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Discovery of Biomarkers for Osteoarthritis Using Proteomics Technologies

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ABSTRACT

Osteoarthritis (OA) is a disease associated with pain and loss of function in numerous diarthrodial joints of the body, and is the most common rheumatic pathology. The diagnosis of OA is commonly based on patient reports of pain and stiffness, and the presence of osteophytes and joint space narrowing as viewed on radiographs, which are not always correlated to severity of disease or joint dysfunction and may be confounded by other factors. Therefore, there is a considerable interest pointed in characterizing new molecules related to OA that may be detected in serum, urine, and/or synovial fluid that would represent repeatable and predictable biomarkers of OA onset and/or progression. To fulfill this goal, new developments have been achieved with "omics" thecnologies (genomic, metobolomic, lipidomic and proteomic). These techniques, coupled with sophisticated statistical methods, permit the simultaneous analysis of multiple targets, and have become very powerful tools in OA research both for etiopathogenesis studies and biomarker discovery. This chapter will focus in the advances accomplished using proteomic

technologies to discover novel OA biomarkers. However, appropriate use of the markers is based on compliance to a set of basic requirements such as the BIPED classification system, thus still qualification of these biomarkers is needed before their implementation in clinical practice.

Keywords: Osteoarthritis (OA); Biomarkers; Biological fluids; Proteomics; BIPEDS

CHALLENGES FOR BIOMARKERS IN OSTEOARTHRITIS

Osteoarthritis (OA) is a common slowly progressive condition that may affect the structure of all joint tissues, and is a major cause of pain and chronic disability in the elderly. The lack of a universal definition of OA is probably due to the complexity of processes underlying its pathogenesis [1] and to the diversity in its clinical presentation, rate of disease progression, pattern of joint involvement, and joint tissue affected [2]. A definition for OA has been recently outlined by the OARSI (Osteoarthitis Research Society International) taking into account all of these facts, as a disorder involving movable joints characterized by cell stress and extracellular matrix degradation initiated by micro- and macro-injury that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity. The disease manifests first as a molecular derangement (abnormal joint tissue metabolism) followed by anatomic, and/or physiologic disarrangements (characterized by cartilage degradation, bone remodeling, osteophyte formation, joint inflammation and loss of normal joint function), which can culminate in illness [3]. OA is a disease characterized in most individuals by an initial prolonged phase, which is clinically silent, followed by a radiographic phase with extensive deterioration of cartilage and evident structural joint changes that already exists by the time of diagnosis. Currently, diagnosis of OA is based on radiographic criteria (such as joint space width) and clinical symptoms, as the description of pain or stiffness in the affected joints, which are insensitive to detect small changes and do not allow the visualization of the tissue most associated with the disease (articular cartilage). Therefore, the disease is definitively diagnosed only when destruction of joint tissue is irreversible. These limitations in the diagnostic tests presently available provide impetus for the substantial increase in interest in finding new specific biological markers of cartilage degradation and to know more about OA etiopathogenesis, both to facilitate early diagnosis of joint destruction and to enhance disease prognosis and evaluation of progression.

A biological marker, or biomarker, is a substance or feature used as an indicator of a biological state. It can be objectively measured and evaluated to monitor normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Ideally, biochemical markers are derived from body fluids that are easily available to researchers at the early phase (blood, serum, urine, synovial fluid…). Candidate biomarkers in OA should also have proven validity, reproducibility and predictive value, and there should be information on how they are related to processes occuring in the joint and clinical endpoints (such as structural damage, pain or dysfunction and/or joint replacement). These biomarkers would allow carrying out screenings for early diagnosis, thus enabling the beforehand settlement of procedures directed to

slow disease progression [4]. Furthermore, they would facilitate the discovery of new drugs and the monitoring of their efficacy by providing information about their success in pharmacological trials.

