9-27-2022

Soluble Biomarkers in Osteoarthritis in 2022: Year in review

Francisco Rocha

Shabana A. Ali
Henry Ford Health, sali14@hfhs.org

Recommended Citation
Rocha FA, and Ali SA. Soluble Biomarkers in Osteoarthritis in 2022: Year in review. Osteoarthritis Cartilage 2022.

Osteoarthritis and Cartilage xxx (xxxx) xxx

Osteoarthritis and Cartilage

Review

Soluble biomarkers in osteoarthritis in 2022: year in review

F.A.C. Rocha † *, S.A. Ali ‡ § ||

† Department of Internal Medicine, Faculdade de Medicina, Universidade Federal do Ceará, Fortaleza, CE 60430-270, Brazil
‡ Bone and Joint Center, Department of Orthopaedic Surgery, Henry Ford Health System, Detroit, MI, USA
§ Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA
|| Department of Physiology, Michigan State University, East Lansing, MI, USA

ARTICLE INFO

Article history:
Received 27 July 2022
Accepted 20 September 2022

Keywords:
Osteoarthritis
Biomarkers
microRNA
Cartilage
Extracellular matrix
Non-coding RNA

SUMMARY

Objective: To review articles reporting on the development of soluble biomarkers in osteoarthritis (OA) over the past year.
Design: Two literature searches were conducted using the PubMed database for articles published between April 1, 2021 and March 31, 2022. Two searches were done, one on soluble biomarkers and another on circulating non-coding RNAs in OA. Additional articles were hand-picked to highlight emerging biomarker trends in OA.
Results: Of 348 publications retrieved, we included 20 articles with 3 that were hand-picked for the narrative synthesis. We review recent data on soluble biomarkers and circulating non-coding microRNAs in OA using the BIPED classification system. We highlight studies using proteomics to show that cartilage acidic protein 1 (CRTAC1) is a promising biomarker, helping diagnose and estimate severity in hand, hip, and knee OA. Subtle changes in the structure of glycosaminoglycans from the extracellular cartilage matrix were shown to discriminate OA from non-OA cartilage. C-reactive protein metabolite (CRPM) and collagen metabolites may help discriminate subsets of OA patients as well as disease progression. Additionally, physical activity may impact determination of biomarkers. We also report on circulating microRNAs, lncRNAs, and circRNAs in OA and their predictive accuracy in diagnosis and prognosis.
Conclusions: Biomarkers for routine use are still an unmet need in the OA clinical scenario. Emerging data and novel classes of biomarkers (i.e., non-coding RNAs) show promise. Although still requiring validation in multiple independent cohorts, the past year brought advances towards a ready-to-use, reproducible, cost-effective biomarker, namely CRTAC1, to better manage the OA patient.

Introduction

The burden of osteoarthritis (OA) cannot be overemphasized as it is the most common chronic inflammatory arthropathy and incurs substantial social and economic impacts. Although diagnosing established OA typically does not represent a major challenge even in primary care settings[1], there is still a need to adequately measure disease severity, prognosis, and response to treatment. An effort to unify reporting data on biomarkers led to the "BIPED" classification, which stands for Burden of Disease, Investigative, Prognostic, Efficacy of Intervention and Diagnostic, and represents five classes of biomarkers for OA[2]. Distinguishing OA patients according to clinical phenotypes that may be associated with molecular characteristics, which can be clustered as endotypes, may help discriminate patient groups and possibly influence treatment outcome and prognosis[2–4].

In this year in review article, our aim is to provide a narrative review summarizing publications presenting data that may point to novel trends in the OA biomarker field. This includes work showing that plasma levels of the cartilage acidic protein 1 (CRTAC1) are positively associated not only with an OA diagnosis but also with disease severity[5]. We also review qualitative alterations of the charge of extracellular matrix components, specifically glycosaminoglycans, that may help design biomarkers in OA[6–8].

Additionally, we review recent evidence for a potential novel class of biomarkers for OA, namely non-coding RNAs. Among non-coding RNAs, microRNAs, long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) have been most explored in the context of OA and represent promising biomarkers for minimally-invasive, affordable,

* Address correspondence and reprint requests to: F.A.C. Rocha, Rua Cel. Nunes de Melo, 1315, Rodolfo Teófilo, 60430-270 Fortaleza, CE, Brazil. Tel/fax: 55-85-3366-8243.
E-mail address: arocha@ufc.br (F.A.C. Rocha).

https://doi.org/10.1016/j.joca.2022.09.005
1063-4584/

and reliable identification of disease[9]. Though circulating microRNAs were first explored as potential biomarkers for OA in 2010[10] (Fig. 1), advances in profiling technologies including microRNA-sequencing[11] have prompted a surge in studies seeking to elucidate microRNAs as diagnostic and prognostic biomarkers for OA.

Methods

We conducted two literature searches in the PubMed electronic database for publications describing human studies on biomarkers in the period between April 1, 2021 and March 31, 2022, considering epub articles if the date of availability was within this period of time, as follows: 1. Soluble biomarkers: ((((osteoarthritis) OR (arthrosis)) OR (osteoarthrosis))) AND (biomarker [Title/Abstract] OR serum [Title/Abstract] OR plasma [Title/Abstract] OR urine [Title/Abstract] OR synovial fluid [Title/Abstract]). Filters included Clinical Study, Clinical Trial, Controlled Clinical Trial, English Abstract, Randomized Controlled Trial, Humans, English language; and 2. Non-coding RNAs: (("osteoarthritis"[MeSH Terms] OR "osteoarthritis"[All Fields] OR "osteoarthritides"[All Fields]) AND ("micrornas"[MeSH Terms] OR ("microrna"[All Fields] AND "mirna"[All Fields]) OR "noncoding RNA"[All Fields] OR "mir"[All Fields])) AND ((humans[Filter]) AND (2021/4/1:2022/3/31[pdat])). Results were first screened by title and abstract, then by full-text, applying inclusion and exclusion criteria in a stepwise approach. Hand-picked articles and an abstract presented in the European Alliance of Associations for Rheumatology (EULAR) 2021 meeting were also included. Results are presented using the National Institutes of Health (NIH) proposed classification system "BIPED" as reported previously[2,12].

Results

Literature search results

Our first search strategy returned 228 articles [Fig. 2(A)]. Titles and abstracts were screened for the following key terms = 'osteoarthritis', 'arthrosis', 'osteoarthrosis', 'biomarker', 'serum', 'plasma', 'blood', 'urine', and 'synovial fluid'. We excluded any articles that did not use human samples ($N = 3$), were not related to OA ($N = 174$) and were not related to soluble biomarkers ($N = 36$). This retained 11 articles. All but two studies presented data on knee OA with two articles with data on hip OA, one with hand OA, and another with multiple joints (Table I). We also included $N = 3$ hand-picked articles and/or abstracts presented at the EULAR 2021 meeting with data on soluble biomarkers in OA.

