Understanding Osteoarthritis from Bench to Bedside

Editors

Johanne Martel-Pelletier

Professor of Medicine, University of Montreal, Department of Pharmacology Accredited; Head, Chair in Osteoarthritis, University of Montreal; Director, Osteoarthritis Research Unit, University of Montreal Hospital Research Centre (CRCHUM), Notre-Dame Hospital

Jean-Pierre Pelletier

Professor of Medicine, University of Montreal, Department of Pharmacology Accredited; Head, Chair in Osteoarthritis, University of Montreal; Head, Arthritis Centre, University of Montreal; Head, Arthritis Division, University of Montreal Hospital Centre (CHUM); Director, Osteoarthritis Research Unit, University of Montreal Hospital Research Centre (CRCHUM), Notre-Dame Hospital

Research Signpost, T.C. 37/661 (2), Fort P.O., Trivandrum-695 023
Kerala, India
Foreword

Arthritis and related conditions comprise a large group of disorders that affect the joints, ligaments, tendons, bones and other components of the musculoskeletal system. Arthritic diseases are debilitating illnesses that cause disabling pain and most of the rheumatic diseases involve stiffening and progressive destruction of the joint resulting in functional decline. The increasing burden of bone and joint disorders on patients and health care systems led to the establishment of the Bone and Joint Decade launched in the year 2000. Now, at the close of the decade, one of the conclusions reached from this program is that research remains the most efficient means of tackling these diseases and reducing their medical and financial impact. Osteoarthritis is a main concern for this program endorsing the need for this major musculoskeletal disease to be better recognized and understood, and the magnitude of its health consequences more fully documented.

Osteoarthritis is the most common musculoskeletal disease, affecting millions of individuals worldwide. It evolves slowly and progressively and can affect all the joints of the body although the knees, hips, fingers, and spine are the most commonly affected. Ageing, obesity, genetics, joint injury, and work-related activity are among the most well known risk factors. As the baby boomer population ages and rates of obesity rise, this disease will become more prevalent, increasing the significant socioeconomic burden caused by the decline in quality of life and individual productivity.

Research into the understanding and management of osteoarthritis is currently progressing at an impressive speed, producing numerous advances and innovations. In recent years, studies have shown that osteoarthritis is considerably more complex than previously thought, and although the disease can be described as joint failure and involves biomechanical events, it also implicates dynamic biochemical changes in joint tissue metabolism with increased alterations not only in the cartilage but in all the tissues of the joint. We can speculate that with further research, the future of pharmacological treatments for osteoarthritis will follow what we have observed in other arthritic diseases in that, whether aimed at symptoms or joint structure, the next generation of treatments will be based on specific targets derived from basic and translational research.

This book is dedicated to research scientists in academia and industry, clinicians, residents, graduate students, and postdoctoral fellows who have an interest in expanding their knowledge of this disease. The aim of Understanding Osteoarthritis from Bench to Bedside is to review the current state of the art research, epidemiology, and diagnostics of osteoarthritis.
To this end, we have selected leading researchers from the Osteoarthritis Research Unit of the University of Montreal Hospital Centre (CHUM) and Research Centre (CRCHUM) and their collaborators to write comprehensive overviews of the current perspective, knowledge, and innovations in their particular field of interest. The Osteoarthritis Research Unit specializes in the investigation and treatment of this disease. Our expertise ranges from molecule to man, each researcher focusing on a different but complementary scientific perspective of the disease aiming at a global view. Major progress has been achieved during the last decade, and our team has been at the forefront of the research in osteoarthritis that has contributed substantially to the development of new knowledge in the field. This book is a comprehensive review of the advances in osteoarthritis research from the molecular and cellular mechanisms to the development of therapeutic options and innovative technologies.

We would like to thank all the authors for generously contributing their time and expertise to this book. We greatly appreciate the honour of entrusting us with the responsibility of serving as the editors of this work. Moreover, we are also grateful to Virginia Wallis, Santa Fiori and Lise Giguère for their expertise in preparing each chapter. We further would like to express our gratitude to Roche Canada for their financial support via an unrestricted educational grant which has permitted us to bring this book to publication. We also acknowledge the efforts of the personnel of Research Signpost who produced this book in a timely fashion.

Johanne Martel-Pelletier, PhD
Jean-Pierre Pelletier, MD
Contents

Chapter 1
Epidemiology of osteoarthritis
Frédéric Massicotte

Chapter 2
Cyclooxygenase-2 and microsomal prostaglandin E synthase-1
in the pathophysiology of osteoarthritis
Fumiaki Kojima and Mohit Kapoor

Chapter 3
The complex role of peroxisome proliferator-activated receptor
gamma in osteoarthritis
Hassan Fahmi

Chapter 4
Proteinase-activated receptor-2: An attractive DMOAD target for the
treatment of osteoarthritis
Nathalie Amiable, Steeve Kwan Tat and Christelle Boileau

Chapter 5
Subchondral bone involvement in the pathophysiology of osteoarthritis
Daniel Lajeunesse

Chapter 6
New comprehensive methods for the biomechanical analysis
of knee osteoarthritis
Jacques de Guise, Neila Mezghani, Rachid Aissaoui
and Nicola Hagemeister

1
27
39
53
69
85
Chapter 7
Biomarkers in osteoarthritis
Lukas M. Wildi and Giorgio Tamborrini

Chapter 8
Quantitative magnetic resonance imaging in the evaluation of structural changes in knee osteoarthritis patients
Jean-Pierre Raynauld
Abstract. Osteoarthritis (OA) is a progressive multifactorial disease that not only leads to articular cartilage loss and joint space narrowing, but also to pain, loss of function and physical disability, thus greatly impairing quality of life. The concept of the pathology of OA has evolved from being viewed as cartilage-limited to a multifactorial disease affecting the whole joint. Indeed, all the joint tissues including capsule, ligament, tendons, menisci, subchondral bone, synovium, muscle, and neurological structure are intricately implicated in the disease pathology. This chapter will review, from an epidemiological point of view, the classification and radiological definition of osteoarthritis, its socioeconomic burden, the prevalence of radiographic and symptomatic hand, knee and hip OA, and finally the local and systemic risk factors.

Introduction: Osteoarthritis from past to present

Not many diseases can claim to have a history as rich and ancient as osteoarthritis (OA). From prehistoric times to the present day, OA has proven to be a most challenging disease. OA can be traced back in time from paleopathological findings in skeletal remains, historical depictions, and more
recent pathological concepts. In fact, OA is frequently referred to as the oldest known disease on the planet. Indeed, evidence of its presence can be found in dinosaur skeletons of up to 70 million years old [1]. Some more controversial reports even describe the first example of OA in the spine of a 200 million year old Dimetrodon [2]. Osteoarthritis stigmata can be found in nearly every period and civilization, such as in the Neanderthal [3] and Cro-Magnon skeletons [4], Egyptian mummies [5], and more recent skeletal remains from England [6]. The pathological changes in a 100 million year old bone and a contemporary bone are remarkably similar, suggesting that OA is impervious to evolution [7].

As eloquently reviewed by Buchanan et al. [8], despite the nearly ubiquitous presence of the disease throughout evolution, clinicians did not recognize OA until the late 18th century, possibly due to the disease showing few obvious clinical signs. The first report of this disease in the literature may be found in Heberden’s notice of “Digitorum Nodi” in 1782, which was not published until 1802. In the “Commentaries on the History and Cure of Diseases” in 1793 Heberden the Elder described what is now called Heberden’s node with a clear distinction from gout [9]. Further nomenclature confusion delayed the recognition of OA, and OA and rheumatoid arthritis were even considered the same entity, known as arthritis deformans. It was not until 1859, when Alfred Baring Garrod proposed the name rheumatoid arthritis, that a distinction was made between these two diseases [10].

From an epidemiological perspective, the historical reference to OA is controversial, due in large part to the terminology used and the confusion between primary and secondary OA, as well as mimickers such as gout and rheumatoid arthritis. Today, nomenclature debates persist between osteoarthrosis, degenerative bone disease, and osteoarthritis, reflecting the different ideologies on the importance of inflammation in the pathophysiology of OA. However, evidence of the critical role of inflammation in OA is overwhelming and is discussed in Chapter 4 - Proteinase-activated receptor-2: an attractive DMOAD target for the treatment of osteoarthritis.

**Classification of osteoarthritis**

Considering the high prevalence and socioeconomic impact of OA, well-designed fundamental and clinical studies are of the utmost importance. One of the first steps toward reproducible studies is universally accepted classification criteria. It may seem trivial, but these criteria are continually evolving, for example with the creation of different radiographic scoring systems, which make study comparison a real challenge. To date, the most widely accepted classification criteria are those of the American College of
Table 1. American College of Rheumatology criteria for classification of osteoarthritis.