Although biomarkers are classically thought of as biochemical substances, it is also possible to consider RNA, DNA, their fragments, or a combination or multiplicity of these, as biomarkers. Besides, imaging techniques may themselves be considered biomarkers for the pathologic joint abnormalities that define OA. Nevertheless, this chapter will addresses only protein-based biomarkers in body fluids such as synovial fluid and serum, and the power that large-scale ("omics") analytical techniques (mainly proteomics) have for generating newer candidates that can be useful for early diagnosis, prognosis and drug efficacy studies.

THE PRESENT OF BIOMARKERS IN OA

The best OA biomarker candidates are generally molecules or molecular fragments present in cartilage, bone or synovium that may be specific to one type of joint tissue, or common to them all. (Table 1) lists the biomarkers that have been employed in clinical trials to date. The information generated in these studies has been recently summarized elsewhere [5,6]. The molecules that have been employed as OA biomarkers are proteins directly or indirectly involved in cartilage degradation, or proteins synthesized in an attempt at cartilage repair. Many are associated with the metabolism of collagen in cartilage (type II collagen) or subchondral bone (type I collagen), or to the metabolism of aggrecan in cartilage.

Table 1: Summary of biomarker candidates being investigated for the evaluation of osteoarthritis [5,6].

Bimarkers related to Collagen metabolism	C-terminal telopeptide of collagen type II (CTX-II)
	Type II collagen α chains collagenase neoepitope (α-CTX-II)
	Type II collagen propeptides (PIINP, PIIANP, PIIBNP, PIICP, CPII)
	Pyridinoline and Glc-Gal-PYD
	Type II collagen cleavage product (C2C)
	Collagen type II-specific neoepitope (C2M)
	C-terminal telopeptide of collagen type I (CTX-I, α-CTX-I)
	N-terminal telopeptide of collagen type I (NTX-I)
	Aminoterminal propeptide of collagen type I (PINP)
	Types I and II collagen cleavage neoepitope (C1,C2)
Biomarkers related to aggrecan metabolism	Core protein fragments (aggrecan neoepitopes, ARGS and FFGV fragments)
	Chondroitin sulfate epitope 846 and monoclonal antibody3B3(-)
	Keratan sulfate
Biomarkers related to other non-collagenous proteins	Cartilage oligomeric matrix proteins (COMP and its deamidated form D-COMP)
	Fibulin (peptides of fibulin 3, Fib3-1, Fib3-2)
	Follistatin-like protein 1 (FSTL-1)
	Hyaluronan (hyaluronic acid)
	Matrix metalloproteinases (MMP-1, MMP-3, MMP-9, MMP-13 and TIMPs)
	YKL-40 (cartilage glycoprotein 39)
	Soluble receptor for advanced glycation endproducts (sRAGE)
Biomarkers related to other processes	Inflammatory biomarkers: hs-CRP, IL-1β and IL-6 and COX-2
	Factors indicating fibrosis and complement proteins
	Adipokines (adiponectin, leptin, visfatin)
	Soluble receptor for leptin (sOB-Rb)
	Cellular interactions in bone (periostin)
	Wnt inhibitors (DKKs and SOST)
	Uric acid

COMP: Cartilage Oligomeric Matrix Protein; COX-2: Cyclo-Oxygenase-2; Glc-Gal-PYD: Glucosyl-Galactosyl-Pyridinoline; hs-CRP: Highsensitivity C Reactive Protein; IL: Interleukin; MMP: Matrix Metalloproteinase; PIIANP: N-Propeptide IIA Of Type II Collagen; PIIBNP, N-Propeptide IIB Of Type II Collagen; PIICP: C-Propeptide Of Collagen Type II; PIINP: N-Propeptide II Of Type II Collagen; SOST: Sclerostin; TIMP: Tissue Inhibitor Of Matrixmetalloproteinase

