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OARSI World Congress – Brussels 2010 Year in Review – Biochemical Markers

Virginia Byers Kraus, MD, PhD
Duke University Medical Center, Division of Rheumatology, Department of Medicine, Durham, NC 27278

Abstract

At the 2010 OARSI congress in Brussels I was asked to present on "Biochemical Markers" in the "Year in Review" session. This provided an opportunity to summarize ongoing work and consensus building in the osteoarthritis research community related to osteoarthritis biomarkers, and second, and an opportunity to briefly overview a subset of studies from the previous 12 months related to soluble biomarkers that provided novel insights in the field. This review therefore briefly summarizes the progress in 2010 of the OARSI OA Biomarkers Global Initiative and the OARSI FDA Biomarkers Working Group, and provides a summary of selected osteoarthritis biomarker studies reported over the previous 12 months based on a review of articles from seven musculoskeletal journals and a PubMed search using the terms biomarkers and osteoarthritis.

Keywords

osteoarthritis; biomarkers

Introduction

The field of osteoarthritis (OA) biochemical markers is steadily advancing, leading to the progressive unfolding of the potential role to be played by osteoarthritis biomarkers in basic research, clinical studies and clinical practice. The long-term goal is to advance, through clinical qualification, a subset of useful biomarkers from the realm of the plausible to the realm of practical application. Increasingly the OA disease process is being considered a continuum, beginning with an inciting event, such as genetic variation or injury, progressing through molecular, preradiographic, and radiographic stages, culminating in end-stage disease [1]. With this reclassification of the disease process as a continuum of a series of

Correspondence can be sent to: Virginia Byers Kraus, MD, PhD Box 3416 Duke University Medical Center Durham, NC 27710 Phone: 919-681-6652 ; Fax: 919-684-8907 vbk@duke.edu.

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The author declares that there are no conflicts of interest

stages, it is readily apparent that biomarkers could play a pivotal role in disease detection and monitoring, particularly during the critical early molecular stages when other tools could not readily identify it. These developments are occurring in parallel with efforts to harness biomarkers for applications in a wide variety of diseases and to standardize the regulatory process for biomarker qualification to accelerate development of therapies [2]. To put into perspective the progress on OA-related biochemical biomarkers in the past year, I summarize here the recent history of the networking efforts in the OA biomarkers research community, and highlight some of the biomarker studies published in the last 12 months.

OARSI OA Biomarkers Global Initiative

The OARSI OA Biomarkers Global Initiative grew out of an NIH/NIAMS sponsored biomarkers network of investigators and public biomarkers research meetings funded from 2002 through 2006. In 2008 the NIH/NIAMS funded support for three OA-related biomarkers workshops to continue the investigative momentum in the OA biomarker field. A first workshop was held in April, 2009 (Bethesda, MD) focusing on OA-related biochemical markers with a meeting summary published this year [3]. A second workshop, focusing on OA-related genetic markers, was held in November, 2010 (Atlanta, GA). The slides from these meetings are available through the OARSI website (http://www.oarsi.org/) and a meeting summary is in preparation. A third workshop will be held in 2012 focusing on OA-related imaging biomarkers. These workshops are serving the purpose for which they were designed, namely, to bring together a critical mass of OA biomarkers researchers to advance the knowledge, qualification, and clinical application of biomarkers in OA.

