Osteoarthritis Biomarkers: Year In Review

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Running title: OA biomarkers

Objective: To summarise important findings from biomarker studies relevant to osteoarthritis (OA), published between April 2016 and March 2017; to consider these findings in the context of new discoveries and technologies, and clinical and scientific need in OA.

Design: Studies were selected by PubMed search, conducted between 01/04/2016-01/03/2017. MeSH terms [biomarker] AND [osteoarthritis] were used; the search was restricted to Human, English language and Full Text Available publications, which yielded 50 eligible publications. Any biomarker was considered, including non-proteins and other clinical measurements.

Results: 3 main areas are overviewed: 1) Studies examining qualified biomarkers, in the FNIH OA Biomarkers Consortium and elsewhere, particularly their ongoing application and validation. Control reference intervals, work on predictive validity and other longitudinal studies examining prognostic value of biomarkers in large cohorts are reviewed. 2) Novel studies relating to biomarkers of inflammation are discussed, including complement, the performance of markers of so-called 'cold inflammation' and results from clinical trials including biomarkers. 3) Discovery studies, including whole blood RNA, proteomics and metabolomics are reviewed, with an emphasis on new technologies.

Conclusions: Discovery, characterisation and qualification of various biomarkers is ongoing; several novel protein and non-protein candidate biomarkers have been reported this year. Biomarkers provide us with an opportunity to better diagnose and stratify the disease, via established panels or new discovery approaches. Improving quality of sampling and testing, and measuring large numbers of markers simultaneously in large cohorts would seem likely to identify new clinically applicable biomarkers, which are still much needed in this disease.

Key words: osteoarthritis; biomarker; outcome; prognosis; diagnosis

Introduction

Why do we need a biomarker for osteoarthritis (OA)? There are many answers to this, but probably the most pressing needs are to reliably identify early disease, to stratify those at risk of progression, to enable new interventions(1-3). A biomarker, or biological marker is "any substance, structure, or process that can be measured in the body or its products and influences or predicts the incidence or outcome of disease" (WHO)(4). Ultimately, a biomarker is a medical sign: "an objective indication of medical state observed from outside the patient, which can be measured accurately and reproducibly." This explains the absolute point of a biomarker: there must be clinical meaning, to health, or to disease, either alone, or when combined with other biomarkers or clinical factors. Some biomarkers could even act as much-needed surrogate clinical endpoints, but in OA we still seem some way from this point(5).

The focus of this review is on published peer-reviewed reports in the past year relating to 'wet' biomarkers (imaging and genetic biomarkers are covered separately). Wet biomarkers for OA can be measured in any accessible samples: synovial fluid (SF), blood, urine or connective tissues(6).

There is a well-described biomarker pipeline from discovery to the clinic(7). For any biomarker, a number of measurement characteristics are important, and essential to assess from the outset. Specificity to target (for example, does an assay for FGF-1 also detect FGF-18?); specificity for disease or outcome (for example, as a diagnostic marker); its sensitivity, precision and reliability. Often we focus on the reliability of an assay's performance, but of course variation within individuals over time may be another source of variability which may

only be detected late in the qualification process. In this review, biomarkers at all stages of the pipeline have been included.

Methods

Studies were identified by a PubMed search between 01/04/2016-01/03/2017 (included if they were e-published by 01/03/2017, and visible on PubMed by that date). MeSH terms [biomarker] AND [osteoarthritis] were used. Restrictions were to Human; English language; Full Text Available; excluding Reviews. This yielded 79 publications whose abstracts were then manually screened. 50 reports of proteins, non-proteins and other clinical measurements which could represent novel biomarkers were eligible for inclusion. All 50 identified manuscripts have been referenced in what is a narrative review, but increased emphasis put on studies fulfilling one or more of 3 criteria: quality (size, design, analysis, subsequent publication impact), clinical relevance (to OA diagnosis, or prognosis, or those in at risk groups) and novelty.

Results

1. Progress in Qualified OA biomarkers

In February 2012, the FNIH and OARSI launched its biomarkers consortium, to test associations of 12 selected biochemical markers with clinical outcome. Focussed on bone and cartilage turnover in serum and urine, these fulfilled BIPEDS criteria for highly validated biomarkers(1). Assays were by LabCorp, a CLIA-certified laboratory. In this important methodological paper, marker reference intervals from 129 ‘multi-joint’ controls (no symptomatic or radiographic OA in hand, hip, knee or spine, over time from the Johnson

County cohort) are published(8). Control samples are usually in low numbers or not selected under stringent conditions, risking contamination bias (given OA is common and sometimes asymptomatic). Certain biomarkers needed separate reference intervals based on age, race or gender. Race and physical activity were also identified as confounders for adipokine and insulin levels(9) and for other inflammatory markers respectively(10). Such factors must be considered when collecting and analysing cohort and control biosamples.

An augmented 18 biomarker panel was examined by the consortium for its ability (both individually, and in combination) to predict knee OA progression at 48 months in the Osteoarthritis Initiative (OAI) cohort(11). 194 progressors were defined (by pain and joint space narrowing [JSN]). The 406 non-progressors had not progressed on both measures. Time integrated concentrations (TICs) were expressed, which essentially show each biomarker's concentration over time (24 months). Z scores enabled comparison of the relative effects of different biomarkers. In the primary analysis, the 24 month TIC of 8 biomarkers significantly predicted case status at 4 years on univariable analysis; of these urinary (u)C-terminal cross-linked Telopeptide of type II collagen (uCTX-II) showed the strongest association (OR 1.37). This association for uCTX-II held versus 'pure' (neither pain, nor JSN) non-progressors (OR 1.72[1.36,2.18]). The most predictive, 'frugal' combination model was 24 month TIC for 3 biomarkers: uCTX-II, serum (s)hyaluronan (sHA) and sN-telopeptide of type I collagen (sNTX-I). Adjusted for 7 covariates, this gave an Area Under the [Receiver Operating] Curve (AU[RO]C) 0.667 (AUC>0.7 being desirable, and achievable with other clinical predictors(12)).

