our knowledge of biomarkers of OA found in different locations.

Another classical proteomic approach using a quantitative methodology (iTRAQ, or isobaric tags for relative and absolute quantitation) was performed in articular cartilage from patients with OA, using patients with femoral neck fracture for controls[8]. This study identified 76 proteins with expression levels in OA patients differing from those of the control group. Among these proteins, LECT2 (leukocyte cell-derived chemotaxin-2), BAALC (brain and acute leukemia, cytoplasmic), and PRDX6 (peroxiredoxin-6), are worthy of note because they have not previously been reported as biomarkers for OA[8].

During the 2014 Osteoarthritis Research Society International (OARSI) meeting in Paris, new and exciting methodologies were presented to assist in the discovery of new biomarkers. One of these was nucleic acid programmable protein arrays (NAPPA) technology[9]; this is a new generation of self-assembled protein microarrays to detect auto-antibodies (Fig. 2). Recent reports show activation of pro-inflammatory pathways by extracellular matrix (ECM) proteins, leading to their being named damage-associated molecular patterns (DAMPs). This abnormal metabolic activity can be specifically detected by the immune system, leading to a humoral immune response producing immunoglobulins (auto-antibodies) against these proteins. Auto-antibodies, which are stable circulating proteins easily measurable in serum, may be detectable before clinical manifestations of disease. Therefore, serum auto-antibody profiling may facilitate the discovery of OA diagnostic, prognostic or progression biomarkers. Utilizing NAPPA, autoimmunity profiles measuring the specificities of the IgG repertoire in serum from OA and RA patients, as well as healthy controls, were presented in Paris as an abstract[9]. The analysis of auto-antibody levels revealed immunoreactivity against seven full-length proteins with significant differences between OA, RA and control samples. The proteins were identified as CD44 (CD44 antigen), CHST14 (carbohydrate sulfotransferase 14), LEP (leptin), PCOLCE (procollagen C-endopeptidase enhancer 1), IGFBP4 (insulin-like growth factor-binding protein 4), IGFBP6 and IL-6 (interleukin-6). Interestingly, although OA is not a classical autoimmune disease, the detection of auto-antibodies is possible and methodologies such as NAPPA could be useful for characterizing disease-specific autoimmunity profiles used as high-value biomarkers.