OARSI FDA Biomarkers Working Group

A second major accomplishment related to OA biomarkers, to come to fruition in 2010, has been production of a comprehensive white paper on the “Application of Biomarkers in the Development of Drugs Intended for the Treatment of Osteoarthritis” by the OARSI FDA Biomarkers Working Group [4]. This group was one of seven that dealt with different aspects of OA, all part of a major initiative on the part of OARSI to assist in overcoming barriers to development of successful disease modifying agents for OA. The forthcoming white papers (to be published in OAC in 2011) represent responses to queries posed by the FDA in an effort to consider revision of guidelines for drug development. Action items posed in the white paper represent a summary of critical needs in the OA biomarkers field as envisioned by the 20 experts of the biomarkers working group. The white paper on biochemical markers included recommendations on biomarker qualification (defined as the evidentiary process of linking a biomarker with biological processes and clinical endpoints), and elucidated unmet needs in the field including articulating a research agenda. A few key recommendations include the need to standardize reporting of biomarker results, to find minimal meaningful differences in biomarkers in the presence and absence of a treatment, to standardize methods of sample collection for biomarker studies (the white paper provides an appendix with suggested methods of sample collection), to collect information on non-signal joints in studies measuring systemic biomarkers, to identify principal tissue sources of a biomarker, and finally, to begin epitope mapping of biomarkers using techniques such as mass spectroscopy (using the antibodies or reagents specific to the particular assay). It is hoped that this white paper will inform and facilitate the process of advancing OA biomarkers for drug development and clinical trial applications.

Summary of Selected Biomarkers Publications (2009-2010)

To identify OA biomarker studies for this year in review presentation, I reviewed the OA biomarker studies of the past 12 months in seven musculoskeletal journals (Osteoarthritis & Cartilage, Arthritis & Rheumatism, Arthritis Care & Research, Arthritis Research & Therapy, Annals of Rheumatic Disease, J Rheumatology, and J Orthopaedic Research),

performed a PubMed search using the query terms of biomarkers and osteoarthritis with the search limited to the prior 12 months, and reviewed the abstracts of the 2010 World Congress on Osteoarthritis OARSI (Brussels). A summary of selected studies is provided in Table 1. In brief, this Table summarizes: a recent major review of human OA biomarker studies fulfilling the need for a means of comparing and contrasting various trials and biomarkers using some specific reporting criteria [5]; 2 trials (one clinical in humans and one preclinical animal study) addressing the need for greater inclusion of biomarkers in OA clinical trials in order to potentiate the effective clinical use of biomarkers in the future; 5 studies related to advances with aggrecanase-generated neoepitopes providing excellent examples of efforts to develop biomarker tools to facilitate dose setting in early clinical studies and to increase confidence in drug mechanism; 2 studies related to prediction of incident OA addressing the need to identify biomarkers that recognize the early molecular stages of OA that may be most susceptible to disease modification [6,7]; 4 studies related to various OA disease subsets addressing the need to study a wide-variety of patient types with varied clinical characteristics and joint-site involvement; and 3 studies related to identifying the joint tissue source of a biomarker addressing the need to understand the principle tissue source(s) of a given biomarker as accurately as possible so that the origin(s) of the epitope(s) is/are clearly understood.

I would like to highlight a few particulars from several of these studies. The paper on OA biomarkers by van Spil and colleagues [5] represents a seminal review on the current qualification status of OA biomarkers for structure and pain outcomes. Van Spil summarized biomarker results from knee and hip OA studies (84 in all), and classified them according to the BIPED classification scheme [8] (denoting Burden of disease, Investigative, Prognostic, Efficacy of intervention and Diagnostic biomarkers). Of note, the OARSI FDA initiative Biomarkers Working Group recently expanded the acronym to BIPEDS with the addition of a Safety biomarker category, to be able to categorize this additional essential use and capability of biomarkers, anticipated to be of increasing importance as the armamentarium of biomarkers expands and becomes more sophisticated. The van Spil review was elegantly organized, and included two monumental supplementary tables that provide a succinct and comprehensive summary of the vast majority of biomarker studies performed to date. The strength (or lack thereof) of evidence for a biomarker to be classified into a BIPED category was scored on a 1-2+ scale. In addition, the precise details were provided regarding the particular assay used (e.g. manufacturer, antibodies, additional references, etc), thus providing a strong paradigm for a method of reporting biomarker results and an example of the level of detail that is not only useful, but increasingly necessary, as we endeavor to further refine our understanding OA-related biomarkers and seek to apply them clinically.