Different studies and development of assays have been undertaken to study type II collagen (COL2), as this protein is relatively specific to articular cartilage and the most abundant in its extracellular matrix [7]. Antibodies recognizing different fragments of type II collagen have been developed. One of the most widely used biomarker of COL2 degradation is a fragment from the C-telopeptide region (CTX-II), which can be measured in serum or urine, and whose urinary levels are strongly associated with radiographic subtypes of OA [8]. Furthermore, two different assays recognizing a sequence - which can be either un-nitrosylated (Coll2,1) or nitrosylated (Coll2,1-NO2) - of the triple helix of COL2 have been developed. An open, non-controlled, pilot clinical trial with 51 patients was recently carried out to evaluate changes in walking pain and serum

levels of Coll2-1 and its nitrated form following visco supplementation, finding that the serum concentration of Coll2-1 was significantly lower at baseline in responders than in non-responders to the treatment [9].

Other molecules that reflect COL2 degradation are Helix-II, C2C and urinary TIINE (type II collagen neoepitope). On the other hand, measuring COL2 synthesis has also been indicative for OA. COL2 is synthesized by the chondrocytes as procollagen, composed of collagen itself and two propeptides located at both edges of the protein: PIINP at the N-term, and PIICP at the C-term. These propeptides are cleaved during collagen maturation and released into the biological fluids, thus its concentration appears to directly reflect the rate of type II collagen synthesis. The determination of PIIANP levels in sex- and age-matched OA donors showed increased levels in incipient knee OA when compared to healthy controls, whereas patients with later stages of OA had decreased values [10]. These data suggest that a cartilage repair mechanism is effective for biomarker search in the early development of OA, but not with advanced disease.

Among the non-collagenous biochemical markers of cartilage turnover is the Cartilage Oligomeric Matrix Protein (COMP), which is associated with collagen, regulating its fibril assembly and collaborating in the maintenance of the network. A systematic review and meta-analysis indicate that serum COMP (sCOMP) is elevated in patients with knee OA and is sensitive to OA disease progression [11], and a specific deamidation of this protein (D-COMP) has been reported as a novel biomarker with specificity for hip OA [12].

A major problem affecting biomarker studies in OA has been the use of small sample sizes and cases-control designs with cases recruited from secondary care settings. To address these limitations, several studies have been performed using larger data sampling. 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 the assessment of their efficacy to measure prevalence, incidence and progression of OA, and to assess their prognostic value. Levels of uCTX-II were significantly associated with risk of hand, hip and knee OA, and progression and incidence of knee OA. On the other hand, 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 concluded that the most informative biochemical marker for prediction of OA was uCTX-II, whereas the other makers were suggested to describe disease activity [13]. In another study, it was concluded that the use of potential OA biomarkers (including sCOMP and uCTX2) for the diagnosis and monitoring of OA should take into account metabolic properties and chronological age of the patients into consideration for the development of effective disease-modifying treatments [14]. Furthermore, a cross-sectional study of hand OA was recently carried out to determine the associations between multiple joint metabolism biomarkers and hand radiographic OA, symptoms, and function in 663 participants. Metacarpo phalangeal (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 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 [15]. Finally, a very interesting paper that studied similarities between CTX-II and bone markers in 1002 individuals from the CHECK cohort suggested that this unique relationship may indicate bone rather than cartilage metabolism [16].

Other molecules employed as biomarkers for OA have been cartilage glycoprotein 39 (YKL-40), pentosidine, and other proteoglycans such as keratan sulfate (KS) and chondroitin sulfate (CS) [17]. Enzymes involved in the breakdown and turnover of collagens have been also assayed. Finally, analogous to the evaluation of cartilage turnover, bone and synovium metabolism has been also under investigation to broaden the list of biomarker candidates. These processes are largely mediated by matrix metalloproteinases (MMPs), whose activity and inhibition is controlled by a variety of tissue inhibitors (TIMPs), pro-inflammatory cytokines and growth factors. Among the large group of known MMPs, those that exhibit collagenolytic activity (mainly MMP-1, -8 and -13), the stromelysin MMP-3, and their main tissue inhibitors (TIMP-1, and -2) have been evaluated in clinical trials as biomarkers of OA [18,19].