<table>
<thead>
<tr>
<th>Idiopathic Localized</th>
<th>Secondary</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hands: e.g., Heberden’s and Bouchard’s nodes (nodal), erosive interphalangeal arthritis (non-nodal), scaphometacarpal, scaphotrapezial</td>
<td><strong>Congenital or developmental diseases</strong></td>
</tr>
<tr>
<td>2. Feet: e.g., hallux valgus, hallux rigidus, contracted toes (hammer/cockup toes), talonavicular</td>
<td>1. Localized</td>
</tr>
<tr>
<td>3. Knee</td>
<td>a. Hip diseases: e.g., Legg-Calve-Perthes, congenital hip dislocation, slipped capital femoral epiphysis, shallow acetabulum</td>
</tr>
<tr>
<td>a. Medial compartment</td>
<td>b. Mechanical and local factors: e.g., obesity (?), unequal lower extremity length, extreme valgus/varus deformity, hypermobility syndromes, scoliosis</td>
</tr>
<tr>
<td>b. Lateral compartment</td>
<td>2. Generalized</td>
</tr>
<tr>
<td>c. Patellofemoral compartment (e.g., chondromalacia)</td>
<td>a. Bone dysplasias: e.g., epiphyseal dysplasia, spondylo-apophyseal dysplasia</td>
</tr>
<tr>
<td>4. Hip</td>
<td>b. Metabolic diseases: e.g., hemochromatosis, ochronosis, Gaucher’s disease, hemoglobinopathy, Ehlers-Danlos disease</td>
</tr>
<tr>
<td>a. Eccentric (superior)</td>
<td>Calcium deposition disease</td>
</tr>
<tr>
<td>b. Concentric (axial, medial)</td>
<td>1. Calcium pyrophosphate deposition disease</td>
</tr>
<tr>
<td>c. Diffuse (coxae senilis)</td>
<td>2. Apatite arthropathy</td>
</tr>
<tr>
<td>5. Spine (particularly cervical and lumbar)</td>
<td>3. Destructive arthropathy (shoulder, knee)</td>
</tr>
<tr>
<td>a. Apophysal</td>
<td>Other bone and joint disorders:</td>
</tr>
<tr>
<td>b. Intervertebral (disc)</td>
<td>E.g., avascular necrosis, rheumatoid arthritis, gouty arthritis, septic arthritis, Paget’s disease, osteopetrosis, osteochondritis</td>
</tr>
<tr>
<td>c. Spondylosis (osteofytes)</td>
<td>Other diseases</td>
</tr>
<tr>
<td>d. Ligamentous (hyperostosis [Forestier’s disease, or DISH])</td>
<td>1. Endocrine diseases: e.g., diabetes mellitus, acromegaly, hypothyroidism, hyperparathyroidism</td>
</tr>
<tr>
<td>6. Other single sites: e.g., shoulder, temporomandibular, sacroiliac, ankle, wrist, acromioclavicular</td>
<td>2. Neuropathic arthropathy (Charcot joints)</td>
</tr>
<tr>
<td><strong>Generalized: includes 3 or more areas listed above (Kellgren-Moore)</strong></td>
<td>3. Miscellaneous: e.g., frostbite, Kashin-Beck disease, Caisson disease</td>
</tr>
<tr>
<td>1. Small (peripheral) and spine</td>
<td>DISH, diffuse idiopathic skeletal hyperostosis</td>
</tr>
<tr>
<td>2. Large (central) and spine</td>
<td>From Altman RD et al. [11]</td>
</tr>
<tr>
<td>3. Mixed (peripheral and central) and spine</td>
<td>Rheumatology (ACR). As shown in Table 1, the ACR has classified OA into two broad categories: idiopathic, which can be localized or generalized, and secondary [11]. Secondary OA may further be classified as post-traumatic, congenital, or due to calcium deposition disease or other bone/systemic diseases. As shown in Table 2, specific classification criteria for the hand, hip and knee were also proposed by the ACR, and these criteria are currently used for OA definition in the vast majority of clinical studies [11-13].</td>
</tr>
</tbody>
</table>
Table 2. American College of Rheumatology criteria for osteoarthritis of the hand, hip and knee.

<table>
<thead>
<tr>
<th>Hand</th>
<th>Clinical</th>
<th>Items required for OA presence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Hand pain, aching or stiffness for most</td>
<td>1, 2, 3, 4 or 1, 2, 3, 5</td>
</tr>
<tr>
<td></td>
<td>days of prior month</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. Hard tissue enlargement of ≥ 2 of 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>selected hand joints*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. MCP swelling ≤ 2 joints</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Hard tissue enlargement of ≥ 2 DIP joints</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Deformity of ≥ 1 of 10 selected hand</td>
<td></td>
</tr>
<tr>
<td></td>
<td>joints</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hand</th>
<th>Clinical and Radiographic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Hip pain for most days of the prior month</td>
<td>1, 2, 3 or 1, 2, 4 or 1, 3, 4</td>
</tr>
<tr>
<td></td>
<td>2. ESR ≤ 20mm/h</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Radiographic femoral and/or acetabular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>osteophytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Radiographic hip joint space narrowing</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hip</th>
<th>Clinical</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Knee pain for most days of prior month</td>
<td>1, 2, 3, 4 or 1, 2, 5, or 1, 4, 5</td>
</tr>
<tr>
<td></td>
<td>2. Crepitus on active joint motion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Morning stiffness ≤ 30 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Age ≥ 38 years old</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Bony enlargement of the knee on examination</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Knee</th>
<th>Clinical</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Knee pain for most days of prior month</td>
<td>1, 2 or 1, 3, 5, 6 or 1, 4, 5, 6</td>
</tr>
<tr>
<td></td>
<td>2. Osteophytes at joint margins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Synovial fluid typical of osteoarthritis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Age ≥ 40 years old</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Morning stiffness ≤ 30 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6. Crepitus on active joint motion</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Knee</th>
<th>Clinical and Radiographic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Knee pain for most days of prior month</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. Osteophytes at joint margins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Synovial fluid typical of osteoarthritis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Age ≥ 40 years old</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Morning stiffness ≤ 30 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6. Crepitus on active joint motion</td>
<td></td>
</tr>
</tbody>
</table>

* 10 selected hand joints include bilateral 2nd and 3rd proximal interphalangeal joints, 2nd and 3rd distal interphalangeal joints and 1st carpometacarpal joints.
DIP, distal interphalangeal; ESR, erythrocyte sedimentation rate; MCP metacarpophalangeal. Adapted from Altman RD et al. [11-13].
Table 3. Kellgren-Lawrence radiographic scoring system for osteoarthritis.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Classification</th>
<th>Feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
<td>No feature of OA</td>
</tr>
<tr>
<td>1</td>
<td>Doubtful</td>
<td>Minute osteophyte, doubtful significance</td>
</tr>
<tr>
<td>2</td>
<td>Minimal</td>
<td>Definite osteophyte, unimpaired joint space</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Moderate diminution of joint space</td>
</tr>
<tr>
<td>4</td>
<td>Severe</td>
<td>Joint space greatly impaired with sclerosis of subchondral bone</td>
</tr>
</tbody>
</table>

Adapted from Kellgren and Lawrence [14]

The sensitivity and specificity for the hip (91% and 89%), knee (91% and 86%) and hand (92% and 98%) of these criteria are high enough to differentiate OA from more inflammatory arthritis, but are somewhat limited with regard to the discernment of early OA.

The most commonly accepted grading system for radiographic OA was proposed by Kellgren and Lawrence (KL) in their atlas of standard radiographs more than 40 years ago [14]. As shown in Table 3, this scoring system is based primarily on osteophyte presence, with the more severe grades based on the progressive appearance of joint space narrowing and subchondral bone sclerosis. However, multiple studies have criticized this grading system. Indeed, poor correlation between pain and radiographic OA [15], and between high risk for disease progression and low KL grade [16] have been demonstrated. Magnetic resonance imaging (MRI), a newer imaging modality in quantifying articular structures which can overcome some of the pitfalls of X-rays, may become the modality of choice for future clinical trials. However, high price and limited access are still limiting its use.

Socioeconomic burden of osteoarthritis

Arthritis and other rheumatic conditions are becoming a major public health problem and the foremost cause of disability in the developed countries. For example, in the United States in 2002, nearly 43 million adults were affected [17]. Direct and indirect costs attributable to arthritis and other rheumatic conditions totalled an estimated $86 billion in 1997, accounting for nearly 1% of gross domestic product [18]. In the National Health Interview Study, OA and related disorders were the third leading chronic condition causing work limitation (1.6 million people or 8.3% of main conditions of impairment), just after heart disease (10.9%) and back disorder (21.1%) [19].
Osteoarthritis is the most common form of arthritis, affecting a conservatively estimated 27 million Americans in 2008 [20]. Although OA is one of the most prevalent diseases in our aging population, its socioeconomic impact may not have been accorded adequate attention. Despite being less disabling than rheumatoid arthritis, OA poses a greater socioeconomic impact due to its prevalence estimated to be at least 7 to 20 times greater than rheumatoid arthritis [21, 22]. The impact of arthritis on health related quality of life is major [23]. About 80% of patients with OA have movement limitation. According to the National Health and Nutrition Examination Survey III (NHANES III) data, 25% of patients cannot perform major activities of daily living and about 12% require help with personal care and routine needs. Moreover, the real burden of this disease on society is difficult to evaluate since OA patients often have multiple comorbidities and OA can also be confused with other inflammatory diseases [24]. Given the prediction that, by the year 2030, 25% of adults in the United States will have physician-diagnosed arthritis (predominantly OA) [25], it is imperative to quantify its direct (e.g. health care costs) and indirect (e.g. labour productivity) costs.

Reviews on the direct costs of OA vary greatly, with up to a 10-fold difference reported among studies [26]. Nonetheless, data from a multivariate analysis of the relationship between OA and health care expenditure showed that OA increased aggregate expenditure by $185.5 billion per year [27]. Women accounted for $118 billion and men $67.5 billion. Part of this amount comprises indirect costs due to absenteeism, for which OA expenditure is approximately $500 annually per capita, which is equivalent to approximately 3 lost workdays. Aggregate annual absenteeism costs are $10.3 billion (women $5.5 billion, men $4.8 billion) [28].

In 1997, OA was responsible for 55% of all arthritis-related hospitalizations in the United States, with over 400,000 hospitalizations [29]. Together, total knee and hip joint replacements accounted for 35% of the procedures during hospitalization for arthritis [30]. With increasing prevalence due to an older population, nearly twice as many total knee replacements were performed in 2000 compared to 1990 [31]. In 2004, OA led to 97% of the 455,000 knee replacements and 83% of the 233,000 hip replacements [32]. Furthermore, OA accounts for approximately 6% of all arthritis-related deaths, and 0.3 deaths per 100,000 population (or 500 deaths per year) [33]. Moreover, these numbers are likely highly underestimated since gastrointestinal bleeding due to treatment with non-steroidal antiinflammatory drugs (NSAIDs) was not included.
Prevalence of osteoarthritis

In adults, the leading cause of disability is arthritis [22]. The number of arthritic persons and the ensuing social impact are projected to increase by 40% in the next 25 years [25]. In 2008, the National Arthritis Data Workgroup addressed this issue and produced the best available report on the prevalence of OA in the United States. They estimated that 46 million American adults (21% of the population) had arthritis, of whom nearly 27 million had clinical OA (hand, hip, knee, overall), an increase of nearly 30% since 1995 [20]. In comparison, rheumatoid arthritis affected 1.3 million adults, a decrease of nearly 60% since a 1995 estimate.

As radiographic changes and clinical symptoms do not correlate well, these manifestations must be evaluated separately. In addition, as recently reviewed by Zhang et al. [34], the prevalence of OA is variable depending on the definition, the joint, and the population under study. Nonetheless, advanced age and female gender are major risk factors for OA (Table 4).

**Table 4.** Prevalence of radiographic and symptomatic osteoarthritic joint by age and sex from population-based study*.

<table>
<thead>
<tr>
<th>Anatomic site, age</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Radiographic†</td>
<td>Symptomatic</td>
<td>Radiographic†</td>
</tr>
<tr>
<td>Hands ≥26(^1)</td>
<td>25.9</td>
<td>3.8</td>
<td>28.8</td>
</tr>
<tr>
<td>Knees ≥26(^2)</td>
<td>14.1</td>
<td>4.6</td>
<td>13.7</td>
</tr>
<tr>
<td>Knees ≥45(^3)</td>
<td>18.6</td>
<td>5.9</td>
<td>19.3</td>
</tr>
<tr>
<td>Knees ≥45(^3)</td>
<td>24.3</td>
<td>13.5</td>
<td>30.1</td>
</tr>
<tr>
<td>Knees ≥60(^4)</td>
<td>31.2</td>
<td>10.0</td>
<td>42.1</td>
</tr>
<tr>
<td>Hips ≥45(^5)</td>
<td>25.7</td>
<td>8.7</td>
<td>26.9</td>
</tr>
</tbody>
</table>

Adapted from Lawrence RC et al. [20]

*Estimates represent prevalence per 100 persons age-standardized to the projected 2000 Census population except for National Health and Nutrition Examination Survey III (NHANES III) estimates, which were adjusted to the 1980 Census population.