Our second search strategy focused on non-coding RNAs and returned 124 articles [Fig. 2(B)]. As inclusion criteria, titles and abstracts were screened for the following key terms = 'biomarker', 'marker', 'serum', 'plasma', 'blood', 'circulating', and 'peripheral'. This retained 23 articles. We excluded any articles that were not in English, did not use human samples, were not related to OA, and were not primary research. We also excluded any articles that reported on tissue expression of microRNAs rather than circulating levels of microRNAs. This removed 14 articles, leaving a total of 9 articles which we summarize in a narrative review format (Table II).

Burden of disease biomarkers

A major challenge facing development of appropriate burden of disease biomarkers (i.e., measuring severity of OA) is the lack of sufficiently large studies. This year, a large study comprising 39,155 individuals in Iceland gathered both clinical data and plasma samples and applied a proteomic approach using a SOMAmer platform examining 4,792 proteins (SomaLogic; https://somalogic.com/). SOMAmer stands for *Slow off-rate modified aptamers*, which are single-stranded oligonucleotides that can specifically bind to proteins. This was an exploratory study with no 'hypothesis-driven' strategy, aiming to identify protein biomarkers that could correctly identify hand, hip, or knee OA diagnosis. Disease severity for hip and knee joints was evaluated as a history of joint replacement obtained in electronic data banks. For hand OA, disease severity was evaluated using radiographies that were available for scoring[5].

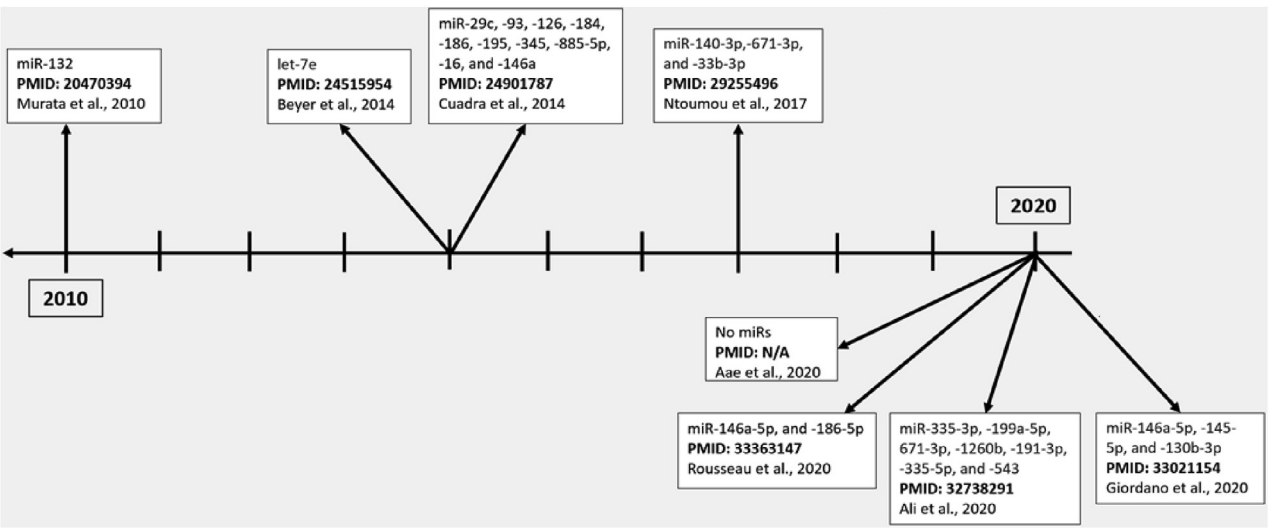

Fig. 1

Osteoarthritis and Cartilage

Timeline depicting the increase in studies performing unbiased profiling of circulating microRNAs (miRs) in OA over the past decade.

Please cite this article as: Rocha FAC, Ali SA, Soluble biomarkers in osteoarthritis in 2022: year in review, Osteoarthritis and Cartilage, https://doi.org/10.1016/j.joca.2022.09.005.

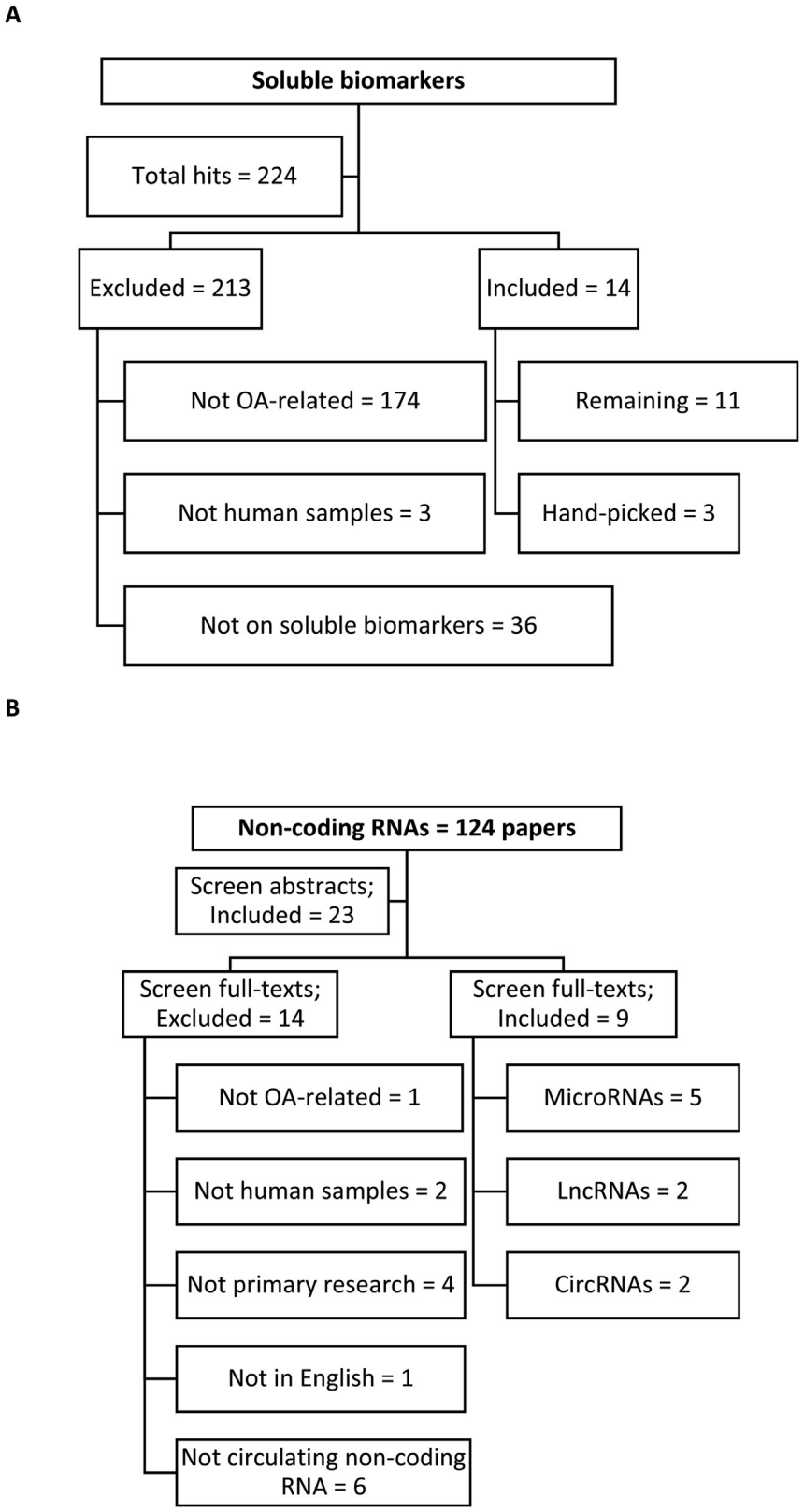

Fig. 2

Osteoarthritis and Cartilage

Flowcharts summarizing the literature search results for A) soluble biomarkers and B) non-coding RNAs in OA.