It is important to highlight those levels of biochemical markers measured in blood or urine (because assessment of synovial fluid is often impracticable) provide information on joint tissue turnover, but is not necessarily specific of the alterations occurring in the symptomatic joint. Moreover, the clearance of the markers from the joint compartment to the blood stream is complex, varies across individuals and can increase with inflammation, after joint mobilization and exercise. In fact, serum and urinary levels of most biochemical markers also vary with sex, age, menopausal status, ethnicity and OA risk factors such as body mass index, as reported for serum HA in a large population-based study [20]. Furthermore, studies of the diurnal variation of various serum and urine biomarkers in patients with radiographic knee OA have been carried out and assessed the variation of their levels. These facts depict how pre-analytical factors contribute to the intra- and inter-subject variability of biochemical markers levels, therefore concluding that patient selection and sampling should be tightly controlled and standardized in future OA clinical trials.

Besides the promising results obtained with these proteins, still useful biomarkers are needed for clinical practice. Some researchers suggest that the most promising strategy in OA would be the combination of different biomarkers. Large-scale analyses, which allow to simultaneously analyze multiple molecules, are thus valuable tools for biomarker discovery and validation. 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 energic efforts to find more biomarkers reaching the qualification phase [21].

PROTEOMIC STUDIES ON OSTEOARTHRITIS

The search for OA disease biomarkers has focused the attention of researchers in recent years. In OA, the lack of complete understanding of its complex etiopathogenesis has hindered this objective, and contributes to the difficulties for early diagnosis and evaluation of drug efficacy. In this field, proteomics has been thrust into the research spotlight for biomarker discovery and several approaches using this technology have been carried out to search for novel protein markers of OA [22]. Proteomic approaches have the advantage over nucleic acid expression profiling in that their interpretation is not limited by a possible disconnection between gene and protein expression levels. As such, this technology has emerged as a powerful method to identify proteins involved in disease etiology and pathogenesis, as well as potential biomarkers. A summary of these efforts is presented in (Tables 2 & 3). Proteomic research includes the characterization of protein mixtures in order to understand complex biological systems and determine relationships between proteins, their function, and protein-protein interactions. Often, the goal of such research is to monitor changes of proteins in perturbed systems, a type of study referred to as differential expression analysis. The next major challenge thus becomes the systematic exploration of protein abundance and structural modification in relation to disease, normal physiological processes, and treatment effects. In this field, several shotgun proteomics studies have been carried out in the last decade for increasing knowledge on the pathogenesis of osteoarthritis and the search of novel protein biomarkers. Pathogenesis studies have been carried out essentially on joint tissues and cells, and also on their secreted fractions [4]. On the other hand, analyses with the aim of novel biomarker discovery have been performed mainly in biological fluids and samples derived thereof, such as synovial fluid, plasma or urine.

Table 2: Summary of proteomic studies of human serum from OA patients adapted from [4].

Serum/ Plasma	Method	Mass spectrometer	Number of proteins identified	Relevant proteins	Reference
OA *vs* HC	DIGE		16	HPT	[23]
RA *vs* OA, PsA, Asthma, Crohn´s and HC	ProteinChip array	SELDI-TOF		MRP-8	[26]
OA *vs* RA *vs* HC	ProteinChip array	SELDI-TOF	4	V65 Vitronectin fragment, C3f peptide, CTAPIII, m/z 3762	[27]
Progressive *vs* non progressive OA	ProteinChip array	SELDI-TOF		APOC-I, APOC-III, TTHY fragment	[28]
OA K/L I/II *vs* K/L IV *vs* HC	iTRAQ + LC-MS/ MS		262	26 differential proteins	[24]
OA *vs* RA *vs* HC autoAb profiling	Antigen and NAPPA arrays			Anti-CHST14 autoantibodies	[29]
Serum and synovial fluid	**Method**	**Mass spectrometer**	**Number of proteins identified**	**Relevant proteins**	**Reference**
RA *vs* OA	2-DE			FIBB, SAA, MRP14 MRP-8, MRP-14	[72,73]