Collagen degradation also predicted progression in other studies: cross-sectionally, higher levels of serum collagen type II cleavage neoepitope (C2C) were seen in individuals with

established radiographic OA compared with pre-radiographic, MR-evident OA(13). However, only baseline uC2C measured using the newer version Human Urine Sandwich Assay (HUSA) was associated with an increased risk of progression of cartilage damage over 3 years (adjusted OR 1.78[1.03,3.09]).

Specific bone biomarkers (sCTX-I,sNTX-I,uNTX-I,uCTX-II,uCTX-Iα/β) were tested by the consortium in 600 OAI participants' serum and urine for associations with bony features on imaging, at baseline and over 24 months(14). Most markers were associated with baseline bone marrow lesions (BMLs); uCTX-II was also associated with large osteophytes (OR 1.39[1.10,1.77]) and bone shape. However, biomarkers were not predictive of changes in BMLs or osteophytes over 24 months, and uCTX-II showed only modest association with bone shape changes, suggesting limited utility here.

4 biomarkers were studied in 1335 Rotterdam cohort participants' serum at baseline: COMP, C-Reactive Protein (CRP), matrix metalloproteinase-dependent degradation of CRP (CRPM) and type I collagen turnover in connective tissue (C1M), plus uCTX-II(15). Cases of hip or knee OA were defined by pain and radiographic data at baseline and 5 years. Incident OA was found in 88/955 cases, associated with sCRP, sCOMP and uCTX-II. In the 170/1125 who progressed, there was no association with C1M, but positive associations with uCTX-II, sCRPM (independent of others), sCRP (OR1.3,P=0.01) and sCOMP. However, after adjusting for predefined covariates (age, sex, Body Mass Index [BMI], joint pain and baseline Kellgren-Lawrence [KL] grade), it became clear that these clinical covariates alone were the main explanatory variables (AUC of 0.68) with only marginal improvement after addition of selected biomarkers to the model.

2. Biomarkers of Inflammation

Synovial inflammation sCOMP and sCRPM are thought to be measures of synovial inflammation(15). The cell population is heterogeneous in OA synovium; CD68+macrophages are maximal in the lining layer but decline less throughout the depth of the tissue in OA than in rheumatoid arthritis (RA)(16). Macrophages, T cells and mast cells were the most abundant: the number of synovial CD4+ T cells was associated with severity of knee pain(17).

CC chemokines are important for macrophage recruitment and are up-regulated after joint injury and in OA in both mouse(18) and human studies(19, 20). CCR2 ligands (CCL2/MCP-1,CCL7,CCL8) but not CCR5 ligands were found to be upregulated in SF from human OA and post-traumatic knees(21). CCR2-expressing cells were increased in OA synovium, and CCR-2 expressing macrophages visualised by immunofluorescence at sites of damage, further implicating this pathway.

Tumour Necrosis Factor (TNFα) is known to be produced by RA synovium, and is persistently elevated after knee joint injury(22). An interesting multi-disciplinary study in a cohort with no knee pain found that Pre-OA cases defined on MR criteria(23) had biomechanical gait differences to controls, and also had a statistically significant difference in sTNFα(24).

Biomarkers of 'cold inflammation' 'Cold inflammation' or 'meta-inflammation' is a term coined to describe low grade inflammation associated with the metabolic syndrome, either systemically or within tissues, which lacks the features of classical inflammation(25). Circulating molecules associated with systemic low grade inflammation have been further

investigated for their effects on OA pathogenesis(26). sIL-6 was again found to be associated with radiological severity of disease, but also IL-4(27).

Strong associations between C-reactive protein (CRP) and incidence and progression of OA were reported (15), but a recent metaanalysis found no association between CRP and OA structural progression, only symptoms(28). A possible reason for this discrepancy was highlighted by a cross-sectional study recording all joint burden in 204 patients scheduled for total hip or knee replacement(29). When adjusting for other important covariates (BMI, age, comorbidity), a paradoxical association was described with increasing sCRP associated with increasing painful joint count in females but reducing painful joint count in males . The effects of gender and hormones on biomarkers but also biological pathways including immune function should be carefully considered(8).

Uric acid is a circulating waste product which can cause systemic and joint-based inflammation via inflammasome activation(30, 31). 88 individuals with medial knee OA, BMI<33 and no gout(32) had baseline serum uric acid (sUA) and semi-flexed knee radiographs at 0 and 24 months. A subgroup also had baseline contrast-enhanced MRI knee synovial volume (SV) assessment. Whether unadjusted, or adjusted (age, gender and BMI), sUA was associated with moderate or fast progression of JSN in this cohort, and also highly correlated with SV. There was a 'step' effect, with the highest 2 quartiles of sUA driving this association. Findings in previous studies regarding the association of uric acid and OA progression have been conflicting; one cross-sectional study of over 4000 individuals showing an association between osteophytes and hyperuricemia, but interestingly only in women(33).

