7. Biomarkers in osteoarthritis

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Abstract. The risk of osteoarthritis (OA), the disease stage, and its progression are generally assessed by basic pain measurements, clinical examination, and conventional X-rays. These techniques lack sensitivity in disease monitoring, especially in the early stages of disease, and need to be further developed using additional variables to optimize clinical studies and disease management. In recent years, biomarker evaluations of joint structural changes using imaging techniques and the measurement of joint tissue turnover in body fluids have greatly evolved. This chapter addresses the question of how the known biomarkers are helpful in the diagnostic assessment and prognosis of OA.

Introduction

In Western countries, osteoarthritis (OA) is the most prevalent of all musculoskeletal conditions, reducing quality of life and imposing a huge financial burden on health care systems. There is therefore an obvious need for optimal management of the disease to reduce symptoms, disability, and costs and, very importantly, for preventive means to retard its onset and progression. To meet these demands, researchers and clinicians depend on
reliable tools to assess risk factors for OA initiation and progression, stage of the disease, and response to treatment. There have been many attempts in the past decades to identify and validate such markers.

To date, the most common clinical markers, disease symptoms and signs, are widely accepted by both clinicians and governmental authorities to reliably allow for the diagnosis of OA. They are included in the diagnostic criteria of the American College of Rheumatology (ACR) [1-3] and are also proposed as outcome measures in clinical trials by the Osteoarthritis Research Society International (OARSI) and the Outcome Measures in Rheumatology initiative (OMERACT) [4]. Functional capacities are also considered to be an important marker and are mainly assessed by questionnaires including the Lequesne Algofunctional Index and the Western Ontario and McMasters Universities Osteoarthritis Index (WOMAC) [5, 6].

With regard to imaging, X-ray is accepted for diagnosis and disease grading, using scales such as the one proposed by Kellgren and Lawrence (KL) [7]. However, all of the aforementioned variables have substantial limitations. For example, questionnaires are prone to subjectivity and X-ray only provides an indirect assessment of the joint structural damage. Importantly, they lack sensitivity not only for evaluating the early stages of the disease, but also for disease monitoring and assessment of structural changes. Therefore, the OA research community has focused on modern imaging techniques and measurement of biochemical markers of joint tissue turnover in body fluids, which are easily accessible and add an important dimension to the known conventional features of OA.

Biomarker definition

In 2001 an expert consortium of the National Institutes of Health (NIH) published an official definition [8] of “biomarker” as a characteristic that is objectively measured and evaluated as an indicator of normal or pathogenic biological processes, and/or pharmacologic responses to a therapeutic intervention. Hence, every measurement, regardless of the technique, is considered a biomarker if it can be objectified. In the field of OA, there are two main groups having such characteristics: the radiological (imaging) means and the biochemical markers. Because questionnaires are prone to subjectivity they are excluded as biomarkers.

Biomarker classification

In 2006, Bauer et al. [9] proposed a classification system, named BIPED, for biochemical markers according to the role they play in OA. BIPED stands
for Burden of disease, Investigative, Prognostic, Efficacy of intervention, and Diagnostic. Table 1 gives an overview of the BIPED classification. Markers, excluding Investigative markers, are considered to meet a criterion if they have shown in a study with the appropriate design significant differences between pathological and control individuals. Putative biomarkers that do not meet the criteria for B, P, E or D fall into the Investigative category.

A sixth category, S for Safety, has been suggested for the more invasive assessments of biomarkers such as exposure to radiation, invasive techniques (arthrography), or administration of contrast agents used in the imaging field [10].

In addition to the above generalized approach, biochemical markers are also categorized according to their physiological nature. Osteoarthritis is nowadays widely accepted as a disease of the whole joint affecting not only the cartilage, which was for many years almost exclusively the focus of clinical trials, but also the subchondral bone, the synovial membrane, the menisci, and the ligaments. Changes in these structures were found to be closely related during the course of the disease and an interesting target in OA research. The most extensively studied types of biochemical markers are those related to tissue metabolism. There are numerous reports in the literature on the enzymatically cleaved molecules as markers of degradation whereas there are much fewer on the markers of tissue formation. Enzymes, cytokines and chemokines have also been assessed as well as systemic markers of inflammation. However, these latter are considered nonspecific for OA.