HC: Healthy Controls; 2-DE: Two-Dimensional Gel Electrophoresis; LC: Liquid Chromatography; MS: Mass Spectrometry; RA: Rheumatoid Arthritis; PsA: Psoriatic Arthritis; K/L: Kellgren And Lawrence Scale; iTRAQ: Isobaric Tag For Relative Quantitation; SELDI: Surface-Enhanced Laser Desorption/Ionization; NAPPA: Nucleic Acid Programmable Protein Array; DIGE: Differential In-Gel Electrophoresis

Plasma or serum is the most generally informative proteome from a medical viewpoint. Almost all cells in the body communicate with plasma directly or through extracellular or cerebrospinal fluids, and many release at least part of their contents into plasma upon damage or death. Through this contact, body fluids pick up proteins secreted or shed by tissues. A major advantage of using plasma and/or serum is their ready availability. However, from the proteomic point of view, an accurate quantization of proteins and peptides in plasma and serum is a challenging problem, because of the high complexity and extreme dynamic range of these type of samples, which makes the detection of low abundance proteins very difficult using conventional proteomic strategies, such as those employing gel electrophoresis with or without or fluorescent labelling as Difference Gel Electrophoresis (DIGE) [23]. Therefore, in order to identify proteins that are differentially present in OA sera, a quantitative proteomic profiling of samples from patients with different grades of OA and healthy controls was carried out after removing the fraction of most abundant proteins (albumin, immunoglobulins and others). Then, a differential labeling with the iTRAQ reagents was performed, followed by Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) analysis [24].

Peptide or protein profiling using Surface-Enhanced Laser Desorption/Ionization (SELDI) is strategies that have been also followed to detect disease biomarkers [25,26]. In a subsequent study specifically focused in OA, the same authors identified four differential MS peaks between OA, RA and controls [27]. Analogous techniques (such as magnetic beads for ion exchange chromatography, followed by Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) analysis for biomarker discovery have been also frequently used due to their high throughput, recently enabling the identification of potential prognostic markers for knee OA [28]. Finally, other kind of high-throughput proteomic approaches that have been performed using plasma or serum are protein microarrays, such as antigen microarrays or Nucleic Acid Programmable Protein Arrays (NAPPA) for profiling the autoantibody repertoire of OA [29].

Besides the analysis of plasma or serum, studying the Synovial Fluid (SF) proteome is highly advantageous in rheumatic diseases, as it is in contact with the site of disease activity. Additionally, alterations in the joint cavity due to injury or disease may be directly reflected in the composition of synovial fluid and could be correlated to disease severity and progression [30]. One of the disadvantages of SF is the difficulty to get samples from healthy controls, thus most proteomic studies performed on SF employed another rheumatic disease as control (Table 3). Using this type of samples, different proteins have been identified using LC-MS/MS tools, and comparing SF from OA patients to healthy controls or RA [31-33]. A more recent study identified 575 proteins in SF, out of which 135 were found to be differentially expressed by ≥3-fold in OA compared to RA [34]. Peptide profiling studies have been also performed on SF by SELDI [35,36], and also employing magnetic beads with a chromatographic surface (such as weak cation exchange) for simplifying the serum complexity prior to MALDI-MS profiling in OA sera vs healthy controls [37]. Furthermore, the combinative technique of Two-Dimensional Electrophoresis (2-DE) and MS has

been widely applied to screen for differentially expressed proteins using SF. Among these, Ritter and colleagues recently evaluated samples from early OA, late OA and healthy controls using the Differential In-Gel Electrophoresis (DIGE) technique, confirmed the alteration of 5 differential proteins by Selected Reaction Monitoring (SRM) and compared these results with transcriptomic data for a comprehensive study of the OA SF proteome [38]. In another study, it was identificated several putative biomarkers of Psoriatic Arthritis (PsA) in synovial fluid using OA samples as control group [39]. Finally, a recent work described a peptide-based equalisation followed by LC/MS/MS for SF proteomics for both discovery and quantification, finding a distinct set of proteins and a catalogue of neopeptides in SF that may act as potential biomarkers between normal and OA joints in horses [40].