Circulating lipopolysaccharide (LPS), associated with 'leaky bowel', may contribute to low grade systemic inflammation via activation of toll-like signalling: it has been associated with a number of other chronic conditions including atherosclerosis and diabetes(34). Measurement of LPS in biosamples is challenging, given its naturally-occurring inhibitors plus potential for contamination. Using samples of human SF and serum, and samples from the Etarfolatide cohort (which included radiographs and location of activated macrophages within the joint), assays for LPS and LPS binding protein (LBP) were optimized(35). SF-LPS and SF-LBP were associated with JSN and self-reported knee pain respectively, and both associated with activated synovial macrophage abundance. sLPS and sLBP were also associated with osteophyte severity. Replication of these findings will be important.

Reports suggest immunoregulatory dysfunction in OA: peripheral blood follicular helper T cells and IL-9-producing 'T helper 9' cells were described by the same group to be elevated in OA compared with healthy controls, and their levels found to positively correlate with CRP and WOMAC scores(36, 37). Increased frequencies of $CD4^{+}CD25^{+}Foxp3^{+}$ regulatory T cells (Tregs) were found in OA patients independent of obesity; however lower IL-10 secretion and Tim-3 expression was seen in OA Tregs than in healthy controls(38).

Can simple haematological metrics represent systemic inflammatory load? In other settings measures such as a raised neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and mean platelet volume (MPV) have been associated with low grade inflammation, and other chronic inflammatory conditions such as RA. In a cross sectional study of 237 patients with hip OA, MPV had an OR of 2.39 for predicting radiographic severity (adj.OR 2.32[1.66,3.25])(39). The association with MPV was greater than for any other factors including age (PLR and ESR were also associated). In another study of 195 patients with knee

OA by the same authors, blood NLR levels were elevated in the severe knee OA group compared with the mild to moderate knee OA group(40). In AUC analysis, a blood NLR≥2.1 gave 50% sensitivity, 77% specificity in predicting severe knee OA. In multivariate analysis, age and blood NLR≥2.1 emerged as independent predictors of severe knee OA. However neither study independently verified these observations or tested them longitudinally.

Clinical trials and inflammation Biomarker responsiveness to experimental treatments is of great interest. Latterman and colleagues report on a small double-blind, placebo-controlled RCT randomizing those with acute anterior cruciate ligament (ACL) tear to 1 of 4 groups (3 groups including intra-articular steroid after injury, 1 group receiving saline injections only)(41). 11 predominantly inflammatory biomarkers were monitored in SF at 3 times after injury, up to a mean of 37 days after injury. CTX-II, COMP and MMP-1 were significantly different between steroid-treated groups and placebo. For example, CTX-II increased over time with placebo, but this increase was suppressed in all steroid-containing treatment arms. There was a trend towards improved knee pain and function for treatment groups compared with placebo; longer term outcomes following surgery are not yet known.

Other investigators defined complex algorithms of mainly qualified biomarkers to attempt to predict cases of progression to knee arthroplasty following anti-NGF in the context of concurrent NSAIDs(42). NSAIDs reduced inflammatory markers such as IL-6 and increased bone remodelling markers (CTX-I,OC,DKK1). These were important in the model, lending support to the hypothesis that the joint safety issues may in part be mediated by effects of NSAIDs on bone turnover and fracture healing. Whether use of other clinical or radiographic factors can be as predictive as this biomarker algorithm, or can be used in combination should be tested in independent studies.

Hand OA remains relatively under-investigated compared with large joint counterparts. DORA (anti-TNF for hand OA) did not reach its primary outcome, but showed some evidence of effect on post-hoc analysis. This was a cross-sectional biomarker sub-study in a low number of patients(18), but studying 144 joints. sIL-1 was associated with self-reported loss of hand function and radiological erosions(43). MRI osteophytes were associated with sCRP (P=0.0026).

Joint Injury and inflammation Joint injury is one of the strongest risk factors for OA and studies in this area are highly important to those studying disease pathogenesis and prognostic biomarkers(20, 22, 44). In an established cohort with 16 years' radiographic data after ACL or meniscal tear, 4 markers (aggrecan,COMP,MMP-3,TIMP-1) failed to predict outcome(45). How similar or different 'post-traumatic' OA is to 'usual' OA is much debated. It is interesting that many molecular changes after acute meniscal tear OA (MMPs,COMP,IL-6,IL-8,TNF) are also be seen after likely degenerative meniscal tear, a lesion often present in those with early OA(44). Greater SF-MMP-10 was seen in the presence of a complex meniscal tear. SF-ghrelin, thought to be an anti-inflammatory growth factor, was inversely correlated with the extent of meniscal and cartilage injury, and with other SF biomarkers such as IL-6. Higher Ghrelin was cross-sectionally associated with improved patient reported outcomes(46). In the last 2 years, several authors have now highlighted the apparent increased utility of SF biomarkers over serum in this setting(20, 22, 44).

Joint injury provides an experimental window on the initiating disease processes in humans, which can be supported by studies in analogous mouse models of post-traumatic OA. One important question for human biomarker research is how much of the murine response is also relevant to man? Following previous pre-clinical studies, evidence is also provided for

activation of the complement system in the human joint after injury and in OA: levels of components including C4d and C3bBbP were elevated in SF after knee injury, the former persisting for several years(47, 48). Complement levels after injury correlate with other classical inflammatory response proteins such as SF-TNFα. We found that of 7 inflammatory response genes previously shown to be strongly upregulated within hours of mouse knee injury, 6 of 7 were also substantially and significantly upregulated at the protein level in SF following human acute knee joint injury(20). This inflammatory gene response, best represented by SF-IL-6, was significantly associated with the Patient Reported Outcome KOOS-4 at the time of injury (a higher IL-6 associated with worse symptoms and pain). However, a greater IL-6 response at baseline did not appear to predict worse outcome at 3 months.