Table 1. BIPED Criteria.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Significant association with</th>
<th>Study design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burden of disease (B)</td>
<td>Extent or severity of osteoarthritis</td>
<td>Cross-sectional or case control</td>
</tr>
<tr>
<td>Investigative (I)</td>
<td>(Do not yet meet criteria for another category of the BIPED classification)</td>
<td>(Do not yet meet criteria for another category of the BIPED classification)</td>
</tr>
<tr>
<td>Prognosis (P)</td>
<td>Onset or progression of osteoarthritis</td>
<td>Longitudinal</td>
</tr>
<tr>
<td>Efficacy of intervention (E)</td>
<td>Treatment effect</td>
<td>Controlled trial</td>
</tr>
<tr>
<td>Diagnosis (D)</td>
<td>Osteoarthritis diagnosis</td>
<td>Cross-sectional or case control</td>
</tr>
</tbody>
</table>

Modified from Bauer et al. [9]
Imaging

X-ray

As the first broadly available radiological technique, X-ray has played a major role in the diagnosis [1] and grading of OA and is still considered to be the “gold standard” in cross-sectional and longitudinal clinical studies. The grading scale proposed by Kellgren and Lawrence [7] more than five decades ago is still widely in use. The OA markers proposed include osteophytes, subchondral sclerosis, subchondral cysts, joint space narrowing (JSN), and altered bone contours (Figure 1). They allow for the diagnosis of radiographic OA and can also be used to assess disease progression over time. X-ray is an affordable technique which is readily available; however, it also has many limitations. Firstly, the X-ray scoring systems are not very sensitive to change and large cohorts in clinical studies are needed to overcome this issue. Secondly, X-ray can only reliably evaluate bone. The other tissues of the joint and synovial effusion are either not visible or the visibility is of poor quality. For example, the cartilage is only estimated by measuring the joint space width (JSW) and JSN over time. Furthermore, in the knee, the JSW is dependent on the shape and position of the menisci. Therefore, meniscal extrusion, an often observed feature of knee OA, greatly influences the JSW assessment and confounds the results. Thirdly, positioning and repositioning the knee in longitudinal studies also poses a major problem. Indeed, in non-weight bearing knee X-rays, the JSW is generally overestimated. In contrast,

Figure 1. X-ray comparison of (a) a normal right knee with (b) end-stage osteoarthritis presenting osteophytes (arrows), subchondral sclerosis (arrowheads), and complete obliteration of the joint space.
Biomarkers in osteoarthritis

in the finger joints, inexact repositioning leads to a JSW underestimation. Acquisition protocols and computer aided methods have, however, further improved the applicability of JSW measurement in clinical studies by reducing the reader dependent variability and improving the reproducibility of the method, but there remains a high measurement error with regards to the very slight annual changes in JSW [11-15]. Moreover, X-rays reduce the 3D bony structure of the joint to the 2D plane of the film. Therefore, in the knee, the JSW only reflects the cartilage thickness of the central weight bearing area of the tibia and the femur. The thickness cannot be estimated for the superimposed peripheral regions of the tibial plateau or for the anterior and posterior regions of the femoral condyle. Finally, this technique is neither sensitive nor specific in early OA [16, 17].

In summary, although structural changes observed in X-ray images serve well as biomarkers for diagnosis in moderate to severe OA, they are not suitable for the diagnosis of early OA and are not optimal for follow-up in clinical studies.

**Computed tomography (Figure 2)**

The advantages of computed tomography (CT) are short acquisition time and excellent depiction of bone and calcified periarticular structures. Modern helical multi-detector systems allow high resolution and 3D reconstruction of bone in any plane. This technique could be used in OA for special indications

![Computed tomography (CT) of the lumbar (L) spine. (a) Axial view and (b) sagittal view presenting severe spinal osteoarthritis on the L4/L5 level including spondylophytes (s), osteophytes (*), joint space narrowing (black arrows), vacuum phenomenon (arrowheads) and subchondral sclerosis (grey arrow).](image-url)
such as the assessment of axial joints which are difficult to assess by conventional X-ray, detection of calcified intraarticular loose bodies [18], or CT guided joint infiltration. CT arthrography also allows assessment of the cartilage surface and meniscal or labral derangements [19]. CT is not suitable for detection of cartilage deterioration, synovial membrane, menisci, or ligaments, making it a modality of limited interest for use in OA. The major disadvantage of using CT lies in the exposure to radiation, especially in longitudinal studies requiring repetitive assessments. In conclusion, the use of CT to assess OA biomarkers remains reserved for special indications.