†Radiographic knee OA based on anteroposterior projections

\(^1\) Framingham OA study [35]

\(^2\) Framingham OA study [36]

\(^3\) Johnston County OA Project [37]

\(^4\) National Health and Nutrition Examination Survey III (NHANES III) [38]

\(^5\) Johnston County OA project [39]

Prevalence of hand osteoarthritis

As with the other joints, the prevalence of radiographic hand OA varies depending on the definition, and in a population-based study, it was as high as 29%-76% [40]. In the Framingham study, the overall prevalence of
radiographic hand OA was 27.2% in subjects over 26 years of age [35]. As with the knee, slightly more women than men were affected. In the Rotterdam study, 67% of the women and 55% of the men over 55 years old were reported to have radiographic OA in at least one hand joint [40]. Distal interphalangeal (DIP), thumb base, and proximal interphalangeal (PIP) joints were affected in 47%, 36%, and 18%, respectively. Moreover, polyarticular hand OA (thumb base, DIP, PIP) was more common than single joint disease.

Interestingly, even though radiographic hand OA is extremely common in older subjects, the prevalence of symptomatic hand OA is much lower. In the Framingham study, 3.8% of men and 9.2% of women over 26 years of age had symptomatic OA [35]. DIP (2.9%) and thumb base (2.8%) were the two most symptomatic joints in men. In women, DIP (9.4%), PIP (7.3%) and thumb base (5.7%) were the most symptomatic. Osteoarthritis was more likely to cluster by row than by ray and occurred in a remarkably symmetrical pattern, especially in women [41]. In contrast to the Framingham study, the Rotterdam study demonstrated that the strongest association of right hand pain was with thumb base (odds ratio [OR] 1.7) followed by PIP (OR 1.4). Radiographic DIP OA was not associated with hand pain (OR 1.1) [40]. In the NHANES III study, the overall symptomatic hand OA prevalence was 8% (8.9% female; 6.7% male) in American adults 60 years of age and older and it significantly increased with age [42].

**Prevalence of knee osteoarthritis**

Depending on the study, overall prevalence of radiographic knee OA in American subjects 45 years of age and older varies between 19.2% and 27.8%, with women more predisposed than men. Similarly, in the largest European survey, the Zoetermeer Community Survey in the Netherlands, the prevalence of radiographic knee OA was higher in women than in men of 45 years and older with 22,800 compared to 14,100 out of 100,000 citizens [43]. A more global perspective in knee and hip OA prevalence has also been reported by the World Health Organization [44]. In NHANES III, the overall prevalence of knee OA worldwide increased to 37.4% in subjects 60 years of age and older. It is likely that the protocol employed for NHANES III knee examination biased the prevalence estimates to be lower than the true population values, since only a single anterior/posterior non-weight-bearing radiograph for each knee was examined [38]. As did the first national Health and Nutrition Examination Survey (HANES I) [45], NHANES III also reported that the prevalence of radiographic knee OA was significantly higher in non-Hispanic African Americans than in non-Hispanic Caucasians or Mexican Americans (52.4%, 36.2%, and 37.6%, respectively) [38]. More
recent studies have demonstrated statistically significant greater severity of knee OA in African Americans than in Caucasians (adjusted cumulative OR of 2.08; 95% confidence interval [CI]: 1.19–3.64, for men and 1.56; 95% CI: 1.06–2.29, for women) [46]. This study evaluated not only the tibiofemoral but also the patellofemoral compartment and demonstrated increased tri-compartmental disease in African Americans. In fact, care must be taken when evaluating radiographic prevalence of knee OA. Indeed, most studies evaluated the tibiofemoral compartment, thus underestimating the tibiopatellar compartment, which can be a major source of radiographic and clinical OA, particularly in a younger population [47].

Classically, knee pain and radiographic OA changes have been discordant [48]. Some studies attribute this discrepancy to the radiographic view chosen to define radiographic OA [49]. Other explanations may be the variation in populations analyzed, the definition of pain in the various studies, and/or that pain can be experienced without visible radiographic alterations. However, other reports estimate that 40% to 80% of subjects with radiographic knee OA are symptomatic [50] and a recent study on two cohorts reported a strong association between radiographic features of OA and knee pain [51]. In the Framingham study, the prevalence of clinical knee OA was 4.9% and 6.7% for subjects older than 26 and 45 years, respectively [36]. In the Johnston County OA project, the prevalence of clinical knee OA was much higher than in the Framingham study, with 16.7% for adults over 45 [37]. NHANES III estimated a 12.1% prevalence of symptomatic knee OA in subjects over 60, which was similar to the Rotterdam study [52]. The remarkably higher prevalence of symptomatic knee OA in the Johnston study [37] could be due to multiple factors. Johnston County, North Carolina, United States, has a high prevalence of sociodemographic subgroups at high risk for OA, with 20% African Americans. As recently demonstrated, both men and women of African American origin are more likely to have osteophytes [46]. Since the presence of osteophytes seems to be predictive of symptoms, at least in the knee, this could explain the higher prevalence of clinical OA in the Johnston study [53]. Recently, the Clearwater Osteoarthritis Study [54] reported that subjects with radiographic knee OA with an elevated body mass index (BMI) had a greater likelihood of knee pain than those with normal BMI, and this rose with each successive elevated BMI category [55]. This concurs with two prior studies [56, 57], but contrasts with a third [58]. A more precise and homogeneous definition of radiographic and symptomatic knee OA between studies, as well as a more sensitive method
for knee assessment, such as MRI, are needed to further clarify the prevalence of and risk factors for this disease. In this line of thought, a recent systematic review of the association between MRI findings (cartilage defects, bone marrow lesions (BML), osteophytes, meniscal lesions, effusion/synovitis, ligament abnormalities, subchondral cysts and bone attrition) and pain in patients with knee OA revealed that BML and effusion/synovitis may indicate the origin of pain in knee OA [59].

**Prevalence of hip osteoarthritis**

In the Johnston County OA project, the prevalence of radiographic hip OA based on KL grade ≥ 2 was 27% in adults 45 years of age and older [39]. As with the other joints, the prevalence increased with age (from 21% at 45-54 years to 43% over 75 years) and was slightly higher in women than men (30% vs 25%). Although some previous studies demonstrated low prevalence of hip OA in African populations [60], this does not seem to apply to Americans of African descent, at least not in the Johnston County OA project or the NHANES I cohort [61, 62]. In both of these studies, no differences were found between the prevalence of hip OA between African Americans and Caucasians. However, the hips of African American men and women had an increased frequency and severity of superior joint space narrowing compared with the hips of Caucasian men and women [32]. In the NHANES I study, the overall prevalence of hip OA in people 55-74 years old was only 3.1%, also based on KL grade ≥ 2. As in the Johnston County OA project, the prevalence increased with age, as indicated by the significantly higher OR in the older group (70-74 years: OR 2.38; 95% CI: 1.15-4.92) compared to the younger group (60-65 years: OR 1.30; 95% CI: 0.6-2.81). In another community-based study in the United States of nearly 5,000 women aged 65 and older, the prevalence was found to be 7.2% [63].

The two first population-based prevalence estimations of symptomatic hip OA were NHANES I (1971-1975) and NHANES III (1988-1994). In NHANES I, the overall prevalence of symptomatic hip OA in adults (55-74 years) was 0.7% and like the radiographic prevalence, the rates increased with age and were similar between Caucasians and African Americans and between sexes [61]. NHANES III reported an increase in overall hip pain prevalence since the previous study, with 14.3% of older adults (≥ 60 years) with significant hip pain [64]. As in the NHANES I study, women were more affected, but age did not influence hip pain, at least in males. Caucasian women had 16% prevalence compared with 14.8% in African
American women, and men had 12.4% prevalence with similar rates for both races. The higher prevalence of hip pain in NHANES III may be related to non-articular sources of pain in older adults or with increased incidence of hip OA [62]. Finally, in the Johnston County OA project, 9.2% of adults were symptomatic (9.3% female, 8.7% male) [39]. Like NHANES I, African Americans were not less affected by symptomatic hip OA than Caucasians.

**Established risk factors for osteoarthritis**

There are numerous studies on risk factors for OA. Classically, they can be divided into two broad categories: systemic factors, which are associated with the development of OA, and local factors, which affect biomechanical loading of the joint [65] (Figure 1). Since the pathophysiology of OA is multifactorial, both systemic and local factors may influence the outcome of OA by increasing the susceptibility of a joint to develop OA upon utilization.

**Systemic and local factors in the pathophysiology of osteoarthritis**

![Diagram showing systemic and local factors in OA pathophysiology](image)

**Figure 1.** Pathogenesis of OA with systemic and local risk factors. Adapted from Figure 1 of Dieppe P. The classification and diagnosis of osteoarthritis. In: Kuettnner K, Goldberg V, eds. Osteoarthritic Disorders. Rosemont, IL: American Academy of Orthopaedic Surgeons; 1995:7.
Systemic risk factors

Age

Even though aging is not sufficient for the development of OA, it is still the strongest risk factor for OA in all joints [66, 67]. However, the mechanism by which age increases the susceptibility to joint degeneration is still largely unknown. Age-related changes in articular cartilage likely play a role. They could be related, among other things, to an alteration within the collagen network crosslinking resulting from the advanced glycation end products [68], a decreased response of chondrocytes to repair stimuli [69] and/or altered levels of cartilage oligomeric protein and hyaluronic acid [70]. Yet, other less cartilage-oriented mechanisms could also be implicated, such as decreased proprioception and muscle strength or unstable articulation with aging [71, 72]. Nevertheless, it appears that the increased prevalence of OA with aging is multifactorial and includes the abovementioned mechanisms as well as, very importantly, increased susceptibility to other OA risk factors [34, 73].