Please cite this article as: Rocha FAC, Ali SA, Soluble biomarkers in osteoarthritis in 2022: year in review, Osteoarthritis and Cartilage, https://doi.org/10.1016/j.joca.2022.09.005

Approach	Joint	Study population	Biomarker(s)	BIPED entry	Results	Conclusion	Reference
Proteomics; plasma levels	Knee, Hip, Hand	- 39,155 individuals; 3 Iceland cohorts; - 3517 individuals from Rotterdam prospective cohort	CRTAC1	Diagnosis/Burden	CRTAC1 strongly associated with Knee > Hip > Hand OA diagnosis; CRTAC1 associated with knee/hip OA arthroplasty risk and x-Ray hand OA severity	CRTAC1 levels: promising biomarker of knee/hip OA progression to arthroplasty	Styrkarsdottir *et al.*[5] Szilagyi *et al.*[14]
Plasma/serum levels vs xRay progression	Knee	253 patients (NYU group/sCT trial SMC021-2301)	Pro-C2 plasma/serum	Burden	Low pro-C2 associated with increased xRay progression	Low pro-C2 serum level may be more prone to progression	Luo *et al.*[15]
Serum levels	Knee	519 patients; MOST cohort	Free fatty acids (FFA)	Burden	FFA not associated with OA development	FFA not suitable as progression marker	Felson *et al.*[16]
Serum levels	Knee	620 patients; MOST cohort	Total cholesterol, LDL, HDL	Burden	No association	Total cholesterol, LDL, HDL not suitable as burden marker	Schwager *et al.*[17]
Serum levels	Knee	30 primary (pOA) vs 16 secondary OA (sOA)	Tumor necrosis factor (TNF)-α, Interleukin (IL)-1β, IL-6	Diagnosis	No association	Serum TNF, IL-1, IL-6 do not distinguish pOA vs sOA	Rankothgedera *et al.*[18]
Chondroitin sulfate (CS) structure (chromatography, electron microscopy)	Shoulder, Hip	20 shoulder, 10 hip	Molar mass (MM), sulphur (S) content	Diagnosis	CS from OA cartilage has >MM and <S	Structural CS changes distinguish OA from non-OA cartilage	Nunes *et al.*[6,7]
Serum levels	Knee; multiple joints	25 patients	C-reactive protein (CRP), CRP metabolite (CRPM), type I/type III collagen MMP-generated metabolites	Diagnosis	Increased CRPM associated with inflammatory markers and MMP-generated metabolites	CRPM may reflect inflammation	Alexander *et al.*[19]
Serum levels	Knee	781 patients	CRP, CRPM	Prognosis	Higher CRPM levels associated with incident OA	Higher CRPM levels may distinguish a subset of knee OA	Bay-Jensen *et al.*[26]
Serum levels	Knee	447 patients	type III collagen degradation (C3M), CRPM, cartilage oligomeric protein (COMP)	Prognosis	C3M and CRPM negatively associated with symptoms	High CRPM/C3M associated with worse OA pain/function	Yang *et al.*[27]
Serum	Knee	200 patients; OAI cohort	inter-alpha trypsin inhibitor heavy chain 1 (ITIH1), complement C3 (C3), and calcyclin (S100A6)	Prognosis	ITIH1, C3, S100A6 associated with xRay progression	High ITIH1 increases chance of incident knee OA	Lourido *et al.*[28]
Urine	Knee	200 patients received either intra-articular hyaluronan or loxoprofen	cross-linked C-terminal type II collagen telopeptide (CTX-II; uCTX-II); baseline/after 5 weeks intervention	Intervention	uCTX-II increased following hyaluronan injection	Hyaluronan may work differently from loxoprofen	Ishijima *et al.*[32]
Serum; synovial fluid	Knee	225 patients (FORWARD sprifermin study)	Pro-C2	Intervention	Sprifermin increased synovial pro-C2; lower baseline pro-C2 associated with increase in cartilage thickness	Patients with lower pro-C2 may benefit more from sprifermin	Bay-Jensen *et al.*[33]
Serum	Knee	64 patients (hydrotherapy/ peloidotherapy vs placebo)	IL-1β, insulin-like growth factor-1 (IGF-1); baseline, 3 and 6 months post intervention	Intervention	No difference in IL-1β or IGF-1		Adıgüzel *et al.*[34]
Serum; urine	Knee	20 patients	type II collagen degradation, C2M, fragment of type VI collagen degradation, C6M, c COMP, PRO-C2, uCTX-II; baseline and post intervention	Intervention	C2M and C6M had trends to increase or decrease after cycling, respectively	Exercise may impact measure of biomarkers	Bjerre-Bastos *et al.*[35,36]