**Magnetic resonance imaging (Figure 3)**

Magnetic resonance imaging (MRI) is one of the most promising fields for the assessment of joint structure in OA. Consequently, a complete chapter of this book is devoted to this subject: Chapter 8 – *Quantitative magnetic resonance imaging in the evaluation of structural changes in knee osteoarthritis patients*. Briefly, in OA, MRI is nowadays used on a regular basis to assess joint structure changes and progression of these changes over time in longitudinal studies. Of special interest are the quantitative determination of cartilage (volume or thickness) and synovial fluid, semiquantitative assessment of subchondral bone marrow lesions (BML), and changes in the synovial membrane. Using this technique, BML were shown to be correlated with disease symptoms, cartilage loss, and risk for total joint replacement.

![Figure 3](image)

**Figure 3.** Magnetic resonance images of the knee. (a) Coronal proton density-weighted sequence showing focal cartilage defects on the lateral tibial plateau and femoral condyle (arrows) and subchondral bone sclerosis in the medial tibial plateau (arrowhead). (b) Sagittal T2 weighted fat saturated sequence showing a large hyperintense bone marrow lesion in the lateral tibial plateau.
replacement [20-23]. However, with regard to the correlation with disease symptoms, the literature remains somewhat conflicting [24, 25]. A possible reason for this is the fact that to date many joint structure changes have been evaluated semiquantitatively using scoring systems [26, 27] that may be insensitive to change as well as being difficult to correlate with continuous parameters such as pain, which is assessed by analogue scales.

**Ultrasound**

High resolution ultrasound (US) can demonstrate structural changes in cartilage, menisci, bone surface, synovial membrane, tendons, ligaments, joint capsule, and bursae in early to late stage OA (Table 2) [28-30]. The osteophytes and cartilage alterations characteristic of OA are considered to be diagnostic markers. Synovial membrane thickening and hyperaemia depicted in the Power Doppler technique as well as effusion reflect synovial inflammation [31, 32] (Figure 4). In combination, these markers allow for an assessment of the extent and severity of the disease, its progression over time, and response to systemic and local treatment [30]. The major advantages lie in the safety and non-invasiveness of the technique, its increasing availability in rheumatology clinics, and the possibility of assessing multiple joints in the same session. An important disadvantage imposed by physics in visualizing joint structures is the limited number and width of acoustic windows. The sound waves used for soft tissue assessment cannot penetrate bone which, in

<table>
<thead>
<tr>
<th>Joint structure</th>
<th>Typical changes in osteoarthritis</th>
<th>BIPED</th>
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</thead>
<tbody>
<tr>
<td>Bone</td>
<td>Osteophytes</td>
<td>BD</td>
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<td></td>
<td>Central erosions in hand osteoarthritis</td>
<td></td>
</tr>
<tr>
<td>Cartilage</td>
<td>Loss of sharpness</td>
<td>BD</td>
</tr>
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<td></td>
<td>Loss of homogeneity</td>
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<tr>
<td></td>
<td>Loss of anechogenicity</td>
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<tr>
<td></td>
<td>Irregularities of the anterior and posterior margins</td>
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<tr>
<td></td>
<td>Focal or diffuse thinning of the cartilage</td>
<td></td>
</tr>
<tr>
<td>Labrum</td>
<td>Labral tears</td>
<td>BP</td>
</tr>
<tr>
<td>Menisci</td>
<td>Meniscal tears, meniscal extrusion</td>
<td>BP</td>
</tr>
<tr>
<td>Synovial tissue</td>
<td>Synovial membrane thickening/hypertrophy</td>
<td>BEP</td>
</tr>
<tr>
<td></td>
<td>Bursitis</td>
<td></td>
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<tr>
<td></td>
<td>Synovial effusion</td>
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</tbody>
</table>
Figure 4. (a) Ultrasound of a knee; medial longitudinal view presenting osteophytes (*) on the femoral condyle (F) and the tibia (T) as well as meniscal extrusion (M). (b) Ultrasound of a knee; axial view of the femoral trochlear cartilage presenting medial thinning (arrows) and loss of anechogenicity (white arrowhead). (c) Ultrasound of a distal interphalangeal finger joint; longitudinal view presenting osteophytes (*) as well as synovial membrane thickening and effusion (black arrowhead).

consequence, obstructs the view of many internal joint structures partially or completely. In addition, intrinsic bone alterations seen in MRI are not accessible with US. Moreover, US is considered to be an operator-dependent imaging technique [33]. This problem has been partially solved by using high-quality machines. Future standardization of the image acquisitions may further improve the validity of the technique. Some progress has already been made with the demonstration of good reproducibility of US measurements of articular cartilage thickness in cadaveric knees [34], which needs to be confirmed in clinical trials. The sensitivity of detection of structural changes in early OA is not yet completely satisfactory but likely to improve with the help of enhanced techniques such as 3D US [35]. Regardless of these limitations, US has proven to be an interesting means to guide therapeutic interventions [36].
**Nuclear medicine**