Gender and estrogens

Women are more susceptible than men to OA as well as to higher disease severity [74]. The significant increase in OA prevalence in women around the time of menopause has led to multiple investigations of the hormonal implication in the pathophysiology of OA. In contrast to other tissues (such as endometrium, breast and brain), joint tissues were traditionally thought to be non-responsive to estrogen or to its deficit. However, although data are conflicting [75], there is now increasing evidence that estrogen influences the activity of joint tissues through complex molecular pathways that act at multiple levels [76]. The Framingham study concluded that estrogen intake in women had a modest but non-significant protective effect on both radiographic OA (OR 0.71; 95% CI: 0.42-1.20) and severe radiographic OA (OR 0.66; 95% CI: 0.33-1.32) [77]. On the other hand, it was also reported that women on estrogen replacement therapy were less likely to require total knee or hip replacement [78, 79]. The benefit seemed to increase with long term use. Indeed, those who had taken estrogen for 10 years or longer had a greater decrease in the risk for hip OA; however, there was no significant reduction in disease symptoms [79, 80]. Moreover, two reviews on the subject by the same group [81, 82] reported that data on endogenous hormones, age at menarche/menopause, and duration of the fertile period, etc. in hand, hip and knee OA, point to no relationship between OA and female
hormonal aspects, or remain conflicting. Nonetheless, there appears to be some evidence that unopposed estrogen use has a protective effect on hip OA. It thus seems that further research is needed to clarify the complex role of estrogen in the pathophysiology of OA.

Race

Numerous studies support the role of ethnicity in the development of OA based on variations among racial and ethnic groups [34]. For example, both hip [83] and hand OA [84] were found to be rare among Chinese in the Beijing Osteoarthritis Study compared to Caucasians in the NHANES I study (80%-90% less frequent hip OA) and Framingham study (about 35% less frequent hand OA) respectively. However, both radiographic and symptomatic knee OA were increased by about 45% in Chinese women compared to the Framingham study [85]. Zhang et al. [86] reported that the higher prevalence of knee OA in Chinese women could be explained by increased manual labour and squatting. As discussed in the previous section, the prevalence of hip OA was similar between African American and Caucasian women, despite a previous report indicating a lower prevalence [60].

Genetics

It has been suspected for nearly seven decades that genetics have a strong influence on the incidence of OA [87]. Osteoarthritis is a multifactorial and polygenic late-onset disease in which environmental factors are key modulators of gene expression [88]. Family studies from the early 1960s reported that first-degree relatives were twice as likely to have radiographic generalized OA [89]. More recently, multiple twin and family studies have reported hand, hip and knee OA. Spector et al. [90] evaluated the genetic influences on OA in twin women and demonstrated a clear genetic impact for radiographic hand and knee OA, with an estimable heritable component ranging from 39-65% with monozygotic twins having a concordance rate nearly 70% higher than dizygotic. Similarly, in another radiographic hip OA study, the same group reported that about 60% of overall hip OA was hereditary in women [91]. These findings from twins are now corroborated with large population-based family studies with closely matched estimates of heritability. For example, the Rotterdam study estimated 56% heritability in hand OA [92]. In a population with clinical and radiographic hand OA and their siblings, the presence of osteophytes was the most sensitive biomarker
of hand OA heritability, with the first interphalangeal joint having 70% heritability in Caucasians [93].

An increasingly popular approach to investigating the genetic etiology of OA is genetic association studies (GAS) [94]. In order to explore each gene variant in OA, Zintzaras et al. [95] analyzed the data from 327 GAS and catalogued them in Cumulative Meta-Analysis of Genetic Association Studies CUMAGAS-OSTEO, a web-based information system (http://biomath.med.uth.gr). They reported that 19 gene variants significantly increased the risk for OA by 30% or more, with GDF5 and LRCH1 being the most important variants. Another means to evaluate the influence of genetics in OA is with linkage analysis. Genetic linkage occurs when a locus involving OA and alleles of a nearby marker are inherited together. A recent meta-analysis summarized linkage of OA susceptibility genes to regions 7q34–7q36.3, 11p12–11q13.4, 6p21.1–6q15, 2q31.1–2q34 and 15q21.3–15q26.1 [96]. Valdes et al. [97] reported that genetic pathways involving the bone morphogenic protein and the wingless signalling are also implicated. Moreover, it is becoming increasingly evident that synovitis and inflammation are also involved in the etiology of OA, and that inflammatory molecules including those related to the metabolism of cytokines, prostaglandins, and arachidonic acid are associated with susceptibility to OA.

**Nutritional factors**

Nutritional factors have long been the subject of considerable interest in OA even though the results are conflicting. One of the most important factors for bone development and remodelling is vitamin D. Since vitamin D influences bone quality, it has long been suspected that its status could have an effect on the risk of the development or progression of OA [98]. In the Framingham study, adults in the middle and highest tertiles of 25-OH-D intake had a 3-fold reduction in risk for radiographic knee OA compared to those with lower intake [99]. In the more recent Rotterdam study, it was also demonstrated that a low dietary vitamin D intake increases the risk for knee OA progression (12.6% in the lowest tertile vs 5.1% in the highest tertile), particularly in subjects with low baseline bone mineral density [98]. Another study also demonstrated that sunlight exposure and serum 25-OH-D levels are both associated with decreased knee cartilage loss (assessed by radiograph or magnetic resonance imaging) [100]. Moreover, the Study of Osteoporotic Fractures reported that women in the middle or lowest tertile of 25-OH-D had a 3-fold increase in incidence of hip OA [101]. However, a study on bone turnover, vitamin D, and calcium regulation in Caucasian twin pairs (66 MZ and 163 DZ) discordant for knee OA, found no difference in
these markers after adjusting for age and BMI [102]. In a review of two longitudinal studies, the Framingham Osteoarthritis Study (Framingham Offspring cohort) and the Boston Osteoarthritis of the Knee Study, it is concluded that vitamin D status is unrelated to the risk of joint space or cartilage loss in knee OA [103, 104].

McAlindon et al. [105] were the first to report that high intake of β-carotene and vitamin C reduced the risk of progression of radiographic knee OA; however, these products had no effect on knee OA incidence. In a well designed two-year randomized placebo controlled study, Wluka et al. [106] concluded that supplementary vitamin E does not affect the loss of cartilage volume in knee OA, corroborating a study on symptomatic relief of knee OA [107].

Multiple other nutritional factors such as ascorbic acid, other vitamins, minerals, fatty acids, flavonoids and ginger have been studied, but also with variable results. Even though some studies suggest a correlation between these factors and OA or symptom improvement, the role of nutrition in slowing down the disease progression remains to be thoroughly evaluated.

**Bone density**

As is now well known, OA is certainly not limited to cartilage degeneration, but involves all components (tissues) of the joint [108]. Literature in recent years has shown increased interest in the implication of bone density and the subchondral bone in the development, or even the initiation, of OA. The bone tissue involvement at the molecular level is discussed in detail in Chapter 5 - *Subchondral bone involvement in the pathophysiology of osteoarthritis*.

**Local risk factors**

The systemic factors previously discussed are key modulators of OA susceptibility but they are frequently associated with local biomechanical and biochemical factors. Following is a review of the biomechanical factors; the biochemical factors are extensively discussed in other chapters.

**Biomechanical factors**

Knee OA progression is found to be often driven by biomechanical forces. The subsequent pathological response of tissues to such forces leads to structural deterioration, symptoms, and reduced knee function. Tetsworth and Paley estimated that as little as 5° of genu varum results in about 80%
higher compressive force on the medial knee compartment [109]. Sharma et al. [54] demonstrated that varus of 5° or more was associated with a 4-fold increased risk of medial knee OA progression, and valgus deformation with a 5-fold increase in lateral progression, and that the severity of the malalignment predicted the decline in physical function at 18 months. Chang et al. [110] subsequently evaluated thrust during ambulation and the progression of knee OA and reported that varus thrust increased 4-fold medial knee OA progression (and varus alignment increased it 3-fold). Using the Osteoarthritis Initiative cohort of knee OA, a recent study demonstrated that African Americans with knee OA had about half the odds of varus thrust compared to Caucasians, and about twice the odds of valgus thrust, adding new insight to the difference in pattern of OA involvement between these groups [111]. It is also well known that concomitant risk factors such as obesity play a role in the impact of the biomechanics in OA development [112]. For example, the genu varum malalignment, having a high bone mass index led to increased OA severity, which is not the case with genu valgum [113]. However, even with strong evidence that malalignment is a risk factor for OA progression [54, 114], data from the Framingham study did not establish a relationship between malalignment and OA incidence [115, 116].

**Muscle strength**

In contrast to high bone mass index or malalignment, muscle strength is a more easily modified risk factor for OA. However, data on the association between the incidence and progression of knee OA and lower quadriceps muscle strength are variable. Some report that 18% lower knee extensor strength in women is associated with a higher incidence of OA [117], while others showed that moderate or high quadriceps strength in women significantly reduced, by about 60%, the risk of hip or knee OA [118]. Moreover, in the largest study to date in this field of research, Segal et al. [119] reported that quadriceps weakness predicted risk for knee joint space narrowing in women but not in men. This study demonstrated that women in the lowest tertile of quadriceps strength had nearly 70% increased risk for whole knee joint space narrowing and tibiofemoral joint space narrowing. The discrepancies between men and women were explained by greater strength in men, thus protecting them against reaching a threshold under which strength becomes a risk factor for OA [120]. However, in another recent longitudinal study by Segal et al. [121], thigh muscle strength failed to predict radiographic knee OA incidence, but demonstrated that higher extensor strength nearly halved the incidence of symptomatic OA in both sexes. Several other studies found no association between muscle weakness
and worsening of OA in women [122, 120]. In contrast, a study by Sharma et al. [72] reported that in the context of malalignment and laxity, greater muscle strength may actually increase the risk for OA progression. In this context, Chaisson et al. [123] reported that men with hand OA with greater grip strength in the Framingham cohort were at increased risk for developing carpometacarpal, metacarpophalangeal and PIP OA. The discrepancies between these studies merit further evaluation since they could have a major impact on future OA management. For example, several studies have shown that quadriceps strengthening exercises may improve joint pain in subjects with knee OA [124, 125].

**Exercise**

Despite the common misconception that exercise is deleterious to one’s joints, there is no evidence to support this notion in the absence of joint injury [126]. In addition to its various well recognized health benefits, exercise is also beneficial to joint tissues [127]. In evaluating the risk of developing OA from sports participation, it is important to discriminate recreational and professional sports participants, since the latter are at higher risk for injury associated with subsequent OA development, especially those in sports involving high impact and repetitive movement [128]. Initial studies on recreational weight-bearing exercise or jogging reported no association between moderate exercise and joint degeneration in the absence of other factors [129-132]. However, some population-based studies have reported an increase in knee and hip OA with higher exposure to sporting activities. Vingard et al. [133, 134] reported a relative risk of 4.5 for men and 2.3 for women of developing hip OA in the higher activity group. Another study demonstrated that male runners below the age of 50 who ran more than 20 miles per week were at greater risk for developing OA (OR 2.4; 95% CI: 1.5-3.9) [135]. Sports requiring high torsional loading, such as in the elbows of professional baseball players or elite javelin throwers, are particularly associated with OA [128, 136] and elite javelin throwers and high jumpers are about three times more at risk for hip OA than non-athletes [126]. Some studies have also demonstrated a prevalence of knee OA in former elite middle and long distance runners [137]; however, this is controversial, since others found no increased prevalence of OA in such groups. It would thus seem that elite athletes performing high impact, repetitive or torsional stress activities are at risk for developing OA but probably secondary to the greater risk of concomitant injury than the activity itself [128]. Indeed, since the joint can be considered a complex organ, the global effect of physical activity on the joint is multifactorial and every aspect including bone density, muscle
strength, proprioception, and risk of injury, must be evaluated to predict the net effect.