Table I

Summary of articles focusing soluble biomarkers in osteoarthritis in 2021

Approach	Joint	Study population	Biomarker(s)	BIPED entry	Results	Conclusion	Reference
Serum levels of miRNAs	Hip	25 osteoporotic fracture; 23 osteoarthritis; 52 control	miR-497	Diagnosis	miR-497 is an excellent discriminator between OA and control with AUC = 0.89 ($P < 0.000$)	Circulating miR-497 represents a putative biomarker for hip OA	Pertusa *et al.*[20]
Serum levels of miRNAs	Knee	3 OA; 3 control	miR-584-5p, miR-183-5p, miR-4435	Diagnosis	miR-584-5p-KRAS, miR-183-5p-NRAS, miR-4435-PIK3R3, and miR-4435-SOS1 are four putative regulatory pathways in OA	Due to the limited sample size independent validation of these results is required	Jiang *et al.*[21]
PBMC levels of miRNA	Hip	30 OA; 26 control	miR-206	Diagnosis	Increased miR-206 was positively correlated with peripheral Th17/Treg imbalance in hip OA	AUC analyses are required to determine the value of circulating miR-206 as a biomarker for hip OA	Ye *et al.*[22]
Plasma levels of lncRNA	Knee	81 OA; 49 control	FER1L4	Diagnosis	AUC of 0.92 indicates there may be good diagnostic value of FER1L4 in OA	FER1L4 efficiently identifies OA cases from control subjects	He *et al.*[23]
Serum levels of circRNAs	Not specified	60 OA; 60 control	circ_0005526 (circ_RUNX2)	Diagnosis	AUC of 0.82 indicates there may be good diagnostic value of circ_RUNX2 in OA	Circulating circ_RUNX2 may be used as potential clinical indicator of OA	Wang *et al.*[24]
Serum levels of circRNA	Knee	10 OA; 10 control	circ_0001103	Diagnosis	Circulating circ_0001103 is reduced in OA compared to controls	Due to the limited sample size independent validation of these results is required	Zhang *et al.*[25]
Plasma levels of miRNAs	Knee, Hip	22 OA RAAK study; 71 OA GARP study	miR-1307-5p, miR-140-3p, miR-181a-3p, miR-221-5p, miR-4326, miR-4443, miR-99a-5p	Prognosis	These 7 miRs have AUC values of 0.86 over 2 years and 0.76 over 5 years in distinguishing progressors from non-progressors	The signature of 7 plasma miRNAs can inform future studies on early biomarkers for prognosticating OA over 2 and 5 years	Ramos *et al.*[29]
Plasma levels of miRNAs	Knee	20 fast-progressors; 35 slow-progressors; 51 non-progressors	miR-320b, miR-320c, miR-320d, miR-320e	Prognosis	miR-320 family members are good predictors of fast-progressors with AUC ranging from 82.6 to 91.9	Circulating miR-320 family members merit further investigation as potential biomarkers of fast-progressing knee OA	Ali *et al.*[9]
Serum levels of lncRNAs	Knee	20 OA	MZF1-AS1, MALAT1, LOC100287846	Prognosis	TKR patients with chronic postoperative pain show preoperative downregulation of these 3 circulating lncRNAs	Due to the limited sample size independent validation of these results is required	Giordano *et al.*[31]

Table II

Summary of articles focusing on circulating non-coding RNAs in osteoarthritis in 2021

Osteoarthritis and Cartilage

Data were cross-referenced against classical OA risk factors including age, sex, and body mass index (BMI). Genetic factors were considered using data from an OA genome-wide association study (GWAS) in the UK databank calculating risk scores for individuals in Iceland that have been genotyped[5].

After adjustment for the traditional risk factors associated with OA and exclusion of other inflammatory arthropathies, serum levels of CRTAC1 displayed the strongest association with a diagnosis of knee OA [odds ratio (OR) 1.46; 95% confidence interval (CI) 1.41–1.52] and was also associated with hip OA [OR 1.36; 95% CI 1.29–1.43] and hand OA [OR 1.33; 95% CI 1.26–1.40][5]. Following CRTAC1, cartilage oligomeric matrix protein (COMP) was the only protein with plasma levels associated with an OA diagnosis, though several orders of magnitude below CRTAC1. Although plasma levels of COMP had a mild correlation with CRTAC1 levels, when adjustment for OA diagnosis was made for CRTAC1 levels, COMP levels were no longer significant whereas CRTAC1 remained significant even when conditioning for plasma levels of COMP. Further, CRTAC1 levels remained significantly associated with an OA diagnosis after adjustment for comorbidities including cancer, cardiovascular, and metabolic diseases. In addition to the strong association with an OA diagnosis, plasma levels of CRTAC1 were also positively and significantly associated with pain and radiological severity in hand OA patients, as well as with the increase in the number of joints affected by OA. Concerning hip and knee OA, joint replacement was taken as a measure of disease severity. Analysis of all proteins indicated that CRTAC1 levels were the most significantly associated with joint replacement; further, stratification of plasma CRTAC1 levels into quintiles showed that those in the highest quintiles had 16 times higher risk of being subjected to knee replacement in a period of 5 years.

Despite the robust associations found, CRTAC1 levels were not considered to be associated with OA pathogenesis and the GWAS analysis rendered no association of variants of CRTAC1 genes with OA[5]. CRTAC1 is a glycoprotein found in the cartilage extracellular matrix as well as in the joint fluid of OA patients that is reportedly not linked to OA pathogenesis[5]. As of today, it has not been isolated in other tissues, though an alternative splicing isoform, CRTAC1b, has been isolated in the eye and brain tissues[13]. Study limitations include a lack of information on time of sampling and disease onset, and a heavy reliance on registry data, yet these do not overshadow its robustness. Although the findings must be replicated in other scenarios, another group of researchers used a similar proteomic approach and reported a strong association of increased plasma levels of CRTAC1 with both the diagnosis and burden of OA in a Dutch cohort[14]. These data point to CRTAC1 as a promising candidate for a reproducible and affordable burden of disease biomarker to be used in the management of OA patients.

Turning to another large-scale burden of disease study conducted in the past year, 106 individuals with OA from New York University (NYU group) and 147 patients from a phase III study on oral salmon calcitonin (sCT trial SMC021-2301) were examined for OA progression[15]. The study tested the association between PRO-C2 plasma and serum levels in the NYU and sCT cohorts, respectively, taken as a marker of type II collagen formation, and OA severity, assessed radiologically based on medial joint space narrowing (JSN) over 24 months, while adjusting for age, sex, BMI, race, baseline pain levels and joint space width. Patients of the NYU group with lower PRO-C2 levels had an increase in JSN progression, while PRO-C2 levels in the sCT trial group were not significantly associated with JSN progression. When combining the 253 patients, low PRO-C2 levels were associated with worsening of JSN and those in the lowest quartiles had increased chance of radiographic progression. Interestingly, patients treated with sCT who had very low to low PRO-C2 levels at baseline showed a decrease in JSN progression as compared to those with higher levels of PRO-C2 (Chi squared $= 6.5$, $P = 0.011$). This study presents a possible distinct OA phenotype, wherein individuals have a low level of cartilage formation. Apart from the need to reproduce these findings in larger and independent cohorts, determination of precise PRO-C2 cut-off values requires validation. Among study limitations, JSN progression appears to be far from an ideal parameter to evaluate structural damage in OA[15].

Two more large-scale studies focusing on burden of disease and diagnostic biomarkers for OA were reported using the Multicenter Osteoarthritis study (MOST) cohort. The first study explored serum levels of free fatty acids (FA) in relation to the risk of developing OA. Among 260 and 259 cases of incident symptomatic and radiographic OA, respectively, after adjusting for classical OA risk factors, the authors found no association[16]. The second study with the MOST cohort included 337 and 283 cases with incident and radiographic OA, respectively. Again, total cholesterol, LDL, or HDL levels were not found to be associated with parameters of OA severity[17]. Although metabolic comorbidities are very common in OA patients, these data argue against the use of those lipid levels to monitor OA severity clinically.