**Scintigraphy**

Scintigraphy allows visualization of bone metabolism with the help of radioactive agents and has been shown to depict structural changes in subchondral bone in OA. Moreover, a good agreement was found between subchondral BML detected in MRI and bone scintigraphy [37]. However, baseline marker uptake was only weakly correlated with radiographic progression [38]. This correlation was not superior to the correlation between baseline X-ray observation and radiographic progression [39]. In turn, absence of marker uptake at baseline was considered to be a good prognostic factor for non-progression of OA [40]. As with CT, the major disadvantage of this technique lies in the exposure to radiation. Moreover, there is a lack of specificity as any type of bone remodelling is prone to a high marker uptake such as seen in primary bone tumours, bone metastases, and fractures.

**Positron emission tomography (Figure 5)**

Positron emission tomography (PET) uses radioactively labeled glucose to visualize elevated metabolism in any type of tissue within the body. Hence, an

![Figure 5](image)

*Figure 5.* (a, b) Fluorodeoxyglucose (FDG) positron emission tomography (PET) showing tracer accumulation in the cervical spine and the right acromioclavicular joint (arrows). (c) Axial computed tomography (CT) image showing osteoarthritis of the right acromioclavicular joint. (d) Fusion of FDG PET image with CT image.
accumulation in zones of active metabolism in the articular bone and synovial tissue make it a possible biomarker for OA. A study by Nakamura et al. [41] showed a good correlation with BML by fusing images obtained by this technique with those from MRI. In addition, periosteophytic accumulation was observed in half of the cases where definite osteophytes were seen. Although PET alone is highly nonspecific, its combination with CT is also available and combines the advantages of both techniques, i.e. demonstration of elevated tissue metabolism and high image resolution of calcified tissue [42]. In brief, the high costs and low availability of this technique, which is limited to a few specialized centres, as well as its low specificity, make it not truly suitable to assess biomarkers in daily practice.

Biochemical markers

Assessment of biochemical markers

Measurement techniques

Most commonly, enzyme linked immunosorbent assays (ELISAs) are used to detect biochemical markers in blood, urine and synovial fluid. The commercially available assays are competitive inhibition and sandwich ELISAs. Other less commonly used techniques are radioimmunoassays (RIA), enzyme immunoassays (EIA), and liquid chromatography-mass spectrometry assays (LC-MS).

Sensitivity and specificity

Normal tissue turnover in all joints of the body is reflected by systemic levels of markers causing a background noise in which a higher turnover in a single small joint may be missed due to insufficient sensitivity. Many of the tests may therefore not be sensitive enough in OA, a typical disease of insidious onset and slow progression, to differentiate between normal metabolism and pathological processes. However, Hayami et al. [43] showed, in an anterior cruciate ligament transection rat model, that OA in a single joint can increase systemic levels to amounts that allow for detection and discrimination from controls. In humans, it has also been shown that biomarker evaluation in serum of patients with OA in multiple body sites could correlate well with a total score reflecting the severity of systemic OA
On the other hand, sensitivity that is too high leads to false positive results in healthy individuals.

As for the specificity, it should be kept in mind that many of the molecules are not only present in articular tissues but also in other sites of the body. Elevated marker levels could therefore reflect mechanisms other than that which is targeted. For example, pathological processes in bone such as osteoporosis and malignant bone disease may mimic elevated subchondral bone turnover in the OA process. In addition, total joint replacement has been shown to have a possible effect on biomarker levels for a significant period of time after surgery, confounding the levels contributed by other OA joints [45].

**Bioavailability**

An elevated level of a given biomarker reflects not only its increased synthesis, but its breakdown or clearance. The data levels are complexified by the molecule distribution, diurnal rhythms, dependence on physical activity, etc [46-50]. Furthermore, the clearance processes can be either linear or nonlinear, and confounded by concomitant treatment with medications that compete for the metabolic and excretion processes, especially in elderly patients [51]. Finally, there is measurement variability leading to inconclusive data. In summary, a great number of factors outside of the disease studied can influence laboratory findings. Unfortunately, only very sparse information on these factors is available in the literature. In addition to this obvious lack of data, probably not all relevant studies are published, further weakening the power of these tools. Nevertheless, biochemical markers can aid clinical assessment and further definition of study endpoints, as long as these important limitations are considered and discussed.