**Joint injury**

Although recreational physical activity and sports alone do not appear to be risk factors for developing OA, it has been clearly demonstrated that injuries can counteract the beneficial aspects of sports and lead to secondary OA. A variety of joint injuries are clearly related to the incidence of OA, particularly in the knee, but nearly all joints can be affected. A recent review of sports injuries reported that approximately 15% of sports related injuries in children and teenagers were associated with growth disturbance [138]. In adults, Felson et al. [139] reported that knee injury is the leading modifiable risk factor for OA in men and the second in women (after obesity). In soccer players, the prevalence of knee OA is higher (26%) than in runners (14%), which can be explained by increased risk of injury in the former group [140]. The most frequently reported injuries are anterior cruciate ligament (ACL) injuries and meniscal tears. As reported by Keays et al. [141], the incidence of OA after ACL reconstruction is alarmingly high, with reports as high as 50% at 6 years. In this study, the highest predictive factor for subsequent knee OA after ACL repair was meniscectomy and chondral damage. Moreover, a recent meta-analysis of knee OA after ACL injury indicated that the prevalence was only between 0%-13% for subjects with isolated ACL injury, but increased to 21%-48% with additional meniscal tear [142]. In their 21-year follow-up study of knee OA after meniscectomy, Roos et al. [143] reported mild radiographic changes in 71% and moderate (≥ KL 2) in 28% of the knees, nearly four to seven times more than healthy control knees. The relative risk for the presence of the more advanced tibiofemoral radiographic changes was 14.0 (95% CI: 3.5-121.2). Even in the 1940s, it was speculated that the increased risk for OA after meniscectomy was probably caused by the increased mechanical stress on the cartilage and subchondral bone due to changes in the knee biomechanics [144]. Unfortunately, to date, ACL reconstruction and meniscectomy cannot completely restore the joint and prevent subsequent OA development.

**Obesity**

Obesity is the strongest modifiable risk factor for OA, possibly due to the high mechanical stresses imposed on the joint. Indeed, it is well demonstrated that OA prevalence in knee [67, 145] and possibly hip [146] and hand OA [147] are increased in overweight people. In the Framingham study, the BMI
at study entry predicted the presence of radiographic knee OA 36 years later [148]. In this context, another study using the Framingham data also demonstrated that a reduction of about 5 kg in women resulted in a 50% reduction in the development of symptomatic knee OA [149] and a meta-analysis of randomized controlled trials reported that patients who reduced their body weight by at least 5% within a 20-week period experienced symptomatic relief of knee OA [150].

Interestingly, since obesity is also a risk factor for non-weight-bearing joints such as the hand [147], which cannot be explained by mechanical factors, an increasing number of studies are focusing their attention on evaluating the presence of possible obesity-related systemic factors in the pathogenesis of OA. Hu et al. [151] recently reviewed the adipokines, one of the emerging classes of molecules implicated in the systemic factors of OA. Discovered by Zhang and Friedman [152] with the cloning of leptin, adipokines or white adipose tissue cytokines, can act via autocrine and paracrine pathways which could explain the systemic effect of obesity in OA and rheumatoid arthritis [153]. To date, the major adipokines implicated in OA are leptin, adiponectin, resistin and visfatin. Although their roles still need to be clarified, it seems that adipokines may soon be pivotal to the diagnosis and prognosis of, and pharmacological approaches to, rheumatoid arthritis and OA [151, 153].

**Occupation**

Studies of occupational groups have demonstrated a clear relationship with the incidence of OA for over 60 years. Indeed, in 1952, Kellgren and Lawrence [154] demonstrated that in adults between 40 and 50, miners showed significantly higher prevalence of knee OA than both manual and office workers. Although OA is a multifactorial disease, there is current evidence that occupational activities requiring repetitive use can be a risk factor for OA. For example, men working in agriculture had a 2.3 (95% CI: 2.1-2.5) increase in prevalence of OA in the overstimulated joint compared to other occupations [155]. In a systematic review, Lievense et al. [156] also found moderate evidence of an association between previous heavy physical workload and hip OA, with an odds ratio of nearly 3. Multiple other studies have demonstrated that heavy physical work involving knee bending or squatting is associated with a higher prevalence of knee OA. More recently, a study from the Johnston County Osteoarthritis Project confirmed an association between physically demanding occupational tasks (lifting over 10 pounds, crawling, heavy work while standing) and both symptomatic knee and hip OA (OR 1.4-2.1), but failed to demonstrate radiographic changes
The absence of radiographic progression could be explained, at least in part, by multiple factors such as the lack of sensitivity of X-rays. Indeed, an MRI study in women recently demonstrated that those physically demanding occupations affect patellar but not tibial cartilage, which is not well evaluated by X-rays [158].

**Conclusion**

Osteoarthritis is the most common form of arthritis and is associated with an alarmingly increasing socioeconomic impact. Even though it is one of the oldest diseases affecting humankind, the pathophysiology of OA is still evolving, from being viewed as cartilage-limited to a multifactorial disease affecting the whole joint. The prevalence of OA varies by site, age, gender and ethnicity. Moreover, an intricate relationship between local and systemic factors modulates the radiological and clinical presentation of OA. Evolving radiological and clinical definitions and a better control and understanding of the various risk factors will improve our knowledge of OA and ultimately lead to new pharmacological and non-pharmacological therapies.

**References**

56. Marks, R. 2007, Obesity (Silver Spring), 15, 1867.
2. Cyclooxygenase-2 and microsomal prostaglandin E synthase-1 in the pathophysiology of osteoarthritis

Fumiaki Kojima¹ and Mohit Kapoor²

¹Department of Pharmacology, Asahikawa Medical University, Asahikawa, Japan
²Osteoarthritis Research Unit, University of Montreal Hospital Research Centre (CRCHUM)
Notre-Dame Hospital, Montreal, Quebec, Canada

Abstract. The exact etiology of osteoarthritis (OA) is not fully understood and as a result no appropriate curative therapeutics are currently available to halt the progression of this disease. Mediators derived from the arachidonic acid (AA) metabolic pathway, especially prostaglandins (PGs), are believed to play a crucial role in the pathophysiology of this disease. Among the various PGs, PGE₂ is the major AA metabolic product involved in inflammatory and destructive mechanisms associated with OA. Two key enzymes involved in the biosynthesis of PGE₂ are cyclooxygenase-2 (COX-2) and microsomal PGE synthase-1 (mPGES-1). Non-steroidal antiinflammatory drugs, which act via COX inhibition, resulting in subsequent inhibition of PGE₂, are extensively used for the management of inflammation, pain, swelling and joint stiffness associated with OA. This chapter will summarize the current knowledge of the role of COX-2 in OA. In addition, the emerging role of mPGES-1 as a potential therapeutic target for OA will be discussed.

Correspondence/Reprint request: Dr. Mohit Kapoor, Osteoarthritis Research Unit, University of Montreal Hospital Research Centre (CRCHUM), Notre-Dame Hospital, Montreal, Quebec, Canada H2L 4M1
E-mail: mohit.kapoor.chum@ssss.gouv.qc.ca
Introduction

Arachidonic acid (AA) is an essential fatty acid which is bound to membrane phospholipid molecules, triglycerides and cholesterol esters. During an event of tissue injury, stress or cell damage, cell membrane phospholipids activate phospholipases to liberate AA which can be further metabolized by at least three different enzymatic systems. One of the most critical enzymes in the AA metabolic pathway is cyclooxygenase (COX), which metabolizes AA into prostaglandin (PG)G\(_2\) by its COX activity and then into the intermediate substrate PGH\(_2\) by its peroxidase activity. PGH\(_2\) is further metabolized by individual PG synthases into PGE\(_2\), TXA\(_2\), PGD\(_2\), PGF\(_{2\alpha}\) and PGI\(_2\) [1] (Figure 1). AA can also be metabolized by the lipoxygenase (LOX) enzymes to generate leukotrienes and hydroxyeicosatetraenoic acids. In addition, recent studies have demonstrated that lipoxins are generated by a unique AA metabolic pathway mediated by both COX and LOX [2].

![Figure 1. Biosynthesis of prostaglandins and thromboxanes.](image-url)
Cyclooxygenases

In 1971, Sir John Robert Vane first demonstrated that the mechanism of action of aspirin and other non-steroidal antiinflammatory drugs (NSAIDs) occurs via the inhibition of PG production [3]. Subsequently, two COX isozymes were identified, namely COX-1 and COX-2 [4-6]. COX-1 is a 69-kd protein, its gene resembles a housekeeping gene and lacks a TATA box [7] and two Sp1 cis-regulatory elements, contributing to its constitutive expression [8]. COX-2, on the other hand, is a 72-kd protein and its promoter contains a TATA box and several inducible enhancer elements, most notably the nuclear factor-κB (NF-κB), cyclic adenosine monophosphate response element and the CCAAT enhancer binding protein.

COX-1, which is constitutively expressed in the majority of mammalian cells and tissues, regulates the production of PGs and thromboxanes (TXs) involved in the regulation of vascular, gastrointestinal and renal homeostasis [9]. In contrast, COX-2, which is induced in response to a variety of proinflammatory stimuli, regulates the production of PGs involved in inflammation, pain, and fever, especially PGE\(_2\) and prostacyclin (PGI\(_2\)) [10-13]. COX-3 was reported to be a novel COX isozyme predominantly expressed in the cerebral cortex and heart [14]; however, its existence in humans has been questioned by a subsequent study [15].

Cyclooxygenases in articular cells and tissues

COX-2 is not expressed in unstimulated normal human articular chondrocytes, but is induced by inflammatory mediators including interleukin (IL)-1β, IL-17, tumour necrosis factor α (TNF-α), leukemia inhibitory factor and bacterial lipopolysaccharide [16-18]. It is expressed in high levels in cartilage from OA patients [17, 19, 20] and its increased expression is associated with the release of PGE\(_2\). Since the production of proinflammatory PGs, especially PGE\(_2\), at sites of inflammation coincides with the upregulation of COX-2 expression in activated articular cells, COX-2 has long been a key target for the treatment of osteoarthritis (OA) and other forms of arthritis.