Other candidate associations Ghrelin is a newly described molecule, reported for the first time in OA, as is adrenomedullin(49). Other cross-sectional studies reporting associations between immunohistochemical, imaging or clinical OA data and candidate biomarkers include those relating to osteopontin and Wnt5A(50); hypoxia inducible factor 1(51); the BMP antagonist, Gremlin-1(52, 53); sclerostin(54); resistin/IL-17(55); macrophage migration inhibitory factor(56) and Apoptosis Signal-Regulating Kinase 1(57).

Other studies sought to distinguish OA from PsA, which can be a clinical diagnostic dilemma. SF-CXCL10, IL-17A, and IFNγ e were all elevated in those with PsA and RA, but not in OA and gout, suggesting a possible diagnostic opportunity(58).

Measurement of SF physical properties could act as a novel biomarker: hyaluronan showed a slower diffusion rate in SF from healthy knees than from joints with chondral damage, by

single molecule microscopy. Only a few microliters of SF are needed, although sensitivity and specificity of this method to detect earlier disease needs be tested(59).

3. OA Biomarker Discovery

Transcriptomics of whole blood Gene expression profiling (GEP) of blood has already been described as having utility in OA(60). This is a potentially important novel source of biomarkers compared with traditional protein measurement in serum or plasma. The same group have investigated whether MRI-graded effusion in knee OA was associated with blood GEP, cross-sectionally in 2 large cohorts (Rotterdam, GARP) measuring GEP in RNA extracted from whole blood, or from PBMCs respectively(61). This was a meta-analysis with adjustment for confounders. Using pathway analysis including STRING, 178 recognised unique genes were described. Pathways strongly represented included Response to stress (45 genes,P=3.44E-02) and Antigen processing/Presentation of exogenous antigens (10 genes,P=3.44E-02). Genes identified included NFATc1, known to be important during the adaptive immune response; and Clorf38, present in monocytes, dendritic, NK, T and cells. It is not clear if these measurements are just a surrogate for effusion itself (as there is correlation with this), or could act as an independent predictor of inflammation.

Proteomics Via mass spectrometry (MS), urinary peptides were sought which acted as diagnostic biomarkers for 3 common joint conditions (RA, Psoriatic arthritis [PsA], OA) with healthy and inflammatory bowel disease control groups(62). There were relatively low numbers (33 in each training group, 85 for validation). ROC curves using 45 biomarkers per classifier were developed, then tested blind in the validation set. In OA, there was an AUC of 0.9; reduced urinary collagen alpha-1 chain fragments were seen. However, these were

individuals with advanced OA: how this finding holds in larger numbers and in earlier stages of OA, where the diagnostic challenge is greater, will be important. A further discovery study using gold-nanoparticle-based MALDI profiling of urine from those with knee OA has also been reported(63).

Multiple Reaction Monitoring (MRM) targeted 14 candidate proteins from Fernandez Puente and colleagues' previous studies(64, 65). MRM is a quantitative proteomic approach, comparing with co-elution of a known isotope-labelled peptide. Following optimisation, 35 peptides representing these proteins were selected. The validation study was carried out in 5 different sample types, including chondrocytes, tissue, synovial fluid and serum (tackling some of the challenges of investigating certain targets or matrices by immunoassay). The candidates haptoglobin, von Willebrand Factor (vWF) and Serum Amyloid P were verified by MRM to be significantly upregulated in 116 OA sera versus controls. Elevation of vWF was also confirmed by bead immunoassay in 38 independent sera.

Proteins damaged by oxidation, nitration and glycation undergo proteolysis to release modified amino acid 'free adducts'. OA cases comprised 2 of the 6 subgroups (also including inflammatory arthritis) of 225 individuals(66). A particular strength was inclusion of early OA (eOA), classified as knee pain, normal X-ray, and arthroscopic Outerbridge cartilage scoring I-II. SF and plasma/serum were analysed using tandem MS with MRM. A 2-stage machine learning approach compared healthy controls to early stage arthritis, and then compared between the 6 disease groups. Unlike traditional proteomics, equilibrium between SF and plasma free adducts meant plasma could be used for all later analyses. In a training set, 10 adducts and anti-CCP status discriminated between groups with a high degree of precision (AUC for eOA 0.98). In a separate test set, early OA was correctly attributed in 18 of 19 cases

(sensitivity 0.83, specificity 0.84, AUC 0.91). There was no adjustment for other demographic or clinical factors, but these are exciting results which would appear to improve on the performance of conventional protein biomarkers.

MRM and immunoassay both have their limitations in terms of high sample throughput and identification of >100 proteins simultaneously in a single sample. Emerging technologies may provide the ability to reliably quantitate many thousand proteins simultaneously in a single sample(67) or quantitate very low levels of proteins in serum(68). Whether these new methodologies could lead to novel OA biomarker identification remains to be established; but they remove some existing obstacles to larger scale discovery.