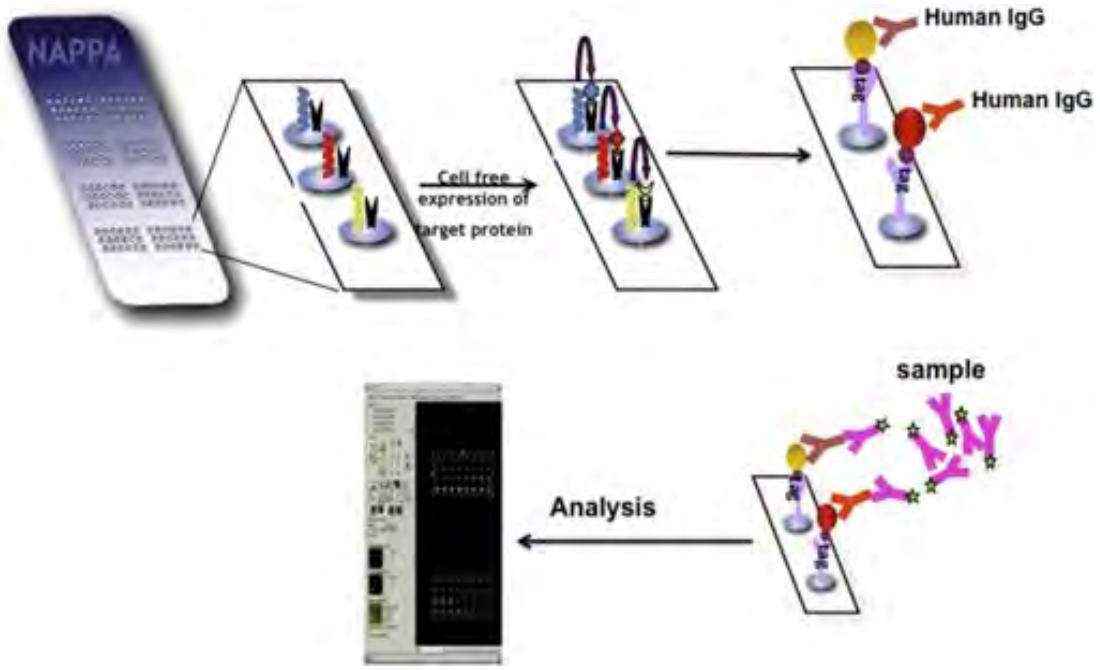

Fig. 2. NAPPA. cDNAs encoding human proteins with a tag, are spotted onto chemically modified surfaces. In the NAPPA, full-length proteins are produced *in situ*, at the moment of assay, using mammalian *in vitro* expression systems. The nascent protein is captured by immobilized antibodies specific for a tag encoded at the carboxy-terminus of the amino acid sequence, which ensures full-length translation of the captured protein. The immunogenicity observed with NAPPA is related to the epitope of the folded protein exposed to its specific auto-antibody. Thus, in NAPPA, the different folding in the expression leads to the detection of different immunogenicity results and also includes the possibility of identifying conformational epitopes.

Another interesting methodology presented in several abstracts at OARSI 2014 was MALDI-imaging MS (MALDI-IMS). MALDI-IMS is a new imaging technology for the study of tissues[10–12] that can determine the distribution of hundreds of unknown compounds in a single measurement and, as such, can assist in the localization and identification of key molecules in OA pathology. In recent years, MALDI-IMS has been used to search for specific peptides, proteins and lipids in precise areas of a tissue cartilage section with a spatial resolution below 50 μm (Fig. 3). This methodology has now been used to study lipidomic distribution and modulation during the chondrogenesis process in human bone marrow mesenchymal stem cells (MSCs) and in the OA synovial membrane. The MALDI-IMS analysis of MSC micromasses at days 2 and 14 of chondrogenesis identified 20 different lipid species, including fatty acids, sphingolipids and phospholipids. In the undifferentiated chondrogenic stage, levels of phosphocholine, several sphingomyelins and phosphatidylcholines were increased. In the OA synovia, proteins, such as hemoglobin subunit alpha 2, hemoglobin subunit beta, actin aortic smooth muscle, biglycan and fibronectin have been identified and localized in areas of high inflammation. In addition, cluster analyses revealed that peaks assigned to diacylglycerols and actin were grouped as molecular signatures specific to normal tissues.

Many “omics” technologies, including microarrays, next generation sequencing and MS, generate large amounts of data. Therefore, bioinformatics tools play an increasingly important role in the analysis of such data and a wide range of methods has been developed for this purpose. Supervised machine learning techniques, based on a training set of labeled samples, are used to build models capable of automatically labeling previously unclassified samples. Samples can be assigned a label (e.g., a treatment group) based on whether they contain a certain attribute (e.g., a protein or group of proteins) and the level of this attribute within the samples. Two very interesting works, a paper and an abstract, have focused on this topic[13,14]. The aim of the paper was to identify suitable bioinformatics methods for the analysis of proteomics data generated from an investigation of cytokine-induced catabolic changes associated with the early stages of OA[13]. This study used an explant model of cartilage to investigate the secretome of canine articular cartilage. BioHEL, a rule-based machine learning and analysis technique, was applied to proteomic MS data to create models that classified samples into their relevant treatment groups by identifying those proteins that separated samples into their respective groups. BioHEL correctly classified eighteen of twenty-three samples, a classification accuracy of 78.3% for the dataset. Among the proteins identified and most frequently used in rules generated by BioHEL, the relevant proteins included MMP-3 (matrix metalloproteinase 3), IL-8 (interleukin-8) and matrix gla protein. These studies support the application of bioinformatics tools for the analysis of proteomic data.

Phase II: development phase

This phase of biomarker development stems from a general understanding of the pathophysiology of a disease. Not surprisingly, biomarker development in OA is escalating as we gain a deeper knowledge of the disease, its stages, and its various phenotypes. A three-part framework for biomarker evaluation has been described that requires answers to specific questions[1]: Assay Development/Analytical validation — Is the biomarker accurately measurable?[2]; Verification — Is the biomarker associated with the clinical endpoint of concern?; and [3] Qualification (Clinical Validation) —What is the specific context of the proposed use and what is the sensitivity and specificity in population-derived human samples?