**Biochemical markers of joint tissue turnover**

The following classification, although reflecting the current findings of the literature (Table 3) [52-56], is subject to ongoing change as new markers are developed on a continuous basis and knowledge of the known markers increases constantly. Of note, the tissue of origin of the measured markers is assumed but not proven in every case; they are most likely derived from many tissues simultaneously and the contribution of each of these to the final levels is, in general, not known.
Table 3. Current biochemical markers of osteoarthritis.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Biochemical marker</th>
<th>Body fluid</th>
<th>Putative process</th>
<th>BIPED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>NTX-I</td>
<td>Serum and urine</td>
<td>Type I collagen degradation</td>
<td>Knee: PE</td>
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<td>Hip: P</td>
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<tr>
<td></td>
<td>CTX-I</td>
<td>Serum and urine</td>
<td>Type I collagen degradation</td>
<td>Knee: BDP</td>
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<td>Hip: -</td>
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<tr>
<td>Osteocalcin</td>
<td>Serum</td>
<td></td>
<td>Anabolic bone turnover</td>
<td>Knee: BPED</td>
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<td>Hip: -</td>
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<tr>
<td>Cartilage</td>
<td>C2C</td>
<td>Serum and urine</td>
<td>Type II collagen degradation</td>
<td>Knee: ED</td>
</tr>
<tr>
<td></td>
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<td>Hip: B</td>
</tr>
<tr>
<td>CTX-II</td>
<td>Urine</td>
<td></td>
<td>Type II collagen degradation</td>
<td>Knee: BPED</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td>Hip: BPD</td>
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<tr>
<td>Coll 2-I and</td>
<td>Serum and urine</td>
<td></td>
<td>Type II collagen degradation</td>
<td>Knee: DBP</td>
</tr>
<tr>
<td>Coll 2-I NO₂</td>
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<td>Hip: D</td>
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<tr>
<td>CS846</td>
<td>Serum</td>
<td>Cartilage aggrecan</td>
<td>Cartilage aggrecan synthesis/turnover</td>
<td>Knee: P</td>
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<tr>
<td></td>
<td></td>
<td>synthesis/turnover</td>
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<td>Hip: -</td>
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<tr>
<td>Keratan sulfate</td>
<td>Serum</td>
<td></td>
<td>Aggrecan degradation</td>
<td>Knee: BPED</td>
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<tr>
<td>PIIANP</td>
<td>Serum</td>
<td>Type II collagen synthesis</td>
<td>Knee: BPD</td>
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<tr>
<td>PIIICP</td>
<td>Serum</td>
<td>Type II collagen synthesis</td>
<td>Knee: D</td>
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<td>Hip: B</td>
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<tr>
<td>TIINE</td>
<td>Urine</td>
<td>Type II collagen neoepitope</td>
<td>Knee: BP</td>
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<td></td>
<td>Hip: none</td>
</tr>
<tr>
<td>Multiple</td>
<td>C1,2C</td>
<td>Serum and urine</td>
<td>Types I and II collagen degradation</td>
<td>Knee: D</td>
</tr>
<tr>
<td>tissues</td>
<td></td>
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<td>Hip: -</td>
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<tr>
<td>COMP</td>
<td>Serum</td>
<td></td>
<td>Cartilage degeneration</td>
<td>Knee: BPED</td>
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<td></td>
<td>Hip: BPD</td>
</tr>
<tr>
<td>HA</td>
<td>Serum</td>
<td></td>
<td>Increased HA turnover</td>
<td>Knee: BPED</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Hip: P</td>
</tr>
<tr>
<td>YKL-40</td>
<td>Serum</td>
<td>Unknown</td>
<td></td>
<td>Knee: BE</td>
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<td></td>
<td></td>
<td></td>
<td>Hip: D</td>
</tr>
<tr>
<td>Proteinases</td>
<td>MMP-1,3,13</td>
<td>Serum</td>
<td>Joint tissue degradation</td>
<td>Knee: E</td>
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<tr>
<td></td>
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<td>Hip: -</td>
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<tr>
<td>Synovium</td>
<td>G1e-gal-PYR</td>
<td>Urine</td>
<td>Collagen fibril degradation in synovium</td>
<td>Knee: BD</td>
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Source [52-56]