Diagnostic biomarkers

Diagnostic biomarkers seek to identify diseased from non-diseased individuals. In a recent case–control study aimed at discriminating primary knee OA (pKOA; $N = 30$) from secondary knee OA (sKOA; $N = 16$) compared to controls and lupus patients, serum levels of the cytokines tumor necrosis factor (TNF)-α, Interleukin (IL)-1β, and IL-6 were evaluated[18]. Study limitations include a low number of patients per group, probable heterogeneity among patients considered to have sKOA, and difficulty distinguishing pKOA from sKOA; resultingly, there was no association with disease parameters. Furthermore, the cytokines that were evaluated are relevant in the pathogenesis of various arthropathies and are also present in comorbidities that are prevalent in OA patients, thereby limiting specificity.

Two studies by Nunes *et al.* reported structural characteristics that distinguish glycosaminoglycans (GAG), namely chondroitin sulfate (CS), extracted from both animals subjected to experimental OA and humans that had undergone arthroplasty secondary to OA or fracture, used as controls. In addition to an increase in the CS content (μg/mg dried cartilage) and in the molar mass of CS in OA cartilage, there was also a significant decrease in the sulphur content in the CS of the OA cartilage, as compared to the non-OA group. That decrease in sulphur was associated with a significant reduction of the negative charge of CS in OA human samples[6,7]. The extent to which these changes (i.e., qualitative alterations of GAG molecules) can be used for diagnosing and/or evaluating the burden of OA remains to be determined.

Interestingly, another study using a GAG chemical exchange saturation transfer [gag(CEST)] protocol derived using 3T magnetic resonance imaging (MRI) was able to discriminate knee OA and control joints, using direct video-arthroscopy images as a gold-standard comparator. The gagCEST technique takes advantage of changes in the saturation transfer of protons bound to solutes[8]. Given that GAG are highly negatively charged and there is cartilage loss at least in late stages of OA, a decrease in cartilage GAG content could be quantitated using the gagCEST technique, thus allowing a reproducible, observer-independent parameter to assess cartilage loss. Coupled to the above data showing that GAG from OA cartilage display decreased sulfation that is associated with a reduced negative GAG charge[7], it might well be possible to determine whether this occurs in early stages of the disease, thus enabling a quantitative method to serve as a biomarker of OA diagnosis and/or burden.

Please cite this article as: Rocha FAC, Ali SA, Soluble biomarkers in osteoarthritis in 2022: year in review, Osteoarthritis and Cartilage, https://doi.org/10.1016/j.joca.2022.09.005.

Another study examined the association of serum levels of C-reactive protein (CRP), CRP metabolite (CRPM), and type I/type III collagen metabolites with radiographic and radionuclide data, using etarfolatide as tracer, in 25 individuals with symptomatic OA[19]. Although the authors investigated the association of CRPM with both isolated and multi-joint inflammation in OA patients, challenges included the low number of patients, adjustment for potential confounders, and definition of site inflammation.

Non-coding RNAs

Within our search timeframe, a total of 6 studies measured circulating non-coding RNAs that could be used to distinguish OA from control individuals. Of these, 3 focused on microRNAs, 1 on lncRNAs, and 2 on circRNAs. In the microRNA studies, miR-497-5p[20], as well as miR-584-5p, miR-183-5p, and miR-4435 were identified in serum[21], while miR-206 was identified in peripheral blood mononuclear cells[22]. A notable challenge is the lack of reproducibility in microRNAs across studies, which could be explained by the different specimens profiled, differences in phenotype definition, or by the low sample sizes with only $N = 3$ per group in one study[21]. Nevertheless, with an area under the curve (AUC) value of 0.89 for miR-497-5p, microRNAs demonstrate utility in discerning OA vs controls[20].

Though other classes of non-coding RNAs have been far less explored as biomarkers for OA, a recent study profiled the lncRNA Fer-1-like protein 4 (FER1L4) in plasma. Showing decreased expression in those with OA ($N = 81$) compared to controls ($N = 49$), predictive models including FER1L4 had an AUC value of 0.92, indicating high accuracy as a diagnostic biomarker[23]. Two other studies measured circRNAs in serum, namely circ_0005526 (circ_RUNX2)[24] and circ_0001103[25]. While circ_0005526 produced an AUC value of 0.82, the predictive ability of circ_0001103 was not reported, likely due to the low sample size ($N = 10$ per group). CircRNAs can function as 'sponges' to bind microRNAs, with circ_0005526 reported to bind miR-498, miR-924, miR-361-3p, and miR-665, each of which regulate gene targets involved in extracellular matrix−receptor interaction pathways[24], and circ_0001103 reported to bind miR-375, which regulates Sirtuin 1 (SIRT1), a regulator of multiple biological processes including inflammation[25]. Regulation of target genes in tissues such as cartilage suggests a connection between these circulating markers and disease pathophysiology, thereby indicating potential specificity to OA.

Prognostic biomarkers

Prognostic biomarkers seek to predict onset or progression of disease. Using data from two phase III rheumatoid arthritis (RA) and OA studies, Bay Jensen *et al.* measured serum CRP and CRPM[26]. While both CRP and CRPM were significantly higher in RA patients, levels in OA patients were marginally higher, as compared to reference levels. However, 20% of OA patients had CRP levels higher than 3 mg/L with 15% having CRP above 5 mg/L, thus suggesting that some OA patients display a more pronounced inflammatory phenotype. In search for a prognostic cut-off, 5% of the OA patients who had CRPM above 9 mg/L and unilateral knee OA at study entry, had increased risk of developing radiographic knee OA in the contralateral knee over 2 years. Moreover, though CRPM levels were associated with incident contralateral knee OA, CRP levels were not. Apart from highlighting "one model fits all" is inadequate when prognosticating OA patients, these data point to a need for larger, prospective, longitudinal studies in order to determine whether CRPM levels can be used as a prognostic biomarker in knee OA[26].

Another recent study examined the association of serum levels of type III collagen degradation (C3M), CRPM, and COMP with OA symptoms and imaging changes in knee OA. There was a statistically significant negative association of serum C3M and CRPM with knee pain and function whereas synovitis and meniscal changes were positively associated with knee symptoms. In addition to finding weak associations, the study design was cross-sectional, without an adequate number of patients to adjust for confounders[27]. Using data from the Osteoarthritis Initiative (OAI) cohort, levels of inter-alpha trypsin inhibitor heavy chain 1 (ITIH1), complement C3 (C3), and calcyclin (S100A6) were shown to be positively associated with incident radiographic knee OA with high predictive capacity (AUC = 0.82). ITIH1 levels, combined with the clinical model, increased the predictability of incident knee OA. Although promising, particularly given the clinical feasibility, these results need to be replicated in larger cohorts with adjustments for confounders, including comorbidities and risk factors[28].