Biomarkers in assay development

This phase is characterized by development of sensitive biomarker assays, essential tools for analysis of patient samples and

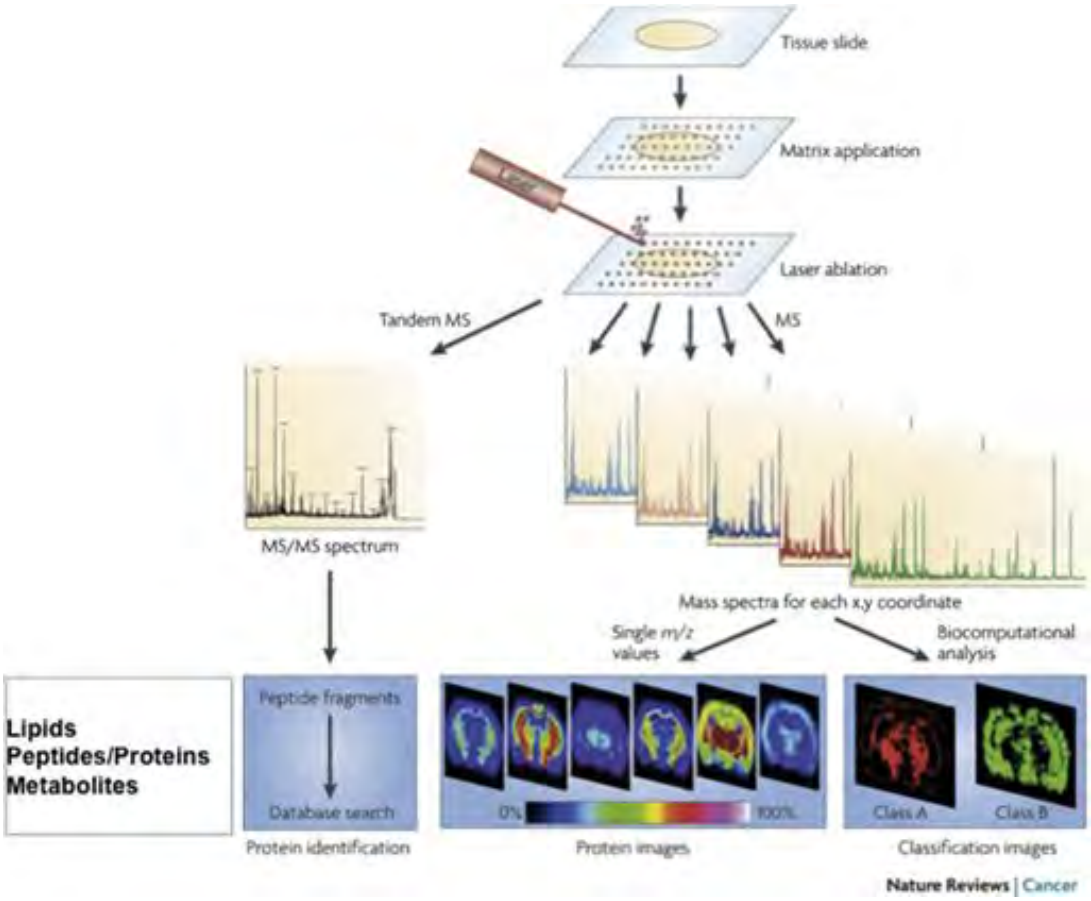

Fig. 3. Matrix assisted laser desorption ionization imaging- Imaging mass spectrometry (MALDI-IMS). After careful preparation, the tissue section is introduced into the mass spectrometer and the proteins, peptides and lipids are desorbed from discrete pixels from the surface in an ordered way. Each pixel is thus linked to the mass spectrum specific from that region. A plot of the intensity of each signal produces a map of the relative abundance of that compound over the imaged tissue.

for our understanding of cartilage degradation in arthritis and in joint injuries. Four studies have developed different tools for detection and quantification of biomarkers in urine, serum and SF[15–18].

Two studies focus on the analysis of aggrecan ARGS neo-epitopes[15,16]. Quantification of ARGS-aggrecan in SF is a more sensitive tool for distinguishing diseased and injured joints from healthy joints than quantification of total aggrecan or other aggrecanase-generated fragments. Aggrecan fragments in SF have been characterized by western blot, but much less is known about the structure of aggrecan fragments in plasma and serum. There are five known aggrecanase cleavage sites, and in terms of destructive loss of sulfated glycosaminoglycans from the cartilage matrix, the most severe cleavage is at the 392Glu-393Ala bond within the aggrecan inter-globular domain (IGD). This cleavage releases N-terminal 393ARGS neoepitope aggrecan fragments into SF. In the first study, the authors describe and characterize an ARGS-aggrecan electrochemiluminescence (ELCL) assay with modifications resulting in a two-fold increased sensitivity compared to a previous version; this technique is capable of quantification of the ARGS neo-epitope in SF, serum, and plasma[15].