Cartilage markers

Articular cartilage consists of an avascular extracellular matrix (ECM) in which only one type of cell, the chondrocyte, is embedded. This tissue’s matrix consists of a network of structural proteins with highly negatively charged molecules that provide water retention. The main matrix macromolecules are aggrecan and type II collagen. In the context of biochemical markers, there is also the cartilage oligomeric matrix protein (COMP).
**Aggrecan**

Aggrecan is a proteoglycan which consists of glycosaminoglycan chains linked to a protein core carrying globular domains. Numerous aggrecan molecules bind to a single hyaluronan chain forming a large negatively charged aggregate. Proteolytic cleavage of aggrecan by members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) enzyme family and matrix metalloproteinases (MMPs), which are produced by articular cells, leads to aggrecan neoepitopes that can diffuse into and be measured in the synovial fluid as well as in serum. Some epitopes are considered to be mainly present during ECM synthesis, such as the epitope 846 [57], which has been shown to aid in the evaluation of treatment effects [58]. Of note, baseline levels of this epitope, however, were not predictive of cartilage loss [59]. A second aggrecan related marker considered to be representative of cartilage degradation is keratan sulfate (KS), the levels of which have been correlated with OA diagnosis [60] and extent of the disease [61].

**Type II collagen**

The hyaline cartilage fibrillar network is primarily composed of type II collagen. This macromolecule is produced as a procollagen triple helix requiring the cleavage of the propeptides at both the C- and N-terminal. The levels of these C- and N-propeptides (PIICP and PIINP/PIIANP) are considered to be markers of cartilage synthesis and have been the subject of many studies in OA patients. While this marker’s synovial fluid levels showed promising results for diagnostic purposes, the serum levels were found to be inconsistent in the same patients [62]. Moreover, in a longitudinal clinical trial, baseline synovial fluid levels were shown to be predictive of OA progression [63].

More extensive research, however, has focused on the type II collagen cleavage products. C-terminal cross-linked telopeptides of type II collagen (CTX-II) measured in urine and serum are the major representatives of this family. Urinary CTX-II (uCTX-II) levels have been extensively evaluated in correlation with the BIPED criteria. In addition to uCTX-II levels being significantly higher in OA than in healthy subjects [64], this marker was also found to be predictive of OA progression, as recently demonstrated by Reijman et al. [65] in a population-based cohort in which OA patients with uCTX-II levels in the highest quartile were more likely to progress than those with levels in the lowest quartile. In another study, Bruyere et al. [66] showed that changes in the uCTX-II level over three months could identify OA progressors at one year. Moreover, uCTX-II levels were also found to
discriminate a placebo group from a treatment group [67]. On the other hand, with regard to the burden of disease, the results are conflicting [64, 68].

Besides CTX-II, there are other post-cleavage neopeptides of type II collagen detectable in serum and urine. There are assays capable of detecting neopeptides of the ¾ length fragment of type II collagen resulting from collagen cleavage by the collagenases. Among them are the C2C assay, which detects the carboxy-terminal, and the C1,2C assay which recognizes, in addition to the neopeptido of type II collagen, the corresponding epitope of the type I collagen fragment. C2C fragments were evaluated in two clinical trials. In the first [69], no significant difference was found between the placebo and treatment groups. However, the second [67] demonstrated a significant decrease in the treatment group compared to placebo. Correlations were found with OA progression and the serum ratio between degradation and synthesis markers of type II collagen, C2C/PIIICP [70], supporting the importance of combinations of biochemical markers. The detection of a specific sequence in the triple helical region of type II collagen (Coll 2-1) and its nitrated form (Coll 2-1 NO₂) were also reported to be predictive of OA progression [71].

Finally, the Helix-II assay [72] recognizes a neopeptido situated on the α1 helix of type II collagen generated by cartilage degrading processes. Interestingly, there seems to be no cross-reactivity with intact type II collagen or with types I or III collagen. Urinary levels of this neopeptido were shown to be significantly higher in OA patients than in healthy controls [72]. An association with disease progression was seen in the upper tertile of baseline levels in rheumatoid arthritis patients while there was no association with OA subjects. Yet, in a retrospective study, the same group found higher levels of urinary Helix-II levels at the end of a follow-up period in OA patients showing rapid progression [73].

**Cartilage oligomeric matrix protein**

Cartilage oligomeric matrix protein (COMP) is a proteoglycan found primarily, but not exclusively, in articular cartilage [74]. This molecule, which is made up of five subunits that bind to five different molecules of types I or II collagen, acts as a catalyst during fibril formation and is abundantly present in cartilage. COMP is found in close proximity to chondrocytes in growing cartilage. In mature cartilage it is predominantly present in the inter-territorial regions of the cartilage matrix where it seems to stabilize the collagen network. In OA, COMP was shown to be released into synovial fluid and subsequently serum, and its levels were suggested to be a
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predictor for OA development, presence and severity, and progression [75, 76]. However, the large between-subject variation in COMP levels precludes the use of its individual values to predict OA progression [45].