Table 3: Summary of proteomic studies of human synovial fluid from OA patients [4].

SF	Method	Mass spectrometer	Number of proteins identified	Relevant proteins	Reference
Early and late OA *vs* HC	1D-PAGE	LC-LCQ Ion trap	135	18	[32]
Early and late OA *vs* HC	2D-DIGE	MALDI-TOF/LC-Triple quadrupole		66/5 verifícate by MRM	[38]
RA *vs* OA	1D-PAGE	LC-MALDI-TOF/TOF	136	MMP1, BIGH3, FINC, GELS	[33]
RA *vs* OA	Protein chip array	SELDI-TOF		3 m/z peaks. S100A12	[36]
RA *vs* OA		LC-LTQ-FT-TOF/TOF LC-triple quadrupole	677	135 differential proteins. CAPG	[34]
RA *vs* OA		SELDI		MRP-8	[35]
OA *vs* HC	UF+LC-MS/MS			COL2, PRG4, SAA, TUB, VIME, MGP	[31]
OA *vs* HC	MB+ MALDI-TOF			Two peptide peaks	[37]
PsA *vs* early OA	LC-MS/MS + SRM			12 proteins quantified by SRM	[39]

MALDI: Matrix Assisted Electrospray Ionization; MB: Magnetic Beads; SRM: Selected Reaction Monitoring; HC: Healthy Controls; RA: Rheumatoid Arthritis; PsA: Psoriatic Arthritis; LC: Liquid Chromatography

Apart from serum or synovial fluid, no other MS-based studies have been carried out for OA biomarker discovery in alternative body fluids, with the exception of the development of an immunoaffinity-LC-MS/MS assay for the determination of a type II collagen neoepitope (TIINE) in urine [41,42]. Being a promising strategy for the accurate quantification of specific markers in biofluids, this approach was applied for the analysis of endogenous aggrecan fragments in synovial fluid and urine [43].

CLINICAL USE OF BIOCHEMICAL MARKERS IN OA

The recorded biochemical markers of joint tissue turnover, and also the novel molecules emerging from large-scale studies, may have various roles in clinical rheumatology. A classification of its utility has been proposed by the Osteoarthritis Biomarkers Network (a consortium of five US

National Institutes of Health designated sites) [44]. This classification employs the BIPED scheme to describe the potential uses of a marker: burden of disease, investigative, prognostic, efficacy of intervention and diagnostic. The BIPED classification, designed specifically for osteoarthritis, provides specific biomarker definitions with the goal of improving our ability to develop and analyze OA biomarkers, capture information in the early stages of development of the disease and to inform the design of future clinical trials within a common framework. A Safety category has been added, thus changing the scheme to BIPEDS classification system [5].

Burden of Disease

These markers assess the severity or extent of OA among affected individuals, and could be considered tools for the staging of the disease. This classification is generally based on investigations of sensitivity and specificity of the biomarker, being typically qualified by comparison to a clinically accepted gold standard. The main challenges for this classification are the definition of a clear gold standard, which remains unclear, and the complex pathogenesis of OA, which involves different tissue types that might exhibit different molecular subsets of disease and thus might require multiple biomarkers for accurate measurement. As clinically evaluated burden of disease markers appear sCOMP levels, uCTX-II or sHA [45,46]. The native form of COMP was found to be more specific for knee osteoarthritis, while its deamidated form was found to be more specific for hip osteoarthritis than for knee [12]. Finally, a number of studies have revealed links between adipokines (adiponectin, leptin and visfatin) and joint disease. High levels of adiponectin and leptin have been measured in synovial fluid of patients with osteoarthritis and were reported to correlate with the severity of osteoarthritis [47].