Two papers provided data showing baseline biomarkers predicting incident knee OA ~7-10 years later [6,7]. I look upon these studies as providing particularly exciting evidence to support the capability of select biomarkers to detect the molecular stage of the disease and to pave the way for gaining insights into the early molecular stages of OA. The first, by Ling, is a case-control study nested within the Baltimore Longitudinal Study of Aging that included 22 incident cases of radiographic OA (both hand and knee together) and 66 age, sex and body mass index matched controls without radiographic hand or knee OA [6]. The samples that were tested were obtained at the time of radiographic classification as either case or control and they had a second sample that was obtained up to 10 years earlier at a time when the participants were free of radiographic hand and knee OA, representing pre-radiographic or molecular earlier stages of the disease. Antibody-based micro-arrays, requiring only 20 μL of serum, were used to screen 169 proteins. Overall, there were 10 differentially expressed proteins predictive of incident OA and 16 proteins that were significantly different between cases and controls at the time of classification at follow-up diagnostic of OA. There were a total of 6 ‘disease initiating event’ biomarkers that were

elevated and differentially expressed prior to radiographic OA but that were not differentially expressed at the time of prevalent OA. There were a total of 4 ‘disease sustaining event’ biomarkers that were elevated both before and at the time of radiographic OA. There were several ‘take-home messages’ from this study: that altered extracellular matrix catabolism plays a central role in OA initiation; that increased expression of inflammatory cytokines and chemokines are associated with prevalent OA, lending additional support to the notion of OA as an inflammatory disease; and that prevalent OA is associated with ongoing attempts at repair based on the observation of increased expression of TIMPs and growth factors.

The second study by Golightly [7], presented as a poster at OARSI (Brussels), evaluated the predictive ability of biomarkers for incident knee OA. There were no statistically significant biomarker associations with incident knee OA based on using Kellgren-Lawrence (KL) grading, however, serum Cartilage Oligomeric Matrix Protein (COMP) was highly predictive of incident osteophytes, and both COMP and serum hyaluronan (HA) were predictive of incident joint space narrowing. It was also notable that osteophyte and joint space narrowing were detected by different biomarkers, and as presented in the Year in Review session by Wim van den Berg (his own work and that of Chris Little's), these two processes appear involve different pathways, and different pathophysiologies. This suggests that these two aspects of radiographic OA are inappropriately conflated by KL grade, and in biomarker studies, analyses of these features should ideally, be looked at individually.

Three studies addressed the effects of immobilization and activity on biomarkers [9-11] and are remarkable for their novelty and rigor. The first, a crossover trial of 5 individuals, studied the effects of 14 days of enforced immobilization in a 6 degree “head-down-tilt-bed-rest’ position, with and without 2 brief treatments (5 minutes) of whole body vibration [9]. The two-week periods of immobilization were separated by a recuperation period of 5 1/2 months. Serum COMP decreased continuously throughout the bedrest period (with or without vibration), but returned acutely, within 24 hours of resuming activity, back to the its constitutive levels in the adaptation period. The average cartilage thickness increased by 21.9% with vibration, and decreased 8% in the control (non-vibration) phase relative to the adaptation period. The biomarker results would suggest that both structural outcomes may be deleterious, the increase in cartilage volume possibly representing cartilage swelling and the decrease, cartilage atrophy or degradation. The papers by Helmark [10,11] evaluated the production of biomarkers at a local level by microdialysis after an acute bout of activity versus immobilization, by placing two catheters into the knee joints, one intra-articular and the second perisynovial. Microdialysis was performed by perfusing the catheters slowly with liquid; epitopes of interest diffused into the tubing from the surrounding region and could be measured in the dialysate that flowed through the catheter. COMP increased immediately after exercise (leg extensions) and dropped during the subsequent period of immobilization (3 hours after leg extensions). Intraarticular levels of COMP were twice those of the perisynovial tissue and comparable to serum levels. These studies showed consistent effects of activity and immobilization on COMP and provided some of the first evidence to elucidate the potential specific joint site contributions to the systemic concentrations of biomarkers.