Non-coding RNAs

MicroRNAs and lncRNAs have recently been explored for their ability to predict both radiographic and symptomatic OA outcomes. Leveraging the unparalleled profiling power of sequencing[11], both Ramos *et al.*[29] and Ali *et al.*[30] identify plasma microRNAs associated with radiographic progression of OA. These studies utilized established OA cohorts including the Research Arthritis and Articular Cartilage (RAAK) study and the Genetics osteoARthritis and Progression (GARP) study[29], and the OAI cohort[30], respectively. Ramos *et al.* report 7 microRNAs (miR-1307-5p, miR-140-3p, miR-181a-3p, miR-221-5p, miR-4326, miR-4443, and miR-99a-5p) to have AUC values of 0.86 over 2 years and 0.76 over 5 years in distinguishing progressors from non-progressors[29]. Ali *et al.* report models with AUC values of up to 0.92 when using the top predictive microRNAs (including members of the miR-320 family: miR-320b/c/d/e) to discern fast-progressors from both slow- and non-progressors over 8 years[30]. The lack of overlap in putative prognostic microRNAs in these two studies could be explained by differences in defining the progression phenotype (e.g., knee and hip[29] vs knee[30]), including the follow-up time frame examined. However, the strong AUC values show promise and suggest validation in independent cohorts is merited.

Another study on circulating non-coding RNAs analyzed 84 lncRNAs in serum from knee OA patients and assessed pain intensity before and 1 year after total knee arthroplasty[31]. Giordano *et al.* report 3 lncRNAs, namely Myeloid Zinc Finger 1 Antisense RNA 1 (MZF1-AS1), Metastasis associated lung adenocarcinoma transcript 1 (MALAT1), and Patched 1 pseudogene (LOC100287846), to be downregulated pre-operatively among patients with chronic post-operative pain vs those with normal post-operative pain[31]. These data suggest circulating lncRNAs may be useful markers for predicting pain outcomes following surgery, but also may be contributing to molecular mechanisms underlying pain since 2 of the 3 lncRNAs are known to be involved in neuropathic pain[31]. Given the small sample size in this study ($N = 10$ per group), independent validation is required.

Intervention

Viscosupplementation has been explored as a valuable alternative to treat OA patients. In a group of 200 knee OA patients treated with intra-articular hyaluronic acid (HA) or loxoprofen for 5 weeks, urinary levels of CTX-II (uCTX-II) were shown to be significantly higher in the HA group of patients[32]. Similar to the above-mentioned studies, the low number of patients and the time of observation, among other issues, precludes a more in-depth analysis of these results. Sprifermin, the recombinant fibroblast growth factor (FGF)-18, has been studied to treat knee OA, with a cartilage anabolic treatment profile[33]. PRO-C2 was measured as a marker of type II collagen formation in synovial and serum samples of patients from the 2-year phase IIb FORWARD study to evaluate intra-articular (IA) sprifermin over placebo. PRO-C2

Please cite this article as: Rocha FAC, Ali SA, Soluble biomarkers in osteoarthritis in 2022: year in review, Osteoarthritis and Cartilage, https://doi.org/10.1016/j.joca.2022.09.005.

levels significantly increased in the sprifermin group, and patients in the placebo group with low PRO-C2 levels had more cartilage loss as compared to those in this group with higher PRO-C2 levels[33]. Although data are not conclusive regarding the usefulness of PRO-C2 as a biomarker for response to treatment, they suggest an anabolic response following sprifermin use, which needs further validation.

Non-pharmacological treatments are a "must use" recommendation in OA. A study evaluated the benefits of balneological treatment (peloidotherapy + hydrotherapy) in knee OA and the possible association with changes in serum levels of IL-1β, TNF-α, and insulin-like growth factor-1 (IGF-1). Sixty-four patients were divided into two groups, either receiving balneotherapy or a control therapy, and were evaluated up to 6 months after treatment. There were neither clinically relevant differences in response to treatment nor between serum cytokine levels, potentially due to methodological issues that impaired data comparison[34]. Physical activity can influence levels of extracellular matrix and/or bone degradation/neoformation products, as well as inflammatory mediators in biologic fluids. At least two studies addressed this issue, one with healthy young individuals and another with knee OA patients, showing variation of collagen markers following exercise[35,36]. This should be taken into consideration as the level of physical activity is typically not specifically addressed or measured when conducting protocols aimed at associating soluble biomarkers with clinical parameters in OA patients. As such, physical activity could represent a critical, yet underappreciated, confounding variable in biomarker studies.

Endotype

The possibility that different OA phenotypes combined with molecular characteristics constitute particular endotypes has gained much attention. The IMI-APPROACH cohort has gathered data that allow discriminating different OA phenotypes using a machine learning approach. Based on these data, three dominant phenotypes associated with three endotypes were characterized, as follows: C1) low tissue turnover (low repair and articular cartilage/subchondral bone turnover), C2) structural damage (high bone formation/resorption, cartilage degradation), and C3) systemic inflammation (joint tissue degradation, inflammation, cartilage degradation). Results were consistent when applied to the FNIH/OAI cohort[3]. This paper was included in order to put forth a recommendation that interventions in OA patients be designed with phenotyping and endotyping parameters in mind.

Discussion

Identifying reliable biomarkers for OA has been a challenge for some years despite concerted effort from the research community. As outlined above, making an OA diagnosis typically does not present a major issue[1]. On the other hand, although radiographies are widely used to assess disease severity, imaging parameters lack a direct correlation with joint pain and function, which represent the most pressing issues for the individual OA patient[1]. Thus, having biomarker(s) to assess disease burden in terms of symptoms, function, and progression, as well as changes secondary to intervention is a major unmet need in OA management.

This last year brought data on a promising biomarker to be used in the clinical scenario, namely CRTAC1 plasma levels[5]. Levels of this protein were associated with OA diagnosis in various joints as well as with disease severity. Data on non-coding RNAs may also be useful in clinical research as a tool for discriminating patients according to OA status or progression. There is perhaps reduced enthusiasm for lipid compounds given the data showing a lack of association with OA parameters. There remains a need for studies evaluating alterations to biomarkers following interventions, and emerging data reinforce that physical activity might impact serum levels of inflammatory mediators, thus indicating we should control for sedentary vs active behavior when comparing treatment modalities[35,36]. Although still in the early preclinical stage, qualitative changes, namely modification of the charge of extracellular components of the cartilage, may better serve as a biomarker rather than measuring increase or decrease of cartilage and/or bone components[6,7].

Notable themes pertaining to study limitations arose during our evaluation of the literature included in this review. Looking at endpoints, joint replacement status and outcomes are frequently used as surrogates for OA severity but clinicians and surgeons, not to mention patients, are well aware that many patients either do not want or are not eligible for surgery, which can bias evaluation. Across studies, small sample size and discrepancies in phenotype definition contribute to the lack of reproducibility, thereby limiting clinical translation. Going forward, well-powered studies with precisely defined cohorts are required. Additionally, there is opportunity to explore composite biomarkers, particularly with respect to non-coding RNAs and their downstream gene targets which may have functional roles in OA pathology.