Metabolomics Tandem MS with MRM gave data on 168 plasma metabolites in Newfoundland OA study samples(69). In the discovery set (64 OA undergoing joint replacement, 45 controls), 18 metabolites were associated with knee OA, which dropped to 6 in the subsequent validation set (72 OA, 76 matched controls). All metabolites were lower in OA plasma compared with controls, lowest P value $<6.5x10^{-4}$. Arginine was 65% lower in OA (AUC 0.98). Arginine is a semi-essential amino acid and collagen precursor. This finding was felt most likely due to over-activity of arginase catabolism rather than dietary. Arginine is a natural inhibitor of cathepsins; increased cathepsin activity in late stage osteoarthritic cartilage is shown in support of this proposed mechanism. In a follow on study, lysophosphatidylcholine to phosphatidylcholine ratio (identified from this metabolomics approach) was shown to predict knee OA. Subjects with a ratio >0.09 were twice as likely to undergo joint replacement as those with a low ratio, over 10 years(70). Two other independent reports support a role for cathepsin K in OA pathogenesis, relating it to knee pain(71) and to effective degradation of collagen II in vitro and in vivo(72).

Loeser reports on the first progression metabolomics study(73). A metabolite panel was measured in fasted second void urine in a subgroup of IDEA participants. Using Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA), a clear separation of progressors from non-progressors was possible on baseline urinary samples with the 3 metabolites: glycolate, hippurate and trigonelline. The latter are gut-flora- derived, implicating the gut microbiome. Interestingly, plasma IL-6 dropped in non-progressors but was unchanged in progressors.

Conclusions

This has been an exciting year for OA biomarkers: several novel potential diagnostic or prognostic biomarkers have been demonstrated in discovery studies. These include less traditional biomarkers including those from metabolomics, and Post-Translational Modification Proteomics, with urinary metabolomics looking a promising area. Cellularly-produced markers, particularly relating to inflammation continue to be highly represented. Novel approaches are much needed: highly validated biomarkers in recent high quality studies are shown to perhaps lack the additional specificity needed to bring OA biomarkers into a new age of clinical utility. Good international pipelines are needed to move promising markers from discovery to qualification, linking laboratories with strengths at one end or other of the pipeline, and considering early other known clinical predictors. The growth in large collaborative international consortia in OA, fostered in some cases by the pharmaceutical collaborations, should drive exciting discoveries into large scale qualification and validation programmes.

Strength is seen in studies with large numbers, prospective sampling, high quality clinical and imaging data, robust biomarker measurement and considered bioinformatic/statistical approaches. Big Data is impacting all areas of medicine, and OA is no different. Combinations of different types of biomarkers may hold promise, but require larger, collaborative approaches to achieve necessary power. Close working with bioinformaticians is necessary to ensure progress. Much should be learned from genetics when approaching array/large panel testing: that insufficient numbers, phenotyping and replication may mean early discovery not being verified; false discovery will dilute our efforts.

Challenges which are being addressed include a perhaps excessive focus on the best-studied serum biomarkers of matrix degradation, and a lack of gold standard definition for early OA (needed if diagnostic markers are to be achieved). Whilst serum markers such as in the FNIH studies may or may not prove to be the ultimate solution, they have provided a paradigm for qualification of OA-related biomarkers and the best means of establishing regulatory approval pathways, enabling the field to progress. Ultimately a laboratory biomarker will only be useful if it tells us something we cannot glean from speaking to, or examining a person with OA, or perhaps performing an X-ray. In this age of precision medicine and a re-emergence of novel therapeutics in OA, we need OA biomarkers more than ever.

Competing interest statement

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References

1. Kraus VB, Burnett B, Coindreau J, Cottrell S, Eyre D, Gendreau M, et al. Application of biomarkers in the development of drugs intended for the treatment of osteoarthritis. Osteoarthritis Cartilage. 2011;19(5):515-42.

2. Kraus VB. Osteoarthritis year 2010 in review: biochemical markers. Osteoarthritis Cartilage. 2011;19(4):346-53.

3. Lotz M, Martel-Pelletier J, Christiansen C, Brandi ML, Bruyere O, Chapurlat R, et al. Value of biomarkers in osteoarthritis: current status and perspectives. Annals of the rheumatic diseases. 2013;72(11):1756-63.

4. Strimbu K, Tavel JA. What are biomarkers? Current opinion in HIV and AIDS. 2010;5(6):463-6.

5. Lohmander LS, Eyre DR. From biomarker to surrogate outcome to osteoarthritis--what are the challenges? The Journal of rheumatology. 2005;32(6):1142-3.

6. Mobasheri A, Henrotin Y. Biomarkers of (osteo)arthritis. Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals. 2015;20(8):513-8.

7. Mobasheri A. Osteoarthritis year 2012 in review: biomarkers. Osteoarthritis Cartilage. 2012;20(12):1451-64.

8. Kraus VB, Hargrove DE, Hunter DJ, Renner JB, Jordan JM. Establishment of reference intervals for osteoarthritis-related soluble biomarkers: the FNIH/OARSI OA Biomarkers Consortium. Annals of the rheumatic diseases. 2016.

9. Gandhi R, Sharma A, Kapoor M, Sundararajan K, Perruccio AV. Racial Differences in Serum Adipokine and Insulin Levels in a Matched Osteoarthritis Sample: A Pilot Study. Journal of obesity. 2016;2016:8746268.

10. Hyldahl RD, Evans A, Kwon S, Ridge ST, Robinson E, Hopkins JT, et al. Running decreases knee intra-articular cytokine and cartilage oligomeric matrix concentrations: a pilot study. European journal of applied physiology. 2016;116(11-12):2305-14.

11. Kraus VB, Collins JE, Hargrove D, Losina E, Nevitt M, Katz JN, et al. Predictive validity of biochemical biomarkers in knee osteoarthritis: data from the FNIH OA Biomarkers Consortium. Annals of the rheumatic diseases. 2017;76(1):186-95.