Finally there has been substantial progress in the last year related to the aggrecanase-generated neopepitope biomarker assays, summarized in an editorial by Fosang [12]. This has culminated, at long last, in an ELISA assay sensitive enough to detect an aggrecanase-generated neoepitope in both human serum and urine [13]. The BC3 antibody, that detects the aggrecanase-generated ARGS neoepitope in the aggrecan interglobular domain, was reverse engineered to create BC3 C-2; BC3 C-2 is much more sensitive for the ARGS neoepitope, with a 10,000-fold increased affinity over BC3, and unlike the parent antibody,

does not require aggrecan deglycosylation, thereby lessening the sample manipulation that could potentially cause loss of the epitope. This is a very exciting development in that it has enabled detection of the aggrecanase-generated neoepitope for the first time in human serum and urine, and could therefore greatly facilitate patient selection, and dose setting, and provide an early indication of drug activity in clinical trials that target aggrecan degradation.

In the last year, Aebersold [14] depicted the biomarker development process as consisting of steps such as sample handling, complexity reduction, chromatography, mass spectrometry, and bioinformatics. Two pathways of biomarker qualification were depicted as two trails up a mountain, one a ‘guided and targeted trail’ representing the process of validating and qualifying candidate biomarkers, and the other a ‘discovery trail’ representing an unbiased approach to identification of biomarkers. Although the latter was depicted as the trail at the ‘higher altitude’, both trails were long and arduous with plenty of mountain to tackle, which I take as plenty of room for discovery and improvement of biomarkers for disease detection, prognosis and monitoring. I also like this analogy as it engenders thoughts of a steady march and a prospect of exciting vistas ahead. In summary, as the qualification of biomarkers yields more specific and/or more sensitive biomarkers, it is expected that clinical applicability will become increasingly feasible, and, as stated at the outset, transition from the realm of the plausible to the realm of the practical.

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Table 1

Summary of select biomarker studies (2009-2010).

Reference	Follow-up	Population/Controls (N)	BIPEDS	Results Description and Comments	Assay
REVIEWS					
Van Spil [5]	N/A	Review of 84 studies	ALL	Comprehensive review of hip and knee OA studies that evaluated biomarkers; this seminal work distinguishes associations with structural versus symptom outcomes.	See the primary article for details
TRIALS					
Pelletier 2010 [15]	2-years	161 knee OA patients (81 licofelone treated, 74 naproxen treated)	P, E	Clinical trial of licofelone versus naproxen; in multivariate analyses adjusted for age, sex, and treatment (but not BMI) baseline lower serum MMP-1, higher MMP-3 and higher COMP predicted risk of MRI cartilage loss in the medial, medial, and lateral compartments respectively; adjusted for age, sex, and treatment (but not BMI) change in serum CRP predicted change in medial cartilage volume loss; adjusted for baseline biomarker level, MMP-3 increased significantly less over 2-years in the licofelone compared with the naproxen group; unadjusted for BMI, baseline CRP predicted symptomatic response to treatment; baseline serum CTXI correlated with increase in size of bone marrow lesions on MRI.	Fasting serum: MMP-1, MMP-3, IL-6, CRP (Fluorokine MAP, R&D Systems, MN, USA); COMP (Biovendor, NC, USA); CTX-I (serum Crosslaps, IDS, Boldon, UK), urinary CTX-II (Cartilaps, IDS, Boldon, UK)
Settle 2010 [16]	Yes, 28 days following medial meniscectomy	60 skeletally mature female beagles	E	A highly selective MMP-13 inhibitor exhibited chondroprotective effects in an *in vitro* cartilage explant model and in an *in vivo* canine medial meniscectomy (MM) model which was reflected in the media and biological samples: urinary TIINE (type II collagen) and synovial fluid aggrecan (ARGS and AGEG epitopes) degradation epitopes; ELISA and LC-MS/MS detected TIINE were comparable and 2-fold increased following MM; Neither ARGS nor AGEG were detected in urine following MM; biomarkers PIINP and CPII did not change in untreated control (meniscectomized) animals; per the authors: 'fragments of both collagen and aggrecan are potential and possibly complementary markers of disease progression'; nicely characterized change in 45- mer TIINE peptides with dog age.	TIINE assayed by sandwich ELISA with 5109/9A4 monoclonal antibodies and by LC-MS/MS; aggrecan neoepitopes by immunoaffinity LC-MS/MS using BC3 anti-ARGN antibody and an in-house anti- ^{1772}AGEG antibody; PIINP (Pfizer); CPII (Ibex, Montreal, Canada)
AGGRECANASE NEOEPITOPE RELATED					
Madsen 2010 [17]	N/A	*In vitro* bovine articular cartilage stimulated with cytokines	N/A	In cytokine stimulated cartilage explant media, glycosylated (ADAMTS-4 aggrecanase-generated) ^{374}ARGSVI fragments detected after 7 days and a deglycosylated low-molecular weight (MMP-generated) ^{342}FFGVG was detected after 21 days; unable to conclude on basis of *in vitro* studies whether glycosylation played an important role in enzymatic cleavage events.	Western blots with BC3 anti-ARGSVI antibody (Caterson 2000)[18]; AF28 anti-FFGVG antibody(Fosang 1995)[19]
De Jong 2010 [20]	N/A	32 OA and 60 RA patients	I	A modest but consistent antigenic response (proliferation and cytokine production) was observed by *ex vivo* stimulation of peripheral blood mononuclear cells of RA and OA patients with aggrecan G1 epitopes.	Serum cytokines by LabMAP Technology (Luminex, TX, USA)
Swearingen 2010a [13]	Yes-1 week	24 Lewis rats for 7 day timecourse; ~66 Lewis rats for treatment efficacy evaluation; 18 human	E	Monoiodoacetate intraarticular injection into the rat knee resulted in a time dependent release (over 7 days) of aggrecanase-generated neoepitopes in synovial fluid (collected by lavage) that was	Sandwich ELISAs with anti-HABR antibody (Invitrogen CAHP0022, Carlsbad, CA,