Investigative

These are markers on which there is insufficient information to allow their inclusion in one of the other categories. Markers derived from new large-scale methodologies, such as proteomics or metabolomics, could be classified in this category, since advances in 'omics' research are uncovering a range of new candidate biomarkers. Although only some of the data have been validated independently, this is a field of active research and of major importance in OA. Metabolomics studies, such as the ratio between branched chain amino acids and histidine in tissue [48] have provided a number of promising candidates. On the other hand, recent proteomics studies described pronounced differences between cartilage from different joint sites [49]. Finally, research in lipidomics suggests altered lipid metabolism in osteoarthritis, which may also be a source of novel biomarkers [50].

Prognostic

Prognostic markers may provide information about the likely clinical course of disease, and also how quickly the progression will occur. Similarly, they could indicate who is at risk for developing symptomatic OA. The main challenges for qualification of new prognostic markers involve the design of large prospective trials, with a refined selection of subjects. uCTXII, sCOMP

or sHA stand among those biochemical markers with putative prognostic value [51]. Elevated levels of uCTX-II have been demonstrated to be associated with radiographic progression [52,53], although recent data suggest that CTX-II may be more a marker of bone turnover than cartilage breakdown [16], as mentioned before. There have also been promising results for prognosis with sCOMP and uCTX-II in a cohort of patients with knee osteoarthritis [54]. Furthermore, in a community-based cohort of 800 individuals, high levels of sCOMP and hyaluronan were reported to predict incident knee osteoarthritis and symptoms over a follow-up period of 6 years [55]. Baseline CRP was found to be a good predictor of the symptomatic response to treatment in a study of 161 patients with knee osteoarthritis over 2 years evaluated by quantitative MRI [56].

Regarding preradiographic OA, collagen type II-specific neoepitope (C2M) and C2C have been found to be promising for the prediction of cartilage loss, as they were found increased in OA serum vs no OA [57]. Also K/L grade 1 patients with knee pain exhibited biomarker features of early OA [58], suggesting that panels of biomarkers may be a better choice for predicting outcomes. Adiponectin [59], visfatin [60], leptin and leptin's soluble receptor (OB-Rb) have been correlated with disease progression in hand osteoarthritis [61] cartilage volume loss in knee osteoarthritis and radiographic changes in hip osteoarthritis [62].

The presence of inflammatory biomarkers has also been shown to predict outcomes in OA. Elevated high sensitivity C Reactive Protein (hs-CRP) predicts cartilage loss associated with osteoarthritis, as well as poorer outcomes after total knee joint replacement [63]. Similar effects have also been found for interleukin 6 (IL-6) and TNF-alpha [63]. However, the specificity of inflammatory biomarkers for OA may be limited since they are implicated in a range of inflammatory conditions and the utility and significance of hs-CRP are generally limited in osteoarthritis when body mass index is taken into account.

Efficacy of the intervention

These biomarkers may range from target evaluation or pharmacodynamic assays (which would be very useful for drug development) to strict surrogate endpoints that indicate the drug is having an impact on the disease (at least, on its clinical manifestations). There have been a number of studies linking the efficacy of interventions in osteoarthritis to levels of uCTX-II [51]. The use of biomarkers for efficacy of intervention has been considerably limited by the absence of proven DMOADs (disease-modifying OA drugs), and most agents used in osteoarthritis have given rather inconsistent results with various biomarkers. Strontium ranelate has shown to have DMOAD properties [6], thus further analysis of these trial results may be expected to shed more light on this issue.

