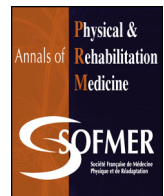




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Update article

## Osteoarthritis biomarkers derived from cartilage extracellular matrix: Current status and future perspectives

Y. Henrotin<sup>a,b,c,\*</sup>, C. Sanchez<sup>a</sup>, A.C. Bay-Jensen<sup>d</sup>, A. Mobasheri<sup>e,f,g</sup>

<sup>a</sup> Bone and Cartilage Research Unit, Arthropôle Liège, University of Liège, CHU Sart-Tilman, 4000 Liège, Belgium

<sup>b</sup> Artialis SA, GIGA Tower, CHU Sart-Tilman, 4000 Liège, Belgium

<sup>c</sup> Physical Therapy and Rehabilitation Department, Princess Paola Hospital, Marche-en-Famenne, Belgium

<sup>d</sup> Rheumatology, Biomarkers and Research, Nordic Bioscience, Herlev, Denmark

<sup>e</sup> Faculty of Health and Medical Sciences, University of Surrey, Surrey, GU2 7XH United Kingdom

<sup>f</sup> Arthritis Research UK Centre for Sport, Exercise and Osteoarthritis, Arthritis Research UK Pain Centre, Medical Research Council and Arthritis Research UK Centre for Musculoskeletal Ageing Research, Queen's Medical Centre, Nottingham, NG7 2UH United Kingdom

<sup>g</sup> Center of Excellence in Genomic Medicine Research (CEGMR), King Fahd Medical Research Center (KFMRC), Faculty of Applied Medical Sciences, King Abdulaziz University, 21589 Jeddah, Saudi Arabia

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### ABSTRACT

Specific soluble biomarkers can be powerful tools for the diagnosis, prognosis and personalized management of osteoarthritis (OA). Biomarkers are potential indicators of the effect of a drug on cartilage metabolism and provide crucial information about the mechanisms of drug action. In this review, we address key questions concerning the use of biomarkers in OA management: Why do we need soluble biomarkers? What are the most widely investigated biomarkers derived from cartilage extracellular matrix? What are the most common pitfalls in interpreting soluble biomarker measurements? What are the perspectives and future research directions in this field? We review current evidence to propose that cartilage-derived soluble biomarkers are complementary “drug development tools” that can be applied during drug development from preclinical research to clinical evaluation. In the future, such biomarkers could be surrogate markers of clinical and/or imaging outcomes. Successful standardization and implementation of automated biomarker assays will facilitate their use in companion diagnostics in the context of personalized medicine for enhanced management of OA.

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### 1. Introduction

Osteoarthritis (OA) is one of the major causes of pain and disability in the adult population. OA is now considered a severe joint disease affecting all articular tissues (i.e., cartilage, synovial membrane, meniscus and ligaments) and also periarticular tissues including tendons, adipose tissue and muscles. These joint tissues undergo metabolic, structural and functional alterations that contribute to the initiation and increased chronicity of pain and synovitis, activating pro-inflammatory pathways of innate immunity, facilitating disease progression and leading to patient disability. OA is a risk factor for some other age-related co-morbidities such as

diabetes or cardiovascular diseases [1,2]. Therefore, OA must be better managed to prevent these co-morbidities.

Pain is a key determinant of kinesiophobia in OA patients; it is responsible for physical deconditioning and a sedentary lifestyle, which is probably a decisive factor in the association of OA and metabolic syndrome, obesity and cardiovascular disorders [3]. Low-grade chronic systemic inflammation is the link between articular and periarticular tissues via pro-inflammatory mediators. This low-grade systemic inflammation may result from physiological aging (inflammaging) or metabolic disorders (meta-inflammation) [4,5] (Fig. 1).

The challenge for the next decade will be to find better remedies and management strategies for OA and to identify tools that can help in diagnosis and monitoring disease progression as well as assessing the efficacy of new therapeutic interventions. These tools need to be accurate for monitoring the structural progression of the disease and sensitive enough to identify early events at the molecular level and objectively assess the efficacy of novel or

\* Corresponding author at: Bone and cartilage Research Unit, Arthropôle Liège, Institute of Pathology, level +5, CHU Sart-Tilman, 4000 Liège, Belgium.  
Tel.: +32 4 242 77 27.

E-mail address: [yhenrotin@ulg.ac.be](mailto:yhenrotin@ulg.ac.be) (Y. Henrotin).