12. LaValley MP, Lo GH, Price LL, Driban JB, Eaton CB, McAlindon TE. Development of a clinical prediction algorithm for knee osteoarthritis structural progression in a cohort study: value of adding measurement of subchondral bone density. Arthritis research & therapy. 2017;19(1):95.

13. Poole AR, Ha N, Bourdon S, Sayre EC, Guermazi A, Cibere J. Ability of a Urine Assay of Type II Collagen Cleavage by Collagenases to Detect Early Onset and Progression of Articular Cartilage

Degeneration: Results from a Population-based Cohort Study. The Journal of rheumatology. 2016;43(10):1864-70.

14. Deveza LA, Kraus VB, Collins JE, Guermazi A, Roemer FW, Bowes M, et al. Association Between Biochemical Markers of Bone Turnover and Bone Changes on Imaging: Data From the Osteoarthritis Initiative. Arthritis care & research. 2016.

15. Saberi Hosnijeh F, Siebuhr AS, Uitterlinden AG, Oei EH, Hofman A, Karsdal MA, et al. Association between biomarkers of tissue inflammation and progression of osteoarthritis: evidence from the Rotterdam study cohort. Arthritis research & therapy. 2016;18:81.

16. Mucke J, Hoyer A, Brinks R, Bleck E, Pauly T, Schneider M, et al. Inhomogeneity of immune cell composition in the synovial sublining: linear mixed modelling indicates differences in distribution and spatial decline of CD68+ macrophages in osteoarthritis and rheumatoid arthritis. Arthritis research & therapy. 2016;18:170.

17. Klein-Wieringa IR, de Lange-Brokaar BJ, Yusuf E, Andersen SN, Kwekkeboom JC, Kroon HM, et al. Inflammatory Cells in Patients with Endstage Knee Osteoarthritis: A Comparison between the Synovium and the Infrapatellar Fat Pad. The Journal of rheumatology. 2016;43(4):771-8.

18. Burleigh A, Chanalaris A, Gardiner MD, Driscoll C, Boruc O, Saklatvala J, et al. Joint immobilization prevents murine osteoarthritis and reveals the highly mechanosensitive nature of protease expression in vivo. Arthritis Rheum. 2012;64(7):2278-88.

19. Li L, Jiang BE. Serum and synovial fluid chemokine ligand 2/monocyte chemoattractant protein 1 concentrations correlates with symptomatic severity in patients with knee osteoarthritis. Annals of clinical biochemistry. 2015;52(Pt 2):276-82.

20. Watt FE, Paterson E, Freidin A, Kenny M, Judge A, Saklatvala J, et al. Acute molecular changes in synovial fluid following human knee injury are associated with early clinical outcomes. Arthritis & rheumatology. 2016.

21. Raghu H, Lepus CM, Wang Q, Wong HH, Lingampalli N, Oliviero F, et al. CCL2/CCR2, but not CCL5/CCR5, mediates monocyte recruitment, inflammation and cartilage destruction in osteoarthritis. Annals of the rheumatic diseases. 2017;76(5):914-22.

22. Struglics A, Larsson S, Kumahashi N, Frobell R, Lohmander LS. Changes in Cytokines and Aggrecan ARGS Neoepitope in Synovial Fluid and Serum and in C-Terminal Crosslinking Telopeptide of Type II Collagen and N-Terminal Crosslinking Telopeptide of Type I Collagen in Urine Over Five Years After Anterior Cruciate Ligament Rupture: An Exploratory Analysis in the Knee Anterior Cruciate Ligament, Nonsurgical Versus Surgical Treatment Trial. Arthritis & rheumatology. 2015;67(7):1816-25.

23. Hunter DJ, Arden N, Conaghan PG, Eckstein F, Gold G, Grainger A, et al. Definition of osteoarthritis on MRI: results of a Delphi exercise. Osteoarthritis Cartilage. 2011;19(8):963-9.

24. Edd SN, Favre J, Blazek K, Omoumi P, Asay JL, Andriacchi TP. Altered gait mechanics and elevated serum pro-inflammatory cytokines in asymptomatic patients with MRI evidence of knee cartilage loss. Osteoarthritis Cartilage. 2017;25(6):899-906.

25. Calay ES, Hotamisligil GS. Turning off the inflammatory, but not the metabolic, flames. Nature medicine. 2013;19(3):265-7.

26. Courties A, Sellam J, Berenbaum F. Metabolic syndrome-associated osteoarthritis. Current opinion in rheumatology. 2017;29(2):214-22.

27. Mabey T, Honsawek S, Tanavalee A, Yuktanandana P, Wilairatana V, Poovorawan Y. Plasma and synovial fluid inflammatory cytokine profiles in primary knee osteoarthritis. Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals. 2016;21(7):639-44.

28. Jin X, Beguerie JR, Zhang W, Blizzard L, Otahal P, Jones G, et al. Circulating C reactive protein in osteoarthritis: a systematic review and meta-analysis. Annals of the rheumatic diseases. 2015;74(4):703-10.

29. Perruccio AV, Chandran V, Power JD, Kapoor M, Mahomed NN, Gandhi R. Systemic inflammation and painful joint burden in osteoarthritis: a matter of sex? Osteoarthritis Cartilage. 2017;25(1):53-9.

30. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature. 2006;440(7081):237-41.

31. Denoble AE, Huffman KM, Stabler TV, Kelly SJ, Hershfield MS, McDaniel GE, et al. Uric acid is a danger signal of increasing risk for osteoarthritis through inflammasome activation. Proc Natl Acad Sci U S A. 2011;108(5):2088-93.