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Reference	Follow-up	Population/Controls (N)	BIPEDS	Results Description and Comments	Assay
		synovial fluids from time of joint replacement for assay specificity evaluation		inhibited in a dose-dependent manner by an aggrecanase-inhibitor; ELISA specificity demonstrated by peptide competition in human synovial fluid; NITEGE assay measured bovine, human and rat aggrecan without need for aggrecan deglycosylation; ARGS assay required aggrecan deglycosylation in bovine and human measurements but not rat (that lacks KS residues).	USA) coupled with anti-NITEGE (681-3 antibody) or anti-ARGS (BC3 antibody)
Swearingen 2010 b [21]	Yes-baseline, 2 and 4 weeks	42 individuals (14/group) with no, mild or moderate OA on clinical exams & radiographic analysis of knee, hip and spine		Development of an optimized anti-ARGS antibody (BC3-C2) able to detect aggrecan neoepitope in human cartilage explant culture media and human serum, urine and synovial fluid (that does not require sample deglycosylation).	Sandwich ELISA with BC3-C2 capture and anti-HABR detection antibodies
Dufield 2010 [22]	no	113 OA patients with symptoms (n=65), knee/hip (n=30), or hand/ spine (n=18), and a no OA reference control group (n=12); additional group (overlap with first group not-specified) of 40 healthy controls and 40 OA patients	D	Description of development of a sensitive immunoaffinity-based liquid chromatography-tandem mass spectrometry method to detect aggrecan cleavage by aggrecanases and metalloproteinases; first report of detection of aggrecanase-generated ARGS neoepitope in urine; method worked on human urine and synovial fluid and several other species (rat, dog, and bovine); pilot results showed higher levels in OA patients than controls and an ARGS/ AGEG ratio in synovial fluid of OA joints of 19.	Immunoaffinity based on BC3 anti-ARGS antibody [23]; and an in-house polyclonal antibody to the ^{1820}AGEG epitope of aggrecan [24]
INCIDENT OA					
Ling 2009 [6]	Up to 10 years (9.3-11.9 years)	Nested case-control study within the Baltimore Longitudinal Study of Aging - 22 incident cases of knee OA and 66 age, sex and body mass index matched controls	P	Tested 169 proteins using 20 μL of serum; identified 10 differentially expressed OA predictive proteins (IL-15, MMP-7, PAI-1, VAP-1, DD5, DD6, Eot-2, ICAM-1, MMP-2, P-selectin) and 16 differentially expressed OA associated proteins (present at the time of OA diagnosis of IL-15, MMP-7, PAI-1, VAP-1, 6Ckine, BLC, FGF-7, GM-CSF, ICAM-3, IGFBP-2, IL-1α, IL-2, MIP-1α, NT-4, TIMP1, VE-cadherin).	Antibody based microarrays using Rolling Circle Amplification assays of Molecular Staging, Inc.
Golightly 2010 [7]	Average follow-up 6 years	542 knees were at risk for incident OA by KL grades (KL grade of 0-1 at baseline), 349 knees at risk for incident OST formation (OST=0 at baseline), and 440 knees at risk for incident JSN (JSN=0 at baseline)	P	In adjusted models (age, gender, BMI, bilateral clustering of knees), the hazard of incident knee OST and incident JSN increased with higher baseline COMP levels. The hazard of only incident knee JSN increased with higher HA levels. Baseline CRP and KS did not predict incident knee outcome.	COMP (in house ELISA with 16F12 and 17C10 monoclonal antibodies [25]); HA (Corgenix, Westminster, CO, USA); hsCRP (UBI Magiwel Enzyme Immunoassay, United Biotech Inc. Mountain View, CA); KS (in-house competitive ELISA with 5D4 monoclonal antibody [26])
OA SUBTYPES					
Filkova 2009 [27] – HAND OA	Yes, Baseline blood & bone scintigraphy; hand x-rays at baseline and 2 years	55 erosive and 33 non-erosive hand OA female patients	B, P	Serum HA correlated with age and disease duration of hand OA but not BMI nor treatment with NSAIDs; baseline serum HA was higher in erosive than non-erosive hand OA groups, correlated with number of hand joint deformities, correlated with Kallman radiographic hand OA scores (at baseline and follow-up), and correlated with level hand joint bone remodeling by late phase bone scintigraphy but not with numbers of inflamed joints by blood pool phase bone scintigraphy; CRP was neither elevated not associated with scintigraphic findings	HA (Corgenix, USA); CRP (immuno-tubidometric technique, biochemical analyzer Olympus 400, Japan)