In an observational study, the authors evaluated the ARGS neo-epitope in patients with knee OA to support development of GSK2394002 and other drugs that may affect cartilage degradation[16]. This study focused on the assessment of the performance of the ARGS neo-epitope assay and the determination of relative concentrations of the ARGS neoepitope in blood, SF and urine of patients with knee OA. These patients had either been managed conservatively with OA confirmed by X-ray but not scheduled or anticipated to require joint replacement in the coming year, or had end-stage knee OA and were undergoing total knee replacement. Correlations between serum and SF ARGS neo-epitope concentrations with other demographic or clinical factors were assessed. A set of samples obtained from age- and sex-matched healthy volunteers served as a control group. Data from this study inform and facilitate biomarker strategy and study design of disease-modifying treatments of OA. In addition, ARGS neo-epitope measurements have potential to serve as prognostic or stratification markers to identify patient subsets more likely to respond to and benefit from specific treatment regimes[16].

The third study quantified the activity of an arthritis-associated HA binding protein, tumor necrosis factor-α (TNF-α)-stimulated gene-6 (TSG-6) in SF[17]. Because a TSG-6 ELISA adequately sensitive and specific to be used with biological fluids is not currently available, these authors developed an assay to measure TSG-6 activity in SF under conditions very close to *in vivo* conditions. The association of TSG-6 activity, determined retrospectively in SFs collected at baseline, was analyzed with data from a prospective natural history study of OA progression. The authors suggest that TSG-6 activity is a promising independent biomarker for OA progression that may be particularly useful for identifying those patients at low risk for rapid disease progression and for providing guidance for the timing of arthroplasty[17].

At OARSI 2014, a very interesting abstract was presented that established the reference interval range of 18 biomarkers. Traditionally, reference ranges for biomarkers are determined using commercially purchased blood from normal donors. This source does not provide adequate controls for OA studies because the normal donor OA status is not ascertained. The authors defined the concept of "supercontrol" subjects, those who have no radiographic evidence of OA of the knee, hip, hand, and spine and no knee or hip symptoms (pain, aching or stiffness on most days)[18].

Biomarkers in verification phase

Verification studies must determine whether the biomarker is associated with any clinical endpoint. Relative to this requirement, several published studies have analyzed the role of inflammatory biomarkers in knee pain[19,20]. Knee pain is associated with body mass index (BMI) and knee structural changes, such as cartilage defects, bone marrow lesions (BML), synovitis, joint effusion and osteophytes. The positive associations between synovitis/effusion and knee pain indicate that inflammation may be involved in its genesis. One of these studies examined the association between levels of inflammatory biomarkers and changes in knee pain in community-based older adults over a 5-year time interval[19]. The results of this study indicate that changes from baseline in high-sensitivity C-reactive protein (hs-CRP) over 5 years and TNF-α over 2.7 years were associated with increased knee pain as assessed by the total Western Ontario and McMaster Universities Arthritis Index (WOMAC) score. Interestingly, hs-CRP woas found to be associated with increased knee pain when lying in bed and sitting, while TNF-α and IL-6 predicted greater knee pain when standing[19].

A translational study compared the effects of diet-induced weight loss plus exercise (D + E), diet-induced weight loss only (D), and exercise only (E) interventions on mechanistic (knee-joint compressive force, IL-6 levels) and clinical outcomes (pain, function, mobility, health-related quality of life [HRQL]) in overweight and obese adults with knee OA[20]. The results of this study showed that after 18 months, those overweight and obese adults with knee OA in the diet and exercise group had more weight loss and a greater reduction in IL-6 levels than those in the exercise only group[20].

Another series of studies examined the role of bone and cartilage biomarkers in OA. OA is a disease of the whole joint, affecting, besides articular cartilage, the juxta-articular bone, including osteophyte formation and subchondral changes that finally lead to sclerosis. Historically, two decades ago the suggestion was made that bone rather than cartilage may be responsible for the initial pathophysiological events in OA. Moreover, the potent inhibitors of bone resorption, bisphosphonates, have been shown to reduce cartilage degeneration, osteophyte formation and bone resorption in both experimental animal models of OA and in human OA.