32. Krasnokutsky S, Oshinsky C, Attur M, Ma S, Zhou H, Zheng F, et al. Serum Urate Levels Predict Joint Space Narrowing in Non-Gout Patients With Medial Knee Osteoarthritis. Arthritis & rheumatology. 2017;69(6):1213-20.

33. Ding X, Zeng C, Wei J, Li H, Yang T, Zhang Y, et al. The associations of serum uric acid level and hyperuricemia with knee osteoarthritis. Rheumatology international. 2016;36(4):567-73.

34. Tilg H, Moschen AR. Microbiota and diabetes: an evolving relationship. Gut. 2014;63(9):1513-21.

35. Huang ZY, Stabler T, Pei FX, Kraus VB. Both systemic and local lipopolysaccharide (LPS) burden are associated with knee OA severity and inflammation. Osteoarthritis Cartilage. 2016;24(10):1769-75.

36. Shan Y, Qi C, Liu Y, Gao H, Zhao D, Jiang Y. Increased frequency of peripheral blood follicular helper T cells and elevated serum IL21 levels in patients with knee osteoarthritis. Molecular medicine reports. 2017;15(3):1095-102.

37. Qi C, Shan Y, Wang J, Ding F, Zhao D, Yang T, et al. Circulating T helper 9 cells and increased serum interleukin-9 levels in patients with knee osteoarthritis. Clinical and experimental pharmacology & physiology. 2016;43(5):528-34.

38. Li S, Wan J, Anderson W, Sun H, Zhang H, Peng X, et al. Downregulation of IL-10 secretion by Treg cells in osteoarthritis is associated with a reduction in Tim-3 expression. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2016;79:159-65.

39. Tasoglu O, Sahin A, Karatas G, Koyuncu E, Tasoglu I, Tecimel O, et al. Blood mean platelet volume and platelet lymphocyte ratio as new predictors of hip osteoarthritis severity. Medicine. 2017;96(6):e6073.

40. Tasoglu O, Boluk H, Sahin Onat S, Tasoglu I, Ozgirgin N. Is blood neutrophil-lymphocyte ratio an independent predictor of knee osteoarthritis severity? Clinical rheumatology. 2016;35(6):1579-83.

41. Lattermann C, Jacobs CA, Proffitt Bunnell M, Huston LJ, Gammon LG, Johnson DL, et al. A Multicenter Study of Early Anti-inflammatory Treatment in Patients With Acute Anterior Cruciate Ligament Tear. The American journal of sports medicine. 2017;45(2):325-33.

42. Arends R, Karsdal MA, Verburg KM, West CR, Bay-Jensen AC, Keller DS. Identification of serological biomarker profiles associated with total joint replacement in osteoarthritis patients. Osteoarthritis Cartilage. 2017;25(6):866-77.

43. Roux CH, Foltz V, Maheu E, Baron G, Gandjbakhch F, Lukas C, et al. MRI and serum biomarkers correlate with radiographic features in painful hand osteoarthritis. Clinical and experimental rheumatology. 2016;34(6):991-8.

44. Liu B, Goode AP, Carter TE, Utturkar GM, Huebner JL, Taylor DC, et al. Matrix metalloproteinase activity and prostaglandin E2 are elevated in the synovial fluid of meniscus tear patients. Connective tissue research. 2017;58(3-4):305-16.

45. Neuman P, Dahlberg LE, Englund M, Struglics A. Concentrations of synovial fluid biomarkers and the prediction of knee osteoarthritis 16 years after anterior cruciate ligament injury. Osteoarthritis Cartilage. 2017;25(4):492-8.

46. Zou YC, Chen LH, Ye YL, Yang GG, Mao Z, Liu DD, et al. Attenuated synovial fluid ghrelin levels are linked with cartilage damage, meniscus injury, and clinical symptoms in patients with knee anterior cruciate ligament deficiency. Discovery medicine. 2016;22(123):325-35.

47. Struglics A, Okroj M, Sward P, Frobell R, Saxne T, Lohmander LS, et al. The complement system is activated in synovial fluid from subjects with knee injury and from patients with osteoarthritis. Arthritis research & therapy. 2016;18(1):223.

48. Wang Q, Rozelle AL, Lepus CM, Scanzello CR, Song JJ, Larsen DM, et al. Identification of a central role for complement in osteoarthritis. Nature medicine. 2011;17(12):1674-9.

49. Liu L, Huang R, Ma D, Cheng W, Feng W, Xing D, et al. Correlation of Adrenomedullin Concentrations with Knee Osteoarthritis Grade. Medical science monitor : international medical journal of experimental and clinical research. 2016;22:2775-8.

50. Li Y, Xiao W, Sun M, Deng Z, Zeng C, Li H, et al. The Expression of Osteopontin and Wnt5a in Articular Cartilage of Patients with Knee Osteoarthritis and Its Correlation with Disease Severity. BioMed research international. 2016;2016:9561058.

51. Qing L, Lei P, Liu H, Xie J, Wang L, Wen T, et al. Expression of hypoxia-inducible factor-1alpha in synovial fluid and articular cartilage is associated with disease severity in knee osteoarthritis. Experimental and therapeutic medicine. 2017;13(1):63-8.

52. Yi J, Jin Q, Zhang B, Wu X, Ge D. Gremlin-1 Concentrations Are Correlated with the Severity of Knee Osteoarthritis. Medical science monitor : international medical journal of experimental and clinical research. 2016;22:4062-5.

53. Zhong L, Huang X, Karperien M, Post JN. Correlation between Gene Expression and Osteoarthritis Progression in Human. International journal of molecular sciences. 2016;17(7).