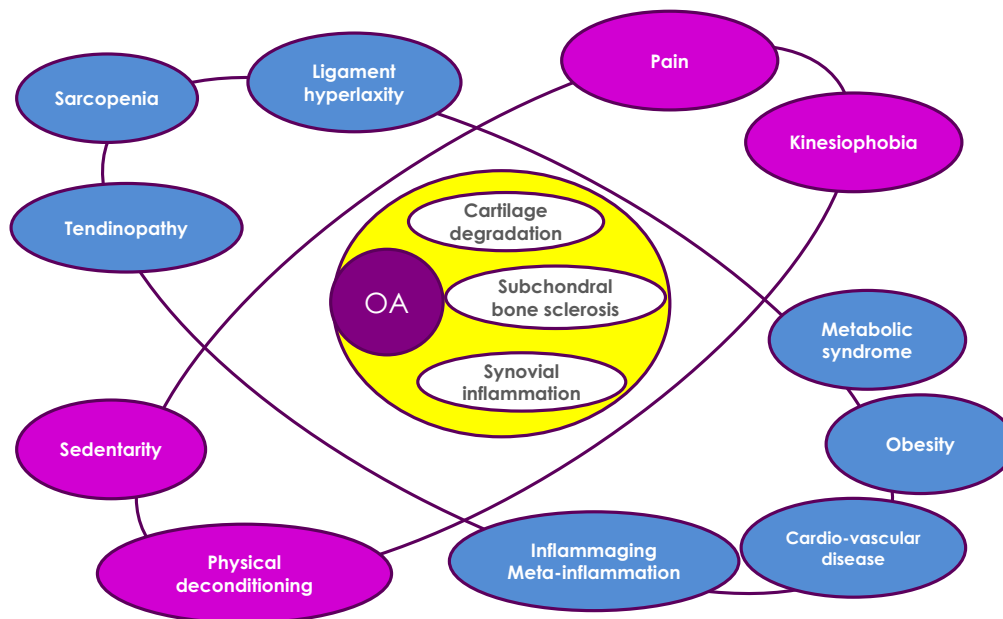


Fig. 1. Global representation of the systemic contributors and co-morbid situations associated with the pathogenesis of osteoarthritis (OA).

preexisting therapeutic modalities. Soluble biomarkers are among these tools. This review addresses the following key questions concerning the use of biomarkers in OA management.

## 2. How to define and classify OA biomarkers?

The National Institutes of Health (NIH) Biomarkers Definitions Working Group defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [6]. Existing biomarkers can be categorized by the OA process targeted, as markers of cartilage degradation/synthesis, bone remodeling, or synovitis. They can also be classified as “dry” or “wet” biomarkers. “Dry” biomarkers may include imaging features, visual analog scales or questionnaires and “wet” biomarkers may include proteins, protein fragments, metabolites or microRNAs. The BIPEDS system classifies the major types of biomarkers according to their clinical background into 6 categories corresponding to burden of disease, investigational, prognostic, efficacy of intervention, diagnostic and safety [6]. The adoption and use of this classification system has been encouraged to communicate advances within a common framework and so that OA biochemical marker research is more transparent and efficient, offering suggestions on optimal study design and the development of analytical methods for use in OA-focused investigations. In 2011, the Osteoarthritis Research Society International/Food and Drug Administration (OARSI/FDA) Biomarkers Working Group classified biomarkers into 4 categories (exploration, demonstration, characterization and surrogacy levels) by their level of qualification for drug development [7]. More recently, the OARSI RCT working group published guidelines for soluble biomarker assessment in OA clinical trials [8]. This document summarizes the use of biomarkers at 5 stages, including preclinical development and phase I to IV trials.

## 3. Why do we need soluble biomarkers in OA?

The management of OA often begins too late during the course of the disease. It is generally initiated after the patient complains about joint pain and loss of function, which is confirmed by the presence of radiographic changes [9]. Unfortunately, by the time the disease is diagnosed radiographically, joint tissue degeneration

is already well established and in most cases irreversible. Clinical OA is now considered to be preceded by a “silent” pre-radiographic phase during which extensive metabolic changes occur in joint tissues, without any pain. One challenge is the detection of these early metabolic changes that are early indicators of abnormal joint changes before the occurrence of structural changes.

OA is a heterogeneous syndrome with different clinical phenotypes defined by risk factors, progression profiles, co-morbidities, signs and symptoms. Although one goal is to have clearly defined and demarcated OA phenotypes, the classification and identification of phenotypes of OA is difficult in clinical studies or in clinical practice because of all these factors. Thus, we need a better subgrouping of OA, especially when several processes may overlap and various tissues are dominant during different phases of the disease. In real life, different OA phenotypes likely overlap significantly and thus are difficult to be separated into distinct clusters. However, establishing better-defined biological profiles specific to each phenotype may help in clustering the phenotypes.

Another key concern in OA management is the absence of effective treatment or cure. We lack standard treatments that allow for objective assessment of the sensitivity of a biomarker to a particular intervention and innovative treatments that efficiently address symptoms and disease progression. The reasons for the lack of effective treatments are the lengthy follow-ups and large sample sizes required for phases II and III clinical trials. We need sensitive and reproducible variables to accelerate drug development and reduce costs and attrition in the pharmaceutical pipelines. Soluble biomarkers could be considered “drug development tools” accompanying drugs from screening to post-marketing phases [9].