The significance of COX-2 in arthritis has also been demonstrated in various experimental models involving cartilage destruction. A selective COX-2 inhibitor significantly inhibited the pathophysiological symptoms including paw edema, spontaneous pain, and hyperalgesia in an adjuvant induced arthritis (AIA) rat model [21]. However, in another study in the same model, a COX-1 selective inhibitor did not reduce the inflammation or PGE\(_2\) production [22]. In addition, a selective COX-2 inhibitor, but not a selective
COX-1 inhibitor, reduced the severity of symptoms in a type II collagen-induced arthritis (CIA) mouse model [23]. Furthermore, COX-2-deficient mice, but not COX-1-deficient mice, displayed a significant reduction in both clinical and histological signs of CIA [24].

COX-2 expression is mediated by several signalling pathways depending on the type of tissue/cell and on the stimulus. In chondrocytes and synovial fibroblasts, some of the most important signalling pathways involved in mediating COX-2 expression include the mitogen-activated protein kinase signalling cascades JNK/SAPK and p38 [25]. β-catenin has also been shown to regulate the expression of COX-2 in articular chondrocytes [26]. NF-κB is another key mediator involved in the regulation of COX-2 in articular cells [27]. The latter appears important as the majority of COX-2-inducing mediators also activate NF-κB, and the COX-2 promoter contains 2 consensus NF-κB binding sites [27-29].

**Prostaglandin E synthases**

To date, various terminal enzymes acting in the conversion of PGH₂ to the active prostanoids downstream of COX have been cloned and characterized. These enzymes include PGE synthase (PGES) for PGE₂, PGDS for PGD₂, PGFS for PGF₂α, PGIS for PGI₂, and TXS for TXA₂. These terminal PG synthases are known to be functionally linked with preferential COX isozymes [30]. In the final step of PGE₂ biosynthesis downstream of COX, PGES specifically catalyzes the conversion of PGH₂ to PGE₂. To date, at least three individual forms of PGES, cytosolic PGES (cPGES), microsomal PGES (mPGES)-1, and mPGES-2, have been cloned and characterized [31-33]. cPGES is largely believed to contribute physiologically to the production of PGE₂ for the maintenance of homeostasis based on the fact that it is constitutively expressed and functionally coupled with COX-1 in the cytosol under basal conditions in various cells and tissues [32]. mPGES-1, on the other hand, shows coordinated induction with COX-2 by inflammatory stimuli in various cells and tissues [31, 34]. Because of its inducible nature, it has been of interest to investigate the role of mPGES-1 in inflammatory diseases. mPGES-2 has a catalytic glutaredoxin/thioredoxin-like domain and is activated by various thiol reagents. Like cPGES-1, mPGES-2 is also constitutively expressed in various cells and tissues. However, the fact that it is functionally coupled with both COX-1 and COX-2 [35] indicates that mPGES-2 may play a role in the production of PGE₂ not only in homeostasis but also in pathological conditions.
Microsom al prostaglandin E synthase-1 in articular cells and tissues

mPGES-1, originally known as microsomal glutathione S-transferase 1-like 1 (MGST1-L1), is a glutathione-dependent enzyme that shows coordinated induction with COX-2 by inflammatory stimuli in various cells and tissues [31, 34]. mPGES-1 has been considered the most prominent PGES isozyme to be targeted in inflammatory diseases including OA. We and others have reported that articular chondrocytes from OA patients express mPGES-1 after stimulation with the proinflammatory cytokines IL-1β or TNF-α [36, 37]. In addition, the pattern of mPGES-1 expression by cytokine-activated synovial fibroblasts was shown to be similar to that observed in OA chondrocytes [38]. mPGES-1 protein (immunoreactivity) was also detected in both the chondrocytes and synovial lining cells in OA patients. These observations indicate that overexpression of mPGES-1 in articular tissues such as synovium and cartilage may be a crucial contributing factor to the development of chronic articular inflammation in OA patients.

The roles of mPGES-1 have also been demonstrated by several studies using mPGES-1-deficient mice in experimental models of arthritis. We and others have demonstrated that mPGES-1-deficient mice are resistant to CIA [39] and show a marked reduction in anti-type II collagen antibodies [40], and decreased pain response in models of inflammatory pain and neuropathic pain [39-41]. A study using several mouse models of skeletal disorders with mPGES-1-deficient mice revealed that mPGES-1 was indispensable for bone repair via proliferation of chondrocytes [42]. Conversely, studies have also shown that mPGES-1 was not essential for the skeleton under normal physiological conditions, nor did it play a role in the pathophysiological conditions of bone loss (due to ovariectomy or to unloading) or during stress-induced OA. Since studies using mPGES-1-deficient mice reveal resistance to some symptoms of inflammatory arthritis, selective mPGES-1 inhibitors could be promising targets for the treatment of OA and other forms of arthritis.

Prostaglandin E$_2$ and its receptors

Prostanoids exert a variety of physiological and pathophysiological actions via their respective receptors expressed on target cells. The PG and TX receptors are G protein-coupled receptors with seven transmembrane domains. They are comprised of EP for PGE$_2$, DP for PGD$_2$, FP for PGF$_{2\alpha}$, IP for PGI$_2$ and TP for TXA$_2$ [43]. In addition, another PGD receptor, CRTH$_2$ (also known as DP$_2$), was identified as the chemoattractant receptor-
homologous molecule expressed on Th2 cells [44]. Expression of these receptors depends on the cell type they are expressed on, leading to alterations in the specificity and physiological functions of the final active products.

PGE₂ exists in a wide variety of cells and tissues, and plays important roles in various physiological functions in addition to its role as a major mediator of inflammation. High concentrations of PGE₂ have been detected in the synovial fluid of OA patients and it is well established that it is a key mediator of cartilage degradation. In growth plate chondrocytes, PGE₂ has been shown to increase DNA synthesis and inhibit collagen synthesis [45]. It has also been shown to increase matrix metalloproteinase (MMP) production in human OA cartilage explants [19], articular chondrocytes [46] and synovial fibroblasts [47]. PGE₂ also potentiates inflammation by promoting the expression of the proinflammatory cytokine IL-1β [48].

As mentioned above, the preferential production of PGE₂ results from the sequential activation of two cytokine-inducible enzymes, COX-2 and mPGES-1. We previously reported that increased production of PGE₂ in primary cultured chondrocytes from OA patients [36] and human rheumatoid arthritis synovial fibroblasts [49, 50] is due to induction of mPGES-1 by proinflammatory cytokines.

PGE₂ exerts its effects via four EP subtypes, EP₁, EP₂, EP₃, and EP₄. There are differences in the downstream signalling pathways of the EP subtypes. EP₁ is coupled with PLC/PI3 signalling and stimulates the release of intracellular calcium, while EP₂ and EP₄ increase cAMP by activation of adenylate cyclase via coupling to Gₛ-type G protein [51]. Although EP₃ has variants that mediate multiple signalling pathways, it generally causes the inhibition of cAMP via Gᵢ-type G protein [52].

EP₂ and EP₄ have been shown to be expressed at higher levels in knee cartilage of OA patients [53]. Specific agonists and antagonists targeting each EP have been developed and used for research [54]. A study using selective EP agonists for individual EP₁-₄ subtypes clearly demonstrated in OA synovial fibroblasts that PGE₂ regulates the production of IL-1β-induced IL-6, macrophage colony stimulating factor, and vascular endothelial growth factor through the activation of EP₂ and EP₄ with an increase in intracellular cAMP [55]. A similar regulation of TNF-α-induced IL-6 and MMP expression was also recently reported in synovial fibroblasts [56].

In OA chondrocytes, PGE₂ has been shown to inhibit proteoglycan synthesis and stimulate matrix degradation via EP₄ [57]. A recent study also showed that COX-2-derived PGE₂ signals via upregulation of EP₂ and downregulation of EP₃ to increase intracellular cAMP, and activate the
protein kinase A and phosphatidylinositol 3-kinase/Akt pathways, regulating shear stress-induced IL-6 expression in chondrocytes [58]. Hence, PGE2-EP signalling may play a pivotal role in the pathophysiology of chronic inflammation related to OA.

Apart from EPs, recent studies suggest the possible impact of the PGI2-IP system in experimental models of acute pain [59]. There is a need for detailed roles of other prostanoid receptors in OA to be further investigated.

**Prostaglandin E2 inhibition in osteoarthritis**

PGE2 inhibition has long been the prime therapeutic target to overcome the destructive and inflammatory mechanisms associated with OA. Inhibitors of COX-2 exert antiinflammatory effects and pain relief by inhibiting both PGE2 and PGI2. Traditional NSAIDs such as indomethacin and ibuprofen are clinically effective for the management of pain associated with OA and related diseases such as rheumatoid arthritis. However, traditional non-selective NSAIDs have been associated with a number of gastrointestinal and renal side effects due to their inhibitory effects on both COX-1 and COX-2, resulting in inhibition of PG and TX production [60]. Selective COX-2 inhibitors were therefore developed with the aim of reducing these side effects of non-selective NSAIDs [61]. Selective COX-2 inhibitors, or COXIBs, have improved gastrointestinal toxicity and similar efficacy to the non-selective NSAIDs in OA patients [62-64]. The VIGOR (Vioxx GI Outcomes Research) study showed that treatment with rofecoxib resulted in significantly less clinically important upper gastrointestinal events than treatment with naproxen [62]. However, long term use of these COXIBs has been shown to be associated with increased risk of myocardial infarction and thrombosis [65]. Therefore, rofecoxib was withdrawn worldwide in 2004, and in 2005 valdecoxib was withdrawn by the United States Food and Drug Administration (FDA) with concerns regarding increased cardiovascular side effects. Another COXIB, celecoxib, is presently under the FDA alert [66].

A possible primary mechanism related to the cardiovascular side effects associated with selective COX-2 inhibitors is their ability to inhibit not only PGE2 production but also PGI2 (derived from endothelial COX-2), which plays a key role in the regulation of thrombogenesis [67]. Hence, a more specific inhibition of PGE2 production via inhibition of mPGES-1 would seem to be a more relevant approach. Indeed, within the AA metabolic pathway, COX-2 is located relatively higher than MPGES-1, resulting in the inhibition of all PGs. By inhibiting mPGES-1, hypothetically only PGE2 can be blocked. Studies performed in COX-2 and mPGES-1 transgenic mice fully support this hypothesis. Indeed, mice with COX-2 genetic deletion, mutation,
or treatment with celecoxib develop thrombosis and hypertension. However, mPGES-1-deficient mice exhibit significant reduction in PGE\(_2\) production and increased PGI\(_2\) production with no alterations in blood pressure or thrombogenesis when fed a normal or high salt diet [68]. In addition, loss of mPGES-1 in mice retards atherogenesis associated with decreased PGE\(_2\) production, increased PGI\(_2\) production and no change in TX production [69]. Furthermore, mPGES-1-deficient mice in experimental models of arthritis are resistant to cartilage destruction associated with marked reduction in inflammation and pain response [39-41].