Bone markers

There is increasing awareness of the important role of subchondral bone in OA, a subject that is discussed in depth in Chapter 5 – Subchondral bone involvement in the pathophysiology of osteoarthritis. In brief, the bone tissue is made up of a matrix containing mainly fibrillar type I collagen fibers, a ground substance of glycoproteins and proteoglycans, and hydroxyapatite crystals and osteocytes. In general, markers of bone turnover have received far less attention in the context of OA than markers of cartilage turnover.

Type I collagen

Type I collagen molecules, like type II collagen, are made of polypeptide chains that form triple helices which are subsequently assembled to collagen fibers. Crosslinking pyridinoline (PYR) groups stabilize the bonds between the molecules within and between the fibers. Upon degradation of bone tissue, these cross-links can be measured in urine and were shown to be significantly elevated in OA patients compared to healthy controls [77], ascribing this marker diagnostic properties. They were also shown to correlate with radiographic extent of disease [78].

Degradation of type I collagen during bone resorption is also reflected by elevated levels of N- and C-terminal cross-linked telopeptides (NTX-I and CTX-I). Although studies revealed no difference between healthy and non-progressive OA subjects [79, 80], a significant difference was found between non-progressive and progressive OA patients [79]. The latter suggests the detection of increased subchondral bone resorption in progressive OA.

Osteocalcin

Osteocalcin is a non-collagenous protein which is tightly bound to hydroxyapatite and is believed to play a major role in the formation of mineralized bone. It was found to be elevated in serum subsequent to an elevated bone turnover. However, in OA studies, determination of osteocalcin levels generally presented inconclusive data [77, 81, 82].
Synovial tissue markers

Type III collagen

The synovial membrane is composed of synovial cells and a loose supporting network of types I and III collagen fibers. Type III collagen is mainly synthesized during growth, healing processes, and inflammation as a procollagen molecule. After cleavage, the N- and C-terminal propeptides (PIIINP and PIIICP) diffuse into the synovial fluid and subsequently into serum. PIIINP and PIIICP are therefore considered to reflect the rate of type III collagen production. Disease processes associated with the proliferation of synovial membrane, such as OA, rheumatoid arthritis, and psoriatic arthritis have shown elevated levels of serum PIIINP compared to healthy controls [83]. However, a significant difference between the diseases was not observed.

Glycosylated pyridinolin crosslinks (Glc-gal-PYR)

The molecules of fibrillar type I, II, and III collagen consist of three α chains that form a triple helix. In the extracellular matrix of cartilage, synovium and bone, these molecules are crosslinked by pyridinoline (PYR) and deoxypyridinoline (D-PYR) to form the fibrils. In these tissues, the collagen degradation releases the PYR and D-PYR crosslinks which diffuse into the body fluids and are excreted in the urine. While type I collagen is mainly seen in bone, type II in cartilage, and type III in synovial tissue, the crosslinks are non-specific, although the glycosylated form of the PYR seems to be present in great amounts in synovial tissue only, absent in bone, and in very small amounts in cartilage [84]. The relevance of this marker has mainly been studied in rheumatoid arthritis trials. In OA, there have only been a few studies that showed an association between urinary levels and symptoms, knee swelling, JSN and osteophytes [85, 86]. However, the applicability in daily practice remains to be proven.

YKL-40

YKL-40 has been detected in many organs of the body including articular cartilage and synovial membrane. Its biological function is not yet completely known. Elevated levels have been found in synovial fluid of end-stage OA compared to controls [87]. Moreover, its serum levels seem to correlate with the serum level of C-reactive protein (CRP) in hip OA patients, suggesting that YKL-40 might be a marker of inflammation [88]. In view of
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its low specificity, its future role in OA as a biochemical marker remains to be determined.

**Hyaluronic acid**

Hyaluronic acid (HA), also called hyaluronan, is an extremely long glycosaminoglycan made up of several thousand repeat disaccharides of glucuronic acid and N-acetyl-galactosamine and serves as backbone for multiple aggrecan molecules. HA binds to the cell surface receptors CD-44 which are present on various cell types. It is predominantly produced in cartilage but also synthesized by cells of the synovial membrane such as synoviocytes, macrophages and fibroblasts. Elevated serum levels of HA have been associated with progression of inflammatory arthritic diseases as well as OA [89].