Reference	Follow-up	Population/Controls (N)	BIPEDS	Results Description and Comments	Assay
Goekoop 2010 [28] – HAND, HOP or KNEE OA	Yes, blood at age 85 and 86 years; radiographs at age 90 years	82 individuals from the Leiden 85-plus Study	D	Low innate production of IL-1β and IL-6 upon whole blood stimulation (4 hours versus 24 hours not specified) with LPS was associated with increased likelihood of being "free from OA" (no radiographic hip or knee OA defined as KL<2 and no more than 2 hand joints with radiographic OA); low innate IL-6 and IL-1Ra associated with lack of hand OA; low innate IL-1β associated with lack of hip OA; no associations for TNF-α or IL-10.	Morning blood: cytokine analysis by non-specified commercially available ELISAs performed in a central laboratory
Rousseau 2010 [29] – KNEE OA	no	64 osteogenesis imperfecta patients and 64 healthy age-matched controls; 87 knee OA patients and 291 age-matched controls	D	Both osteogenesis imperfecta (OI) and knee OA associated with elevated CTX-II as well as lower type I collagen maturation (α/βCTXI ratio) indicative of turnover of recently synthesized collagen I, i.e. 'lower type-I collagen maturation'; results suggest cartilage degradation related to bone resorption but causality could not be inferred from this cross-sectional study; Helix-I was higher in OI but not OA patients compared with controls.	Urinary α and βCTXI and CTX-II (IDS, Boldon, UK); urinary Helix-I (Metra, Quidel Corp, Mountain View, CA, USA)
Chayanupatkul 2010 [30] – KNEE OA	no	36 knee OA patients and 15 healthy controls (all non-diabetic)	B	Plasma sRAGE was significantly lower in OA patients than in healthy controls; sRAGE in plasma and synovial fluid was inversely correlated with knee OA severity, possibly due to decreased production or increased consumption or clearance of sRAGE with disease severity; sRAGE in plasma correlated with and was higher than paired synovial fluid concentrations.	sRAGE (Quantikine ELISA, R&D Systems, MN, USA)
TISSUE LOCALIZATION					
Liphardt 2009 [9]	Yes-2 weeks	8 young (mean age 26 years) healthy males	I	Crossover trial of vibration therapy for immobilization: 14 days of 6 degree head down tilt with or without twice daily whole body vibration (5 mins); serum COMP declined 10-15% during bedrest despite vibration; serum COMP declined within 24 hours of bedrest and returned to baseline within 24 hours of recovery; MRI cartilage thickness of the weight bearing tibia decreased 8% following control intervention and increased 22% following vibration intervention but portent of this is unknown.	Fasting blood: COMP (AnaMar Medical AB, Sweden)
Helmark 2010 [11]	no	7 patients with knee OA scheduled for arthroscopy	I	Intraarticular (IA) and subsynovial (SS) microdialysis catheters retrieved samples for biomarkers showing: COMP IA>SS, aggrecan IA=SS. and Glc-Gal-PYD IA>SS.	COMP (AnaMar, Sweden); Aggrecan (IDS, Nordic, Denmark); Glc-Gal-PYD (Synarc)
Helmark 2010 [10]	no	29 women (13 non-exercise and 16 acute bout of exercise) with symptomatic knee OA	I	Samples obtained after exercise (one-legged knee extension) or no-exercise from blood, urine, intraarticular IA) and subsynovial (SS) sites by microdialysis; mean COMP and aggrecan were higher IA than SS; IA COMP decreased, and IA and SS IL-10 increased following exercise compared with non-exercise.	COMP (AnaMar Medical AB, Sweden); Total Aggrecan for Culture ELISA (IDS Nordic, Denmark); CTX-II (serum and urine Preclinical Cartilaps, IDS Nordic, Sweden); IL-6, IL-8, IL-10, TNF-α (MilliplesMAP, Millipore, Bioiberica, MA, USA)