**Conclusion**

mPGES-1 seems to be an attractive therapeutic target for counteracting OA while avoiding the cardiovascular side effects associated with COX-2 inhibition. However, these assumptions can only be proven when a specific potent inhibitor of mPGES-1 is tested in a human clinical setting. It will be very interesting to see how effectively an mPGES-1 inhibitor is able to mimic the results observed in mPGES-1-deficient mice. In addition to mPGES-1, targeting EP receptor subtypes is another promising alternative approach.

**References**

3. The complex role of peroxisome proliferator-activated receptor gamma in osteoarthritis

Hassan Fahmi

Osteoarthritis Research Unit, University of Montreal Hospital Research Centre (CRCHUM)
Notre-Dame Hospital, Montreal, Quebec, Canada

Abstract. Osteoarthritis (OA), a widespread chronic human health disorder, is the most common form of arthritis and a major cause of disability in developed countries. To date, treatment for OA has been largely palliative, as no treatment exists that can stop the disease from progressing. Although several potential therapeutic approaches have been tested, recent studies suggest that the activation of the transcription factor peroxisome proliferator-activated receptor gamma (PPARγ) is a promising target. PPARγ, a member of the nuclear receptor superfamily, is a ligand-activated transcription factor. Its activation inhibits inflammatory and catabolic responses both in vitro and in vivo, and reduces the development and progression of cartilage lesions in OA animal models. This chapter summarizes recent findings on the effects of PPARγ activation on inflammatory and catabolic responses in chondrocytes and synovial fibroblasts. The role of PPARγ polymorphism in the pathogenesis of OA will also be discussed.
Introduction

Osteoarthritis (OA) is the most frequent musculoskeletal disorder and the most widespread chronic form of arthritis. It affects approximately 15% of the population and 60% of those over the age of 60, affecting women more frequently than men. OA treatment has become a serious medical concern along with the aging world population. As incidences of the disease are expected to increase over the next few decades, the economic burden related to its medical and social costs is imposing. Improving treatment efficacy and the development of therapeutic strategies to stop the disease progression is becoming increasingly important.

Cartilage damage is a critical event in OA [1]. The articular cartilage is typically regarded as the primary diseased tissue, with increasing deterioration and insufficient tissue repair. During the initiation and progression of OA, chondrocytes, stimulated by the proinflammatory factors, of which interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) appear to be key cytokines, express cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and microsomal prostaglandin E synthase-1 (mPGES-1) as well as matrix metalloproteinases (MMPs), resulting in the degradation of the extracellular matrix components. Moreover, during the disease process, the expression of anabolic factors is substantially downregulated, causing increased cartilage damage.

Synovial inflammation has also been shown to occur in the early stages of OA and can be subclinical [2]. However, synovitis, which is evident at the clinical stage of the disease, can be the cause for a patient to consult a physician. It is the belief that synovitis is induced by the cartilage matrix degradation products that produce wear particles and soluble cartilage-specific neo-antigens, as well as other factors including microcrystals and abnormal mechanical stress. When released into the synovial fluid, these components are phagocyted by synovial lining macrophages, perpetuating synovial membrane inflammation via the synthesis of mediators. These mediators, in turn, are diffused into the cartilage through the synovial fluid, creating a vicious circle in which cartilage increasingly disintegrates and causes further inflammation. The inflammatory mediators in the synovial membrane are synthesized by several cell types; however, in OA, data suggest the synovial fibroblasts to be the principal producers of catabolic factors.

There is no effective treatment for OA and new therapeutic approaches with the potential to stop or reduce the disease progression are needed. Among such potential targets, peroxisome proliferator-activated receptor gamma (PPARγ) is a promising one for the treatment of OA. This chapter
will describe the role of PPARγ in the biology of chondrocytes and synovial fibroblasts as well as its complex role in the structural alterations of OA.

**PPARγ**

PPARγ receptors belong to the nuclear hormone receptor superfamily, which includes receptors for steroids, thyroid hormone, vitamin D, and retinoic acid. Three PPAR isoforms have been identified: PPARα, PPARβ/δ and PPARγ [3]. PPARα, primarily present in the liver, heart, and muscle, plays important roles in the catabolism of fatty acid [4]. PPARβ/δ which is ubiquitously expressed, is implicated in various physiological processes, including lipid homeostasis, epidermal maturation and skin-wound healing, and brain development [5]. PPARγ, the most widely investigated member of the PPARs, exists under at least two isoforms: PPARγ1 and PPARγ2. Although derived from the same gene, their production results from the gene alternative promoter and differential mRNA splicing [6, 7]. PPARγ1 is expressed in several tissues including inflammatory and immune cells, whereas PPARγ2 is mainly found in adipose tissues. PPARγ is significantly implicated in glucid and lipid metabolism regulation and has been shown to contribute to diabetes [8], cardiovascular disease [9], carcinogenesis [10], and inflammation [11].

**PPARγ ligands**

PPARγ is activated by a number of natural physiological and synthetic agonists. The first natural endogenous agonist of PPARγ to be identified was the cyclopentanone prostaglandin 15-deoxy-Δ^{12,14}-prostaglandin J_2 (15d-PGJ2) [12], which has since been used extensively in attempts to define the role of PPARγ. Other natural PPARγ agonists include the essential fatty acids arachidonic acid, docosahexanoic acid, eicosapentanoic acid, and the 15-lipoxygenase (LOX) metabolites 13(S)-hydroxy octadecadienoic acid (13-HODE) and 15(S)-hydroxyeicosatetraenoic (15-HETE) [13]. Nitrolinoleic acid (LNO2), an unsaturated fatty acid derivative induced by nitric oxide (NO)-dependent oxidative inflammatory reactions, has also been reported to activate PPARγ [14].

Among the many synthetic compounds that bind to and activate PPARγ are the antidiabetic thiazolidinediones or glitazones, including troglitazone, pioglitazone, ciglitazone and rosiglitazone [8]. Nonsteroidal antiinflammatory drugs such as ibuprofen, indomethacin, fenoprofen, and flufenamic acid have also been reported to bind to and activate PPARγ [15]. Glitazars, which include muraglitazar, tesaglitazar, and farglitazar are dual acting PPARα/γ agonists, currently evaluated in the treatment of type 2 diabetes [16].
Effects of PPARγ activation on inflammatory and catabolic responses

Chondrocytes

Activation of PPARγ has been shown to downregulate several inflammatory responses in chondrocytes (Table 1). Treatment of human OA chondrocytes with 15d-PGJ2 or troglitazone inhibited IL-1β-induced NO and prostaglandin E2 (PGE2) production and iNOS and COX-2 expression [17-19]. PPARγ activation also prevented NO production by the inflammatory cytokines IL-17 and TNF-α, also known for their role in the pathogenesis of OA [18].

In contrast, treatment with the synthetic PPARγ activator rosiglitazone had no effect and the PPARγ antagonist GW9662 did not relieve the inhibitory effect of 15d-PGJ2, suggesting the implication of PPARγ-independent

Table 1. Mechanisms involved in the pathogenesis of OA and effects of PPARγ activation.

<table>
<thead>
<tr>
<th>Mechanism of OA pathogenesis</th>
<th>Effects of PPARγ activation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chondrocytes</strong></td>
<td></td>
</tr>
<tr>
<td>• Enhanced production of MMPs</td>
<td>Reduction in MMP production [17, 18, 26, 27]</td>
</tr>
<tr>
<td>• Reduced proteoglycan synthesis</td>
<td>Prevention of proteoglycan degradation [27, 52]</td>
</tr>
<tr>
<td>• Increased iNOS expression and NO production</td>
<td>Inhibition of IL-1-induced iNOS expression and NO production [18, 52]</td>
</tr>
<tr>
<td>• Increased expression of COX-2 and mPGES-1 and formation of PGE2</td>
<td>Inhibition of COX-2 and mPGES-1 expression and PGE2 formation [19, 21]</td>
</tr>
<tr>
<td><strong>Synovial fibroblasts</strong></td>
<td></td>
</tr>
<tr>
<td>• Increased production of IL-1, IL-6 and TNF-α</td>
<td>Suppression of IL-1, IL-6 and TNF-α production [39]</td>
</tr>
<tr>
<td>• Increased production of MMPs</td>
<td>Prevention of IL-1-induced MMP production [36]</td>
</tr>
<tr>
<td>• Increased production of PGE2, and expression of mPGES-1 and COX-2</td>
<td>Inhibition of COX-2 and mPGES-1 expression and PGE2 formation [37, 38]</td>
</tr>
</tbody>
</table>

COX-2, cyclooxygenase-2; IL, interleukin, iNOS, inducible nitric oxide synthase; mPGES-1, microsomal prostaglandin E synthase; MMP, matrix metalloproteinase; OA, osteoarthritis; PGE2, prostaglandin E2; PPARγ, peroxisome proliferator activated receptor gamma; TNF-α, tumour necrosis factor-α.
Role of PPARγ in osteoarthritis

Increased production of MMPs plays a critical role in cartilage degradation during OA. Interestingly, PPARγ activators were shown to suppress the production of several MMPs by chondrocytes and to prevent proteoglycan degradation. For instance, our group has shown that 15d-PGJ₂ and troglitazone suppressed IL-1β-induced MMP-13 expression by inhibiting the AP-1 and NF-κB pathways in human OA chondrocytes [18]. Similarly, rosiglitazone blocked IL-1β-induced MMP-1 production in rabbit chondrocytes through DNA binding competition on the composite PPRE/AP1 site in the MMP-1 promoter [26]. In rat chondrocytes, 15d-PGJ₂ and a synthetic activator, GI262570, inhibited IL-1β- and TNF-α-induced MMP-3, MMP-9, and proteoglycan degradation [27]. It was recently demonstrated that 15d-PGJ₂ and the synthetic triterpenoid 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO) suppress, in a PPARγ-independent manner, MMP-1 and MMP-13 in the human chondrocytic cell line SW1353 [28].

The 15-LOX metabolites 13-HODE and 15-HETE were shown to dose-dependently suppress IL-1β-induced MMP-1 and MMP-13 expression [29]. They also decreased the degradation of type II collagen in human OA cartilage explants treated with IL-1β [29]. Pretreatment with the PPARγ antagonist GW9662 was shown to prevent the suppressive effect of 13-HODE and 15-HETE, suggesting that their effects are mediated by PPARγ [29].