We conclude our narrative review with an article on endotypes with the goal of emphasizing that the "one size fits all" strategy is counterproductive in the care of OA patients. Moving forward, we anticipate seeing more studies that separate clusters of phenotypes and endotypes in this disease. Although this year produced the largest biomarker study in OA to date[5], there remains a need for multi-center studies with appropriate longitudinal design and inclusion of diverse patients in order to advance this field. As of now, because validation studies are still needed, precisely which biomarker candidates are most promising remains subject to individual interpretation. We anticipate continued effort in OA biomarker research will bring us closer to the day a useful tool will reach the clinical arena.

Contributions

Both authors, namely Rocha FAC and Ali SA, equally contributed to the article, concerning data collection, interpretation, writing and revising the manuscript.

Conflict of interest

The authors declare no competing interests regarding the preparation of this manuscript.

Funding

There was no specific funding for this article. Partial support for Dr. Rocha FAC was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ grants 313860/2021-1 and 403767/2021-0).

Acknowledgments

We are grateful to Dr. Mohit Kapoor for a critical review of the text. We thank Taqwa Khalaff for assistance in preparing Fig. 1.

References

1. Sakellariou G, Conaghan PG, Zhang W, Bijlsma JWJ, Boyesen P, D'Agostino MA, *et al.* EULAR recommendations for the use of imaging in the clinical management of peripheral joint osteoarthritis. Ann Rheum Dis 2017 Sep;76(9):1484–94, https://doi.org/10.1136/annrheumdis-2016-210815. Epub 2017 Apr 7. PMID: 28389554.

Please cite this article as: Rocha FAC, Ali SA, Soluble biomarkers in osteoarthritis in 2022: year in review, Osteoarthritis and Cartilage, https://doi.org/10.1016/j.joca.2022.09.005.

2. Bauer DC, Hunter DJ, Abramson SB, Attur M, Corr M, Felson D, *et al*. Classification of osteoarthritis biomarkers: a proposed approach. Osteoarthritis Cartilage 2006;14(8):723–7, https://doi.org/10.1016/j.joca.2006.04.001. PMID: 16733093.
3. Angelini F, Widera P, Mobasheri A, Blair J, Struglics A, Uebelhoer M, *et al*. Osteoarthritis endotype discovery via clustering of biochemical marker data. Ann Rheum Dis 2022 May;81(5):666–75, https://doi.org/10.1136/annrheumdis-2021-221763. Epub 2022 Mar 4. PMID: 35246457.
4. Rousseau J-C, Chapurlat R, Garnero P. Soluble biological markers in osteoarthritis. Ther Adv Musculoskelet Dis 2021;13:1–22, https://doi.org/10.1177/1759720X211040300.
5. Styrkarsdottir U, Lund SH, Saevarsdottir S, Magnusson MI, Gunnarsdottir K, Norddahl GL, *et al*. The CRTAC1 protein in plasma is associated with osteoarthritis and predicts progression to joint replacement: a large-scale proteomics scan in Iceland. Arthritis Rheumatol 2021 Nov;73(11):2025–34, https://doi.org/10.1002/art.41793. Epub 2021 Sep 28.
6. Nunes RM, Girão VCC, Castro RR, Cunha PLR, Feitosa JPA, Pinto ACMD, *et al*. Increase in molar mass distinguishes chondroitin sulfate from osteoarthritis and normal extracellular cartilage matrix. Connect Tissue Res 2021 Sep;62(5):597–604, https://doi.org/10.1080/03008207.2020.1825700. Epub 2020 Oct 7. PMID: 33028117.
7. Nunes RM, Girão VCC, Cunha PLR, Feitosa JPA, Pinto ACMD, Rocha FAC. Decreased sulfate content and zeta potential distinguish glycosaminoglycans of the extracellular matrix of osteoarthritis cartilage. Front Med (Lausanne) 2021 Apr 29;8:612370, https://doi.org/10.3389/fmed.2021.612370. eCollection 2021. PMID: 33996844.
8. Soellner ST, Welsch GH, Gelse K, Goldmann A, Kleyer A, Schett G, *et al*. gagCEST imaging at 3 T MRI in patients with articular cartilage lesions of the knee and intraoperative validation. Osteoarthritis Cartilage 2021 Aug;29(8):1163–72, https://doi.org/10.1016/j.joca.2021.04.012. Epub 2021 Apr 30. PMID: 33933584.
9. Ali S A, Peffers MJ, Ormseth MJ, Jurisica I, Kapoor M. The non-coding RNA interactome in joint health and disease. Nat Rev Rheumatol 2021 Nov;17(11):692–705, https://doi.org/10.1038/s41584-021-00687-y. Epub 2021 Sep 29. PMID: 34588660.
10. Murata K, Yoshitomi H, Tanida S, Ishikawa M, Nishitani K, Ito H, *et al*. Plasma and synovial fluid microRNAs as potential biomarkers of rheumatoid arthritis and osteoarthritis. Arthritis Res Ther 2010;12(3):R86, https://doi.org/10.1186/ar3013. Epub 2010 May 14. PMID: 20470394.
11. Potla P, Ali SA, Kapoor M. A bioinformatics approach to microRNA-sequencing analysis. Osteoarthritis Cartilage Open 2021;3(1):100131.
12. 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 May;19(5):515–42, https://doi.org/10.1016/j.joca.2010.08.019. Epub 2011 Mar 23.
13. Steck E, Bräun J, Pelttari K, Kadel S, Kalbacher H, Richter W. Chondrocyte secreted CRTAC1: a glycosylated extracellular matrix molecule of human articular cartilage. Matrix Biol 2007 Jan;26(1):30–41, https://doi.org/10.1016/j.matbio.2006.09.006. Epub 2006 Sep 16. PMID: 17074475.
14. Szilagyi I, Vallerga C, Waarsing JH, Schiphof D, Bierma-Zeinstra SMA, Van Meurs J. OP0111 Plasma proteomics identifies CRTAC1 as biomarker for osteoarthritis severity and progression. Ann Rheum Dis 2021;80:61–2.
15. Luo Y, Samuels J, Krasnokutsky S, Byrjalsen I, Kraus VB, He Y, *et al*. A low cartilage formation and repair endotype predicts radiographic progression of symptomatic knee osteoarthritis. J Orthop Traumatol 2021 Mar 9;22(1):10, https://doi.org/10.1186/s10195-021-00572-0. PMID: 3368757.
16. Felson DT, Misra D, LaValley M, Clancy M, Chen X, Lichtenstein A, *et al*. Fatty acids and osteoarthritis: the MOST study. Osteoarthritis Cartilage 2021 Jul;29(7):973–8, https://doi.org/10.1016/j.joca.2021.03.006. Epub 2021 Mar 20. PMID: 33757857.16.
17. Schwager JL, Nevitt MC, Torner J, Lewis CE, Matthan NR, Wang N, *et al*. Association of serum low-density lipoprotein, high-density lipoprotein, and total cholesterol with development of knee osteoarthritis. Arthritis Care Res (Hoboken) 2022 Feb;74(2):274–80, https://doi.org/10.1002/acr.24455. Epub 2022 Jan 8.
18. Rankothgedera S, Atukorala I, Fernando C, Munidasa D, Wijayaratne L, Udagama P. A potential diagnostic serum immunological marker panel to differentiate between primary and secondary knee osteoarthritis. PLoS One 2021 Sep 20;16(9):e0257507, https://doi.org/10.1371/journal.pone.0257507. eCollection 2021. PMID: 34543351.
19. Alexander Jr LC, McHorse G, Huebner JL, Bay-Jensen AC, Karsdal MA, Kraus VB. A matrix metalloproteinase-generated neoepitope of CRP can identify knee and multi-joint inflammation in osteoarthritis. Arthritis Res Ther 2021 Aug 31;23(1):226, https://doi.org/10.1186/s13075-021-02610-y. PMID: 34465395.
20. Pertusa C, Tarin JJ, Cano A, Garcia-Perez MA, Mifsut D. Serum microRNAs in osteoporotic fracture and osteoarthritis: a genetic and functional study. Sci Rep 2021;11:19372.
21. Jiang Y, Shen Y, Ding L, Xia S, Jiang L. Identification of transcription factors and construction of a novel miRNA regulatory network in primary osteoarthritis by integrated analysis. BMC Musculoskelet Disord 2021;22:1008.
22. Ye X, Lu Q, Yang A, Rao J, Xie W, He C, *et al*. MiR-206 regulates the Th17/Treg ratio during osteoarthritis. Mol Med 2021;27:64.
23. He J, Wang L, Ding Y, Liu H, Zou G. lncRNA FER1L4 is dysregulated in osteoarthritis and regulates IL-6 expression in human chondrocyte cells. Sci Rep 2021;11:13032.
24. Wang C, Li N, Liu Q, Su L, Wang S, Chen Y, *et al*. The role of circRNA derived from RUNX2 in the serum of osteoarthritis and its clinical value. J Clin Lab Anal 2021;35:e23858.
25. Zhang M, Mou L, Liu S, Sun F, Gong M. Circ_0001103 alleviates IL-1beta-induced chondrocyte cell injuries by upregulating SIRT1 via targeting miR-375. Clin Immunol 2021;227:108718.
26. Bay-Jensen AC, Bihlet A, Byrjalsen I, Andersen JR, Riis BJ, Christiansen C, *et al*. Serum C-reactive protein metabolite (CRPM) is associated with incidence of contralateral knee osteoarthritis. Sci Rep 2021 Mar 22;11(1):6583, https://doi.org/10.1038/s41598-021-86064-x. PMID: 33753821.
27. Yang X, Thudium CS, Bay-Jensen AC, Karsdal MA, van Starten J, Ardel NK, *et al*. Association between markers of synovial inflammation, matrix turnover and symptoms in knee osteoarthritis. Cells 2021 Jul 20;10(7):1826, https://doi.org/10.3390/cells10071826. PMCID: PMC8307303.
28. Lourido L, Balboa-Barreiro V, Ruiz-Romero C, Rego-Pérez I, Camacho-Encina M, Paz-González R, *et al*. A clinical model including protein biomarkers predicts radiographic knee osteoarthritis: a prospective study using data from the Osteoarthritis Initiative. Osteoarthritis Cartilage 2021 Aug;29(8):1147–54, https://doi.org/10.1016/j.joca.2021.04.011. Epub 2021 Apr 30. PMID: 33933586.
29. Ramos YFM, Coutinho de Almeida R, Lakenberg N, Suchiman E, Mei H, Kloppenburg M, *et al*. Circulating MicroRNAs highly