One of these studies simultaneously investigated the synthetic, resorptive and mineralization aspects of bone metabolism in early-stage progressive and non-progressive knee OA[21]. The study assayed specific markers of bone formation (procollagen I N-terminal peptide [PINP]) and bone resorption (C-terminal cross-linked telopeptides of type I collagen [CTx-I]), as well as a non-collagenous marker of bone mineralization (osteocalcin [OC]), and a novel non-collagenous marker of bone resorption, urinary midfragments of OC (MidOC). Enhanced bone formation (shown by PINP), together with bone formation activation (also shown by PINP) and non-collagenous bone resorption (shown by MidOC), as well as bone mineralization (shown by OC) preceded OA progression. All bone markers assessed, PINP, OC, and MidOC, demonstrated diagnostic value, and PINP also had predictive value for knee OA progression, particularly progressive osteophytosis. Another work collected radiographic knee OA (RKOA) data over 10-years in a large community-based sample of middle-aged British females[22]. This study investigated whether circulating levels of cartilage oligomeric matrix protein (COMP), aggrecan, N-terminal telopeptide (NTx) and cellular inhibitor of apoptosis protein (cIAP) are predictive of the appearance of RKOA-associated phenotypes. Among these biomarkers, the results of this study indicate that the incidence of some RKOA phenotypes could be predicted by circulating levels of aggrecan and COMP. Analyses of joint space narrowing data led these authors to suggest that aggrecan plays a protective role against cartilage loss while the association of COMP circulating levels with Kellgren–Lawrence (K/L) grades suggests that a high COMP level is a risk factor for development of RKOA[22].

One problem affecting biomarker studies has been the use of small sample sizes and case–control designs with cases recruited from secondary care settings. To address these limitations, a meta-analysis combining data from three large population-based cohorts (the Rotterdam Study, the Genetics osteo-Arthritis and Progression (GARP) and the Chingford Study) and one familial study of OA (the TwinsUK) was performed using hand, knee and hip X-rays. Samples from 3582 individuals were measured for three separate cartilage-based biomarkers (urinary C-terminal telopeptide [uCTX-II], serum COMP (sCOMP), and serum MMP degraded type II collagen [sC2M]) to enable assessment of the efficacy of biomarkers to measure prevalence, incidence and progression of OA and to assess the prognostic value of these biomarkers. Because levels of uCTX-II were significantly associated with risk of hand, hip and knee OA, and progression and incidence of knee OA, the authors concluded that the most informative biochemical marker for prediction of OA was uCTX-II. Levels of sCOMP were found to be associated with knee OA and hip and knee OA incidence, while levels of sC2M were associated with OA incidence and progression; the authors suggest that these markers describe disease activity[23].

In 2013, the role of biomarkers for hand radiological OA has also been studied. One study investigated the age-related characteristics of two putative OA biomarkers (sCOMP and urinary crosslinked C-telopeptide of type II collagen [uCTX2]), in healthy aging individuals (healthy agers), in patients with OA at multiple joint sites, and in a control population[24]. The study found that being a middle-aged metabolically healthy ager was associated with less radiographic OA and that the influence of metabolic health on OA biomarker profiles was not age-related. Independent of hand radiographic OA status, the OA biomarker, uCTX2, was influenced by healthy metabolism and the OA biomarker, sCOMP, increased with advancing age. Compared with age-matched controls of similar BMI, the age-related increase in prevalence of hand radiographic OA was lowered in healthy agers. In addition, glucose levels, representing metabolic health, were shown to partially account for this effect. This study concludes that the use of potential OA biomarkers, including sCOMP and uCTX2, for the diagnosis and monitoring of OA, should take important metabolic properties and chronological age into consideration in the development of effective disease-modifying treatments[24].

A cross-sectional study of hand OA determined the associations between multiple joint metabolism biomarkers and hand radiographic OA, symptoms, and function in 663 participants[25]. Metacarpophalangeal (MCP) and carpometacarpal radiographic OA and a higher number of hand joints with radiographic OA were all significantly associated with higher levels of serum HA (sHA). The levels of the biomarkers, sCOMP and sHA, were positively associated with the Australian Canadian Hand Osteoarthritis Index (AUSCAN) scores and hand symptoms, while hand symptoms and higher AUSCAN scores were independently associated with higher levels of both sCOMP and sHA[25].