OA=osteoarthritis; RA=rheumatoid arthritis; N/A=not applicable;

BIPEDS denotes 6 categories of biomarkers: Burden of Disease, Investigational, Prognostic, Efficacy of Intervention, Diagnostic and Safety Biomarkers;

KL=Kellgren Lawrence; OST=osteophyte; JSN=joint space narrowing; KL=Kellgren Lawrence grade of OA; BMI=body mass index; LC-MS/MS=liquid chromatography-mass spectrometry; LPS=lipopolysaccharide; IDS=ImmunoDiagnostics Systems;

IL=interleukin -1α, –1β. -2, -6, -8, -10, -15; MMP=matrix metalloproteinase -2, -7; sVAP-1=soluble vascular adhesion protein-1; PAI-1=plasminogen activator inhibitor-1; D-dimers (DD)5 and DD6; E0r-2=eotaxin-2; ICAM=intracellular adhesion molecule -1, -3; P-selectin; BLC=B-lymphocyte chemokine; 6Ckine =6-chemokine; MIP-1α=macrophage inhibitory protein-1α; FGF-7=fibroblast growth factor-7; IGFBP-2=insulin-like growth factor binding protein-2; GM-CSF=granulocyte macrophage colony stimulating factor; NT-4=neurotrophin-4; VE-cadherin=vascular endothelial cadherin; TNF-α=tumor necrosis factor-α; IL-1Ra=interleukin-1 receptor antagonist; (hs)CRP=(high sensitivity) C reactive protein; CTX-I= C-telopeptide of type I collagen; COMP=cartilage oligomeric matrix protein; Glc-Gal-PYD =glucosyl-galactosyl-pyridinoline; HA=hyaluronan; CTX-II= C-telopeptide of type II collagen; sRAGE=soluble receptor for advanced glycation end products; TIINE=type II collagen neoepitope; ARGN =aggrecan peptide ending in ARGN; AGEG =aggrecan peptide ending in AGEG; CPII =C-propeptide of type II collagen; PIINP =N-propeptide of type II collagen; KS=keratan sulfate