Diagnostic

Diagnostic biomarkers ideally should be sensitive to allow the detection of localized joint damage before it is detectable by imaging technologies or to identify patients at the stage of OA, when treatment may be most effective. uCTX-II, urinary Glc-Gal-Pyd and sPIINP were increased in

patients with OA, and noted as potentially useful biomarkers for the presence of OA since they also correlated with joint surface area [64]. sCOMP and sC2C have also been shown to correlate with knee degeneration in patients with symptomatic knee osteoarthritis [65]. uCTX-II, sC2C, sColl2-1, sCOMP and sHA showed significantly increased levels in individuals with OA, but a substantial proportion of patients had normal levels [45]. Other candidates for diagnostic OA biomarkers have been identified, as follistatin-like protein-1 (FSTL-1) and also peptides of fibulin 3 (Fib3), Fib3-1 and Fib3-2 [66]. In another study, the cartilage synthesis product N-propeptide IIA of type II collagen (PIIANP) was reported to be decreased in knee osteoarthritis compared with healthy controls [10]. The presence of aggrecan neoepitopes was also shown to be associated with the presence of osteoarthritis [43]. Future possible candidate diagnostic biomarkers include sRAGE, the plasma levels of which were significantly lower in patients with knee osteoarthritis than in healthy controls [67], and the presence of the glycoprotein YKL-40 in synovium or cartilage, which is known to correlate with the presence of osteoarthritis and disease severity [17] and was demonstrated to be elevated in patients with OA versus healthy controls [68]. Factors indicating fibrosis and complement proteins also play a role in the pathogenesis of OA and may prove to be useful diagnostic biomarkers [69]. These studies demonstrate a large overlap in single marker levels between OA patients and controls, meaning that they are insufficiently sensitive to be useful as diagnostic tools. Although there have been a number of promising studies in this direction, no single biomarker stands out for use in diagnosis.

Safety

Safety biomarkers could be used in preclinical and clinical applications to monitor the health of the joint tissues, the whole joint organs, or the skeleton in general. Potential complications obviously exist with regard to discriminating toxic or pathological effects from beneficial effects in the case of musculoskeletal biomarkers. So several challenges should be taken into account, as safety biomarkers will need to be qualified against accepted clinical standards, including pain assessments, functional testing, and imaging; The safety threshold for each biomarker might be different across individuals and understanding what 'safe' ranges are for joint tissue biomarkers;. At the moment there are currently no studies exploring specifically this aspect of joint tissue related biomarkers. This will be a growing area that will enhance the goal of personalized medicine and patient safety [5].

CONCLUDING REMARKS

There are a number of promising candidates, notably urinary C-terminal telopeptide of collagen type II and serum cartilage oligomeric protein, although none is sufficiently discriminating to differentiate between individual patients and controls (diagnostic) or between patients with different disease severities (burden of disease), predict prognosis in individuals with or without osteoarthritis (prognostic) or perform so consistently that it could function as a surrogate

outcome in clinical trials (efficacy of intervention). Since there is no effective Disease-Modifying Osteoarthritis Drugs (DMOADs) approved for the treatment of OA, currently non-pharmacological approaches, such as weight loss, aerobic exercise, physical therapy and knee braces, have been recommended by most guidelines [70].

OA has been recognized to have different phenotypes based on various pathological mechanisms; however, the multifactorial nature of the disease presents a challenge for selecting the right therapy for the right OA patient [71]. Future avenues for research include exploration of underlying mechanisms of disease and development of new biomarkers; technological development; the 'omics' (genomics, metabolomics, proteomics and lipidomics); design of aggregate scores combining a panel of biomarkers and/or imaging markers into single diagnostic algorithms; and investigation into the relationship between biomarkers and prognosis. This fact points out the convenience of analyzing whole panels of available biomarkers as putative diagnostic tests. Despite much active research into biomarkers in osteoarthritis, no single biomarker stands out as the gold standard or is sufficiently well validated and recognised for systematic use in drug development [6]. Validation of these markers is needed, but they bring new information about OA pathogenesis that might be useful to develop new markers with a potential clinical use. Therefore, once the assays of these markers are well validated, they should be ideally included in preclinical studies and clinical trials, along with better-qualified biomarkers.

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