Pre-radiographic OA is a very interesting and existent field. It is well known that patients with anterior cruciate ligament (ACL)

deficiency are at an increased risk for the development of OA. The prevalence of knee OA is lower in individuals with an isolated ACL injury (0 vs 13%) and higher in subjects with combined injuries (21 vs 48%). The most frequently reported risk factor for the development of knee OA is meniscal injury. Based on these data, a new study determined whether there was an association between biomarkers measured in SF and damage to articular cartilages and/or menisci[26]. These investigators examined 108 knee joints arthroscopically in the period following ACL injury and before the development of radiographic changes. The biomarkers studied in SF were cartilage type II collagen collagenase-generated cleavage neoepitope (C2C biomarker assay) and disaccharides of the glycosaminoglycans chondroitin sulfate (CS) and keratan sulfate (KS) that are mainly present in articular cartilages on the proteoglycan aggrecan. In this study population, the presence of multi-high grade cartilage lesions was strongly associated with a combination of defined ranges (quartiles) of the C2C and KS biomarkers; this impact was independent of clinical variables and exceeded the impact of the individual biomarkers. The authors suggest the value of the use of combinations of biomarkers and defined quartiles in future clinical trials.

A very interesting paper that studied similarities between CTX-II and bone markers suggested that CTX-II is not only a marker of cartilage degradation[27]. In the CHECK (Cohort Hip and Cohort Knee) study of early OA, ELISAs were used to determine levels of the putative cartilage marker, uCTX-II, and bone markers (urinary C-terminal propeptide of type I collagen [uCTX-I], urinary cross-linked N telopeptide of type I collagen [uNTX-I], serum N-terminal propeptide of type I collagen [sPINP], and serum OC [sOC]) and other cartilage markers (sCOMP, serum CS846 [sCS846], and serum type IIA procollagen amino terminal propeptide [sPIIANP]). Intriguingly, the results of this study revealed that the putative marker of cartilage degradation, uCTX-II was more strongly associated with markers of bone metabolism than with markers of cartilage metabolism; this strong association with bone markers was not found with the other cartilage markers. Additionally, an abrupt menopausal shift in women aged 48–53 years was seen in levels of both uCTX-II and the bone markers, but not with the other cartilage markers, even when adjusted for age and BMI. Although the association between uCTX-II and the bone markers could be ascribed to the metabolic and biomechanical mechanistic links between cartilage and bone metabolism, this association was not shared by the other cartilage markers. This unique relationship of uCTX-II with bone markers may indicate the metabolism of bone rather than cartilage. The authors suggest that further validation of uCTX-II as a biomarker is required[27].

Biomarkers have also been evaluated in a clinical trial. A pilot clinical trial assessed changes in walking pain and serum levels of two OA biomarkers suggested to reflect oxidative-related cartilage degradation: the α-helical region of type II collagen (Coll2-1) and its nitrated form (Coll2-1 NO2), following viscosupplemenation (VS). Knee osteoarthritis patients ($n = 51$) with unilateral symptoms were followed for 3months following three intra-articular injections of HA[28]. At baseline, serum concentrations of Coll2-1 and Coll2-1 NO2, measured using specific immunoassays, were significantly higher in K/L grade III/IV patients compared to K/L grade I/II patients. These levels decreased over time after VS. The effect of VS was most pronounced in patients with KL III/IV, suggesting a rapid slowdown of type II collagen degradation and joint inflammation after VS with HA. The study also revealed that the serum concentration of Coll2-1 was significantly lower at baseline in responders than in non-responders, and the authors suggest that serum levels of Coll2-1 may be to be a predictive factor for response to treatment. This study was an open, non-controlled, pilot clinical trial with only 51 patients, and, although these results are interesting, they must be confirmed by a controlled and double-blind clinical trial using larger numbers.

In summary, in addition to the papers included in this review, several other interesting papers and abstracts in the field of biochemical biomarkers and OA (See review[29]) were published in the year 2013. Novel biomarkers have been discovered and new and exciting methodologies are used every day in the discovery phase, including metabolomics, lipidomics, imaging mass spectrometry and NAPPA. Several innovative assays have been developed to study biomarkers in SF, serum and urine; and various studies have been carried out in the verification phases. But we need more energetic efforts for more biomarkers to reach the qualification phase.

Author contributions

The author was involved in drafting or critically reading the manuscript for important intellectual content. Conception, design data acquisition, analysis and interpretation of data: FJ Blanco.

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Competing interest statement

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