**Proteinases**

**Matrix metalloproteinases**

Matrix metalloproteinases (MMPs) are a family of endopeptidases known to degrade the components of extracellular matrix, both collagen and aggrecan. In the joints, they are produced by cartilage, synovial membrane, and bone. They are divided into four main groups, the stromelysins (MMP-3, -10, -11), collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9) and the membrane type MMPs. They are inhibited by the tissue inhibitors of MMPs (TIMPs). They have been extensively studied in clinical trials in rheumatic diseases but also in some OA trials, and in very few studies investigating a disease modifying drug (DMOAD). In one such DMOAD trial, it was recently demonstrated that MMP-1 and MMP-3 significantly discriminate two treatment groups, suggesting these MMPs to be helpful in further longitudinal clinical trials to assess treatment and change over time [90].

**C-reactive protein**

C-reactive protein (CRP) is a member of the acute phase reactant class of proteins, the levels of which rise in response to inflammation. It is synthesized in the liver and most strongly stimulated by interleukin-6 (IL-6). CRP is an opsonin that assists in enhancing phagocytosis by macrophages and activating the complement system. It is implicated in all forms of inflammation including infectious diseases, autoimmune inflammatory diseases, tissue necrosis, and cancer. In OA, CRP was shown to be mildly
elevated and not able to justify its place amongst useful OA biomarkers, most likely due to its non-specificity [91].

**Combination of biochemical markers**

To improve sensitivity and specificity of biochemical markers concerning the BIPED criteria, particularly to predict OA progression, focus has been drawn to the combination of the abovementioned markers. Garnero et al. [92] demonstrated in a radiographically and arthroscopically controlled trial associated with an index combining the cartilage synthesis marker PIIANP and the degradation marker, CTX-II, that increased cartilage loss over one year could be predicted [93]. A similar ratio of breakdown to synthesis was assessed with the markers C2C/PIICP and C1,2C/PIICP, in which the ratio at baseline was associated with radiographic progression at 18 months [70]. These examples encourage further trials using a combination based approach.

**Genetic markers**

With the help of twin studies, the importance of genetic determination of cartilage volume was demonstrated [94], as well as the heritability of OA progression [95, 96]. Linkage analysis, a method based on the tendency of several loci to be inherited together, revealed a large number of genomic regions on many different chromosomes including the X chromosome that might lead to OA susceptibility [97]. Moreover, genetic association studies exploring either the entire human genome or regions already suspected to be associated with OA, aim to identify OA-specific loci. Summarization of the most recent data revealed that the gene variants identified so far have only a minor effect size and are not suited to be clinically useful biomarkers [97]. However, the combination of several genes might be of help in future studies to identify individuals at high risk for progression and adjust the treatment strategies according to their genetic risk factors.

**Conclusion**

There is great interest in biochemical markers in the field of OA. However, as mentioned in the most recent reviews [52-56], the conclusion is that to date none of the proposed markers meets the criteria to be of use in daily practice, mostly because of lack of information about sensitivity, specificity, normal range, and clinically important differences. In well
controlled homogenous clinical trial populations, these markers may still be useful for understanding the pathophysiological processes of OA and the mechanisms of action of the study drugs. However, even in this ideal setting, the current markers cannot serve as surrogates replacing clinical or imaging endpoints. The increasing knowledge about their distribution and clearance mechanisms as well as the combination of biochemical markers may further improve the meaningfulness of these tools and lead to their acceptance by the regulatory agencies.

Finally, the take home messages could be that according to the NIH definition, biomarkers can be objectively measured; recognized biomarkers in addition to biochemical factors also include imaging markers of joint structural changes such as osteophytes, bone marrow lesions, cysts, JSW, JSN, cartilage thickness and volume, and synovial membrane thickness; numerous biochemical markers reflect the turnover of many articular tissues or pathological progresses; and none of the current biochemical markers is, at present, accepted by the regulatory agencies or sufficiently discriminating to help with the diagnosis, assessment of disease extent, or prognosis of OA. In the coming years, the research agenda for imaging should include better validation and standardization of US and MRI biomarkers in longitudinal studies and for biochemical markers, the determination of distribution and clearance processes, definition of the minimal clinically important differences, exploration of the combination of multiple biomarkers as a set, report of negative results from previous and future trials, comparison of different assays for the same epitope, and standardization of sample collection.

References

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