54. Wu L, Guo H, Sun K, Zhao X, Ma T, Jin Q. Sclerostin expression in the subchondral bone of patients with knee osteoarthritis. International journal of molecular medicine. 2016;38(5):1395-402.

55. Wang K, Xu J, Cai J, Zheng S, Yang X, Ding C. Serum levels of resistin and interleukin-17 are associated with increased cartilage defects and bone marrow lesions in patients with knee osteoarthritis. Modern rheumatology / the Japan Rheumatism Association. 2017;27(2):339-44.

56. Zhang PL, Liu J, Xu L, Sun Y, Sun XC. Synovial Fluid Macrophage Migration Inhibitory Factor Levels Correlate with Severity of Self-Reported Pain in Knee Osteoarthritis Patients. Medical science monitor : international medical journal of experimental and clinical research. 2016;22:2182-6.

57. Zhang QS, Eaton GJ, Diallo C, Freeman TA. Stress-Induced Activation of Apoptosis Signal-Regulating Kinase 1 Promotes Osteoarthritis. Journal of cellular physiology. 2016;231(4):944-53.

58. Muntyanu A, Abji F, Liang K, Pollock RA, Chandran V, Gladman DD. Differential gene and protein expression of chemokines and cytokines in synovial fluid of patients with arthritis. Arthritis research & therapy. 2016;18(1):296.

59. Kohlhof H, Gravius S, Kohl S, Ahmad SS, Randau T, Schmolders J, et al. Single Molecule Microscopy Reveals an Increased Hyaluronan Diffusion Rate in Synovial Fluid from Knees Affected by Osteoarthritis. Scientific reports. 2016;6:21616.

60. Ramos YF, Bos SD, Lakenberg N, Bohringer S, den Hollander WJ, Kloppenburg M, et al. Genes expressed in blood link osteoarthritis with apoptotic pathways. Annals of the rheumatic diseases. 2014;73(10):1844-53.

61. Peters MJ, Ramos YF, den Hollander W, Schiphof D, Hofman A, Uitterlinden AG, et al. Associations between joint effusion in the knee and gene expression levels in the circulation: a meta-analysis. F1000Research. 2016;5:109.

62. Siebert S, Porter D, Paterson C, Hampson R, Gaya D, Latosinska A, et al. Urinary proteomics can define distinct diagnostic inflammatory arthritis subgroups. Scientific reports. 2017;7:40473.

63. Lopez-Cortes R, Formigo J, Reboiro-Jato M, Fdez-Riverola F, Blanco FJ, Lodeiro C, et al. A methodological approach based on gold-nanoparticles followed by matrix assisted laser desorption

ionization time of flight mass spectrometry for the analysis of urine profiling of knee osteoarthritis. Talanta. 2016;150:638-45.

64. Fernandez-Puente P, Calamia V, Gonzalez-Rodriguez L, Lourido L, Camacho-Encina M, Oreiro N, et al. Multiplexed mass spectrometry monitoring of biomarker candidates for osteoarthritis. Journal of proteomics. 2017;152:216-25.

65. Fernandez-Puente P, Mateos J, Fernandez-Costa C, Oreiro N, Fernandez-Lopez C, Ruiz-Romero C, et al. Identification of a panel of novel serum osteoarthritis biomarkers. Journal of proteome research. 2011;10(11):5095-101.

66. Ahmed U, Anwar A, Savage RS, Thornalley PJ, Rabbani N. Protein oxidation, nitration and glycation biomarkers for early-stage diagnosis of osteoarthritis of the knee and typing and progression of arthritic disease. Arthritis research & therapy. 2016;18(1):250.

67. Gawande BN, Rohloff JC, Carter JD, von Carlowitz I, Zhang C, Schneider DJ, et al. Selection of DNA aptamers with two modified bases. Proc Natl Acad Sci U S A. 2017;114(11):2898-903.

68. Yang SY, Chiu MJ, Chen TF, Horng HE. Detection of Plasma Biomarkers Using Immunomagnetic Reduction: A Promising Method for the Early Diagnosis of Alzheimer's Disease. Neurology and therapy. 2017;6(Suppl 1):37-56.

69. Zhang W, Sun G, Likhodii S, Liu M, Aref-Eshghi E, Harper PE, et al. Metabolomic analysis of human plasma reveals that arginine is depleted in knee osteoarthritis patients. Osteoarthritis Cartilage. 2016;24(5):827-34.

70. Zhang W, Sun G, Aitken D, Likhodii S, Liu M, Martin G, et al. Lysophosphatidylcholines to phosphatidylcholines ratio predicts advanced knee osteoarthritis. Rheumatology (Oxford). 2016;55(9):1566-74.

71. Nwosu LN, Allen M, Wyatt L, Huebner JL, Chapman V, Walsh DA, et al. Pain prediction by serum biomarkers of bone turnover in people with knee osteoarthritis: an observational study of TRAcP5b and cathepsin K in OA. Osteoarthritis Cartilage. 2017;25(6):858-65.

72. Mort JS, Beaudry F, Theroux K, Emmott AA, Richard H, Fisher WD, et al. Early cathepsin K degradation of type II collagen in vitro and in vivo in articular cartilage. Osteoarthritis Cartilage. 2016;24(8):1461-9.

73. Loeser RF, Pathmasiri W, Sumner SJ, McRitchie S, Beavers D, Saxena P, et al. Association of urinary metabolites with radiographic progression of knee osteoarthritis in overweight and obese adults: an exploratory study. Osteoarthritis Cartilage. 2016;24(8):1479-86.