Many scientists and clinicians believe that soluble biomarkers could be helpful tools for addressing all these concerns. However, the development of a biomarker is a lengthy process requiring robust and reproducible assays that pass independent validation tests as well as the clinical characterization of the biomarker in large cohorts.

## 4. What are the most commonly investigated biomarkers derived from cartilage extracellular matrix (ECM)?

The cartilage ECM is a rich source of biomarkers in OA. Collagen type II is the most abundant protein in cartilage [10]. Consequently, the most popular biomarkers are epitopes located in the collagen II

molecule. Biomarkers indicative of collagen II degradation are CTX-II, Coll2-1, C2C and C2M and those representing collagen II synthesis are PIIANP, PIIBNP and CPII [11]. Other cartilage ECM-derived markers include cartilage oligomeric matrix protein (COMP) [12], Coll2-1NO2 [13], CS846 [14], aggrecan epitopes (ARGS, TEGE, FFGV) [15] and fibulin-3 epitopes (Fib3-1, Fib3-2, Fib3-3) [16]. COMP is a noncollagenous ECM protein formed of 5 identical glycoprotein subunits, each with epidermal growth factor-like and calcium-binding (thrombospondin-like) domains. COMP is a marker of cartilage ECM turnover [12]. Coll2-1NO2 is the nitrated form of Coll2-1 reflecting the oxidative-related cartilage ECM degradation [13]. CS846 is an epitope located in the chondroitin sulfate chain of aggrecan and an indicator of large or fetal-like aggrecan synthesis [14]. In contrast, TEGE/ARGS and FFGV are derived from degradation by aggrecanases and matrix metalloproteinases (MMPs). Originally discovered by proteomics to be increased in urine from OA patients [16], the fibulin-3 epitopes (Fib3-1, -2 and -3) contain a specific sequence of fibulin-3 (Fib3), an extracellular glycoprotein widely distributed in various connective tissues including blood vessels, bone, ligament and cartilage. Fib3 inhibits angiogenesis and chondrocyte differentiation [17]. More precisely, its overexpression suppresses chondrocyte differentiation by inhibiting cartilage node formation, proteoglycan production and aggrecan gene expression. Furthermore, its overexpression selectively maintains the expression of Sox-9 but suppresses that of Sox-5 and -6. Fib3 also interacts with tissue inhibitor of metalloproteinase 3, a matrix-bound inhibitor of MMPs [18]. Fib-3 is cleaved by MMP-1, -2, -3, -7, -9 and -12 [19] (Fig. 2).

**5. What are the common pitfalls in the interpretation of biomarker measurements?**

There are several limitations in interpreting the results of biomarker assays. A recent review published in the official journal of the OARSI summarizes the best practice for clinical trials using biomarkers [8]. Some limitations are related to the assay itself and type of assay, and others related to environmental conditions such as food intake, physical activity and circadian rhythms can be critical factors. All these conditions must be verified before the use of an assay in clinical study. The techniques should be reproducible and internationally validated by several independent laboratories like replication in genetics; the coefficient of variations of the assay should at best not exceed 10%; and analysis of the value should involve controlling for several confounding factors such as age, sex, body mass index and bone status for markers of bone turnover.

Another important point is the specificity of the biomarker. To monitor the progression of OA or predict its incidence or progression in the general population, we need biomarkers that are specific and not confounded by systemic inflammatory diseases. For example, a biomarker reflecting bone metabolism could be more predictive of osteoporosis rather than OA. Indeed, many biomarkers are shared by subchondral bone and articular cartilage and can be useful for osteoporosis and OA. As well, the conformation of the biomarker in the biological fluid must be determined. Most assays detect an epitope or a neo-epitope, and for assays, antibodies are produced by injecting synthetic peptides. The form of this type of epitope in blood, urine or synovial fluid is not known. The epitope may be part of a protein fragment or present within several fragments of differing molecular weights.

In addition, the presence of severe kidney or liver disease may induce a bias in interpreting a biomarker value. The diseases may strongly modulate the renal clearance of biomarkers independent of the joint disease activity. This point has never been adequately addressed and must be considered in the design of clinical trials.

Another important point to consider in the prognostic value of a biomarker is the dynamic variations in serum levels over time. A study by Sharif et al. [20] showed that serum level of COMP is dynamic and more reflective of upcoming variations in joint space narrowing than the long-term evolution of disease over a period of years. However, the problem might be complicated by the nonlinear relationship between biomarkers and some structural variables. A biomarker may reflect a metabolic change that is not necessarily related to structural or clinical changes. Hence, one may question the relevance of qualifying a biomarker by its relationship with clinical and radiological criteria. A soluble biomarker is primarily used to investigate changes in tissue or organ metabolism instead of a structural change that may result from a one-time traumatic event.