Please cite this article as: Rocha FAC, Ali SA, Soluble biomarkers in osteoarthritis in 2022: year in review, Osteoarthritis and Cartilage, https://doi.org/10.1016/j.joca.2022.09.005.

correlate to expression of cartilage genes potentially reflecting OA susceptibility-towards identification of applicable early OA biomarkers. Biomolecules 2021;11.
30. Ali SA, Espin-Garcia O, Wong AK, Potla P, Pastrello C, McIntyre M, *et al*. Circulating microRNAs differentiate fast-progressing from slow-progressing and non-progressing knee osteoarthritis in the Osteoarthritis Initiative cohort. Ther Adv Musculoskelet Dis 2022;14. 1759720X221082917.
31. Giordano R, Petersen KK, Santoro M, Pazzaglia C, Simonsen O, Valeriani M, *et al*. Circulating long non-coding RNA signature in knee osteoarthritis patients with postoperative pain one-year after total knee replacement. Scand J Pain 2021;21:823–30.
32. Ishijima M, Nakamura T, Shimizu K, Hayashi K, Kikuchi H, Soen S, *et al*. Different changes in the biomarker C-terminal telopeptides of type II collagen (CTX-II) following intra-articular injection of high molecular weight hyaluronic acid and oral non-steroidal anti-inflammatory drugs in patients with knee osteoarthritis: a multi-center randomized controlled study. Osteoarthritis Cartilage 2022 Jun;30(6):852–61, https://doi.org/10.1016/j.joca.2022.03.003. Epub 2022 Mar 22.
33. Bay-Jensen AC, Manginelli AA, Karsdal M, Luo Y, He Y, Michaelis M, *et al*. Low levels of type II collagen formation (PRO-C2) are associated with response to sprifermin: a pre-defined, exploratory biomarker analysis from the FORWARD study. Osteoarthritis Cartilage 2022 Jan;30(1):92–9, https://doi.org/10.1016/j.joca.2021.10.008. Epub 2021 Nov 1.
34. Adıgüzel T, Arslan B, Gürdal H, Karagülle MZ. Evaluation of the therapeutic and the chemical effects of balneological treatment on clinical and laboratory parameters in knee osteoarthritis: a randomized, controlled, single-blinded trial. Int J Biometeorol 2022 Jun;66(6):1257–65, https://doi.org/10.1007/s00484-022-02274-6. Epub 2022 Mar 26. PMID: 35347399.
35. Bjerre-Bastos JJ, Nielsen HB, Andersen JR, Karsdal M, Boesen M, Mackey AL, *et al*. A biomarker perspective on the acute effect of exercise with and without impact on joint tissue turnover: an exploratory randomized cross-over study. Eur J Appl Physiol 2021 Oct;121(10):2799–809, https://doi.org/10.1007/s00421-021-04751-z.
36. Bjerre-Bastos JJ, Nielsen HB, Andersen JR, Karsdal M, Bay-Jensen AC, Boesen M, *et al*. Does moderate intensity impact exercise and non-impact exercise induce acute changes in collagen biochemical markers related to osteoarthritis? – An exploratory randomized cross-over trial. Osteoarthritis Cartilage 2021 Jul;29(7):986–94, https://doi.org/10.1016/j.joca.2021.02.569.

Please cite this article as: Rocha FAC, Ali SA, Soluble biomarkers in osteoarthritis in 2022: year in review, Osteoarthritis and Cartilage, https://doi.org/10.1016/j.joca.2022.09.005.