The final issue that needs to be considered is joint specificity. Some biomarkers have a diagnostic or a prognostic value for knee or hip OA compared to healthy subjects, despite the presence of overlap between groups, so defining a cutoff value is difficult. Biomarkers are often qualified by using cohorts with one particular OA phenotype. Few biomarkers have been validated in the general population that is representative of the most common phenotypes and rarely (if ever) investigated in a well-stratified cohort. For example, a biomarker could predict the progression of an aging phenotype but not an obese/overweight phenotype.

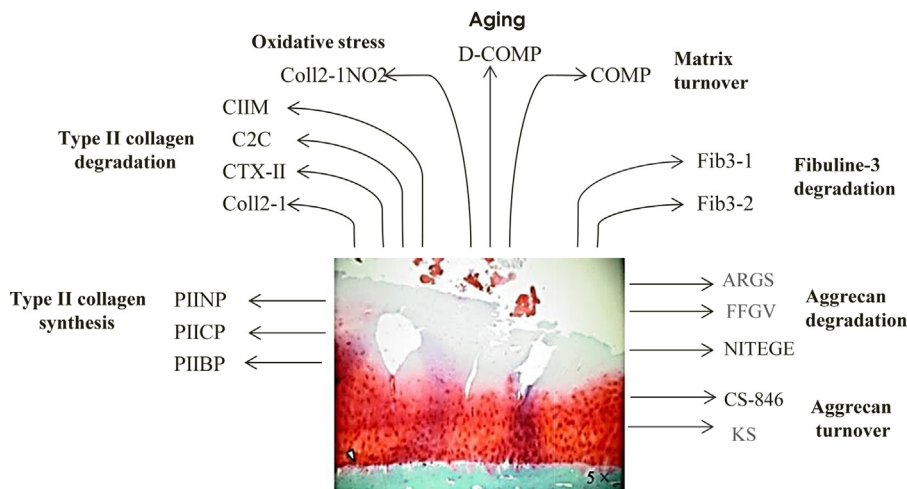


Fig. 2. An overview of cartilage-derived soluble biomarkers.

## 6. Perspectives and future directions

The “omics” approach is a general exploratory approach that be used to investigate alterations in a large number of genes, transcripts, proteins, lipids and metabolites in healthy versus diseased tissues. The challenge with such an approach is to differentiate candidates that are specifically involved in the disease process. The most commonly used omics approaches include genomics (beyond the scope of this review), proteomics, lipidomics, metabolomics and transcriptomics. Omics technologies applied to serum or urine have revealed numerous new biomarkers that are ubiquitous molecules in most cases [21]. A promising research pathway is the development and refinement of omics technologies for specific application to joint tissues (cartilage, bone, meniscus, synovial membrane) and the comparison of omics profiles of tissues at different stages of the evolution of the disease. This approach would provide a range of biomarkers reflecting the metabolic changes in different articular tissues at different disease stages. However, we need to remember that validation, qualification, industrialization and production of an assay are long, arduous and costly processes. Therefore, we must select the most promising of these omics candidates with predefined criteria such as tissue or pathological pathway specificity.

A most promising approach is the multiplexing of biomarkers investigating different tissue metabolisms or pathological pathways. A sensitive multiplex assay could combine several biomarkers, for example, for collagen II synthesis (i.e., PIIANP) and degradation (i.e., Coll2-1 or CTX-II), bone remodelling (i.e., PINP) and synovitis (i.e., HA). Data from multiplex platforms could be integrated in aggregate scores combining imaging, clinical and biological markers. This combination approach is an important step toward personalized medicine.

## 7. Conclusions

Soluble cartilage ECM-derived biomarkers can be used for clinical and imaging outcomes and as objective tools for evaluating responses to specific treatments in clinical trials of OA. Metabolic changes in joint tissues may occur early in OA development, long before the appearance of symptoms and structural changes, and should be considered a therapeutic variable. We have many soluble biomarkers derived from cartilage ECM that can be used as good “drug development tools.” A wide panel of biomarkers should be tested in the preclinical phase of development to better understand mechanisms of drug action and to identify companion marker(s) for subsequent clinical phases. This approach should reduce the length and cost of drug development. In the near future, some biomarkers might qualify as markers of efficacy of treatment and be used in routine follow-up of personalised therapeutic interventions. This situation will require a long-term qualification phase for a particular phenotype or a large panel of OA patients representative of the global population. The routine use of OA biomarkers also requires a successful commercialisation and production of assay techniques. Of note, this target is one of the most challenging for the upcoming years. It may affect the daily practice of primary care physicians and also the economic aspects of disease management by reducing the cost burden of the disease in targeting responders for particular treatments.

## Disclosure of interest

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