15d-PGJ₂ is generated through dehydration of PGD₂, the biosynthesis of which is catalyzed by two PGD synthases (PGDS): lipocalin PGDS (L-PGDS) and hematopoietic-type PGDS (H-PGDS). L-PGDS is glutathione-independent and H-PGDS glutathione-dependent [30]. L-PGDS (or β-trace) is a member of the lipocalin family, a group of secretory proteins that transport small hydrophobic molecules such as retinoids [31].

In an effort to characterize the PPARγ pathway in OA, the expression of PGDS in cartilage was investigated [32]. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry analyses revealed the presence of both H- and L-PGDS in cartilage, with L-PGDS being predominant. In addition, it was demonstrated that the levels of
L-PGDS mRNA and protein were elevated in OA when compared with normal cartilage. The increased expression of L-PGDS in OA cartilage is likely to be mediated by IL-1β, since treatment of chondrocytes with IL-1β enhanced L-PGDS expression in a time-dependent manner. This process requires de novo protein synthesis and involves the mitogen-activated protein kinases (MAPK) c-Jun N-terminal kinase (JNK) and p38, the NF-κB and Notch signalling cascades [32]. L-PGDS, a metabolite of PGD₂, also suppressed IL-1β-induced MMP-1 and MMP-13 production, an effect mediated through the DP1/cAMP/PKA pathway [32]. These data suggest that the increased expression of L-PGDS may represent an attempt to counteract the catabolic effects of IL-1β.

**Synovial fibroblasts**

Early investigations of the role of PPARγ in the biology of synovial fibroblasts demonstrated that its receptor agonists, 15d-PGJ₂ and troglitazone, inhibited the endogenous expression of several inflammatory and catabolic genes including IL-6, IL-8, TNF-α and MMP-3 in human OA synovial fibroblasts [33, 34] (Table 1). PPARγ activators also reduced LPS-induced expression of iNOS, COX-2, IL-1β and TNF-α in rat synovial fibroblasts [35]. In human OA synovial fibroblasts treated with IL-1β, expression of MMP-1, COX-2, and mPGES-1 was inhibited by 15d-PGJ₂ and troglitazone [36-38]. A study in human rheumatoid arthritis synovial fibroblasts [39] demonstrated that 15d-PGJ₂, but not troglitazone, suppressed IL-1β-induced expression of cytosolic phospholipase A₂ (cPLA₂) and COX-2 as well as PGE₂ production. However, pre-treatment with an anti-PPARγ antibody in that study did not reverse the effect of 15d-PGJ₂, suggesting that the effect was mediated via a PPARγ-independent mechanism [39].

**PPARγ-mediated inhibition of gene expression**

A number of mechanisms have been demonstrated to mediate transcriptional suppression by PPARγ, foremost among them its direct binding of key transcription factors and inhibition of their DNA binding and/or transcriptional activity. Indeed, PPARγ has been shown to inhibit transcriptional activity of SP-1, NF-κB, and nuclear factor of activated T cells (NF-AT) via mechanisms involving direct physical protein-protein interactions [40, 41].
PPARγ can also downregulate transcription by competing with general transcriptional co-activators. In this context, PPARγ was shown to interact with cAMP-responsive element binding factor (CREB)-binding protein (CBP), p300, steroid receptor co-activator-1 (SRC-1) and thyroid hormone receptor-associated protein (TRAP220) [42, 43]. These co-factors are required for the transcriptional activity of AP-1, NF-κB, early growth response-1 (Egr-1), signal transducers and activators of transcription (STAT), and NF-AT. Hence, the sequestering of limited amounts of general transcriptional co-activators by activated PPARγ may be responsible for the suppressive effect of PPARγ.

Post-translational modifications in histone proteins are increasingly recognized as critical in the regulation of gene expression via remodelling of chromatin structure. Acetylation, the most studied modification [44, 45], is controlled by the opposing actions of two classes of enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs). Generally, acetylation of histones at target promoters by HATs is associated with transcriptional activation, whereas deacetylation by HDACs is associated with transcriptional suppression. For example, the suppression of IL-1β-induced granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-8 expression by the antiinflammatory agents dexamethasone and theophylline appears to be due to the recruitment of HDACs to these gene promoters resulting in histone deacetylation [46, 47]. In human OA synovial fibroblasts, induction of COX-2 by IL-1β is associated with hyperacetylation of histone H3 and H4 at the COX-2 promoter [38]. Treatment with 15d-PGJ₂ inhibits IL-1β-induced COX-2 expression and histone acetylation at the COX-2 promoter. However, reduction in histone H3 acetylation is not correlated with recruitment of HDACs to the COX-2 promoter. Pretreatment of human OA synovial fibroblasts with trichostatin A (TSA), a specific HDAC inhibitor, did not relieve the 15d-PGJ₂ suppressive effect [38]. PPARγ has also been shown to inhibit transcription by preventing clearance of co-repressor complexes from target gene promoters. Activation of PPARγ was demonstrated to cause a conformational change leading to the sumoylation of its ligand binding domain by the E3 ligase PIAS1 [48]. Sumoylated PPARγ binds to the nuclear receptor co-repressor (NCoR)-histone deacetylase-3 (HDAC3) complex, blocking its release from the inflammatory gene promoter [48]. Finally, activators of PPARγ may also modulate transcription by stimulating specific signalling pathways. Indeed, PPARγ agonists have been shown to activate several intracellular signalling pathways such as the MAPKs extracellular signal-regulated kinase (ERK), JNK, p38, and the MAPK phosphatase-1 [49].
Reduced expression of PPARγ in OA cartilage

Studies from our group have shown that the predominantly expressed isoform of PPARγ in human cartilage is PPARγ1 and its level of expression is reduced in OA [50]. Similarly, Dumond et al. [51] reported reduced PPARγ expression in cartilage from a rat model of mono-iodoacetate-induced OA. Together, these findings suggest that reduced PPARγ expression in OA cartilage may, at least in part, be involved in increased expression of inflammatory and catabolic genes, promoting articular inflammation and cartilage degradation. Treatment of human OA chondrocytes with IL-1β resulted in a decrease in PPARγ protein expression [50]. PPARγ1 expression was also downregulated by TNF-α, IL-17, and PGE₂ [50]. Inhibitors of the MAPK p38, (SB203580) and JNK (SP60012), and of NF-κB signalling, SN-50, MG-132 and cafeic acid phenethyl ester (CAPE), also inhibited IL-1β-induced downregulation of PPARγ1 expression [50]. IL-1β has also been shown to reduce PPARγ protein expression in normal rat chondrocytes [52]. Thus, inhibition of PPARγ expression in chondrocytes by IL-1β may be an important process in the pathophysiology of OA.

PPARγ in experimental models of arthritis

PPARγ has demonstrated its in vivo protective effects in OA animal models. Systemic administration of pioglitazone dose-dependently reduced the size and the depth of cartilage lesions in an experimental guinea pig model of OA (partial medial meniscectomy) [53] in which the histologic severity of cartilage lesions was also reduced. This protective effect appears to be due to reduced expression of MMP-13 and IL-1β, two key mediators in the pathogenesis of OA. Similar reduction in cartilage lesions was found in an anterior cruciate ligament transection canine model of OA [54]. The latter study also showed reduced expression of MMP-1, iNOS, and a disintegrin and metalloproteinase domain with thrombospondin motifs (ADAMTS)-5 as well as a decrease in the activation of the signalling pathways ERK-1/2, p38 and NF-κB [54]. Thus, it seems that PPARγ agonists possess chondroprotective properties by impairing the expression of key genes involved in the pathogenesis of OA and the signalling pathways that mediate their transcriptional activation.

In animal models of rheumatoid arthritis, the protective effects of PPARγ activators have also been demonstrated. The administration of 15d-PGJ₂ and
troglitazone inhibited pannus formation and mononuclear cell infiltration in rat adjuvant-induced arthritis [55]. Treatment with rosiglitazone in a mouse model of type II collagen-induced arthritis (CIA) improved the clinical signs and histological features of the disease and reduced plasma levels of IL-1β, TNF-α and IL-6 [56]. A further study demonstrated that pioglitazone and rosiglitazone reduced synovitis and synovial expression of IL-1β and TNF-α in a rat model of CIA [57]. Both thiazolidinediones prevented bone erosion and bone loss [57]. More recently, Sumariwalla et al. [58] used a murine model of CIA to assess a new PPARγ ligand, CLX-090717, and found that it reduced clinical features of arthritis, paw swelling, and the progression of structural damage. Together these data indicate that PPARγ also demonstrates protective effects in other forms of arthritis. This is strengthened by the observation that antigen-induced arthritis is exacerbated in mice heterozygous for PPARγ deficiency [59].

**PPARγ polymorphism in OA**

There are a number of genetic variants in the PPARγ gene, the most prevalent being Pro12Ala and C161T (also known as C1431T). The Pro12Ala polymorphism results from a CCA-to-GCA missense mutation in codon 12 of exon B of the PPARγ gene. Because this mutation is located in the ligand-independent activation domain of the protein, it may cause conformational changes that affect its transcriptional activity [60]. The Pro12Ala polymorphism is associated with improved insulin sensitivity [61], reduced type 2 diabetes risk [62], myocardial infarction [63], and atherosclerosis [64]. The silent C161T (C1431T) polymorphism in the exon 6 is associated with increased plasma levels of leptin [65], decreased risk of coronary artery disease [66], survival of patients with immunoglobulin A nephropathy [67], and longevity [68].

When the effects of Pro12Ala and C161T were assessed on the prevalence and severity of OA in a French Canadian population, data revealed no significant difference in either polymorphism between OA patients and controls [69]. No significant differences were observed after stratification of patients according to age at disease onset and radiographic or functional severity. Moreover, haplotype analysis of both polymorphisms showed no association with OA or its clinical features. Thus, these PPARγ polymorphisms do not appear to contribute to susceptibility to, or severity of, OA in a French Canadian population. However, further studies in other ethnic populations are necessitated to evaluate the role of these and other polymorphisms in the pathogenesis of OA.
Conclusion

Since to date there exists no disease modifying treatment for OA, the investigation of its pathophysiological mechanisms is crucial. Continued research is necessary to develop and improve strategies aimed at reducing or arresting the disease progression. Improved knowledge of the physiological and pathological mechanisms of OA will lead to the development of specific targets in new therapeutic approaches.

The increased inflammatory and catabolic activity in chondrocytes and synovial fibroblasts is believed to contribute to the pathogenesis of OA and related chronic arthritic diseases. Over the last decade, in vitro and in vivo studies have shown that PPARγ agonists inhibit several of these catabolic and inflammatory responses, suggesting their potential benefit in the treatment of arthritis. The thiazolidinedione class of PPARγ agonists is already used in the treatment of type 2 diabetes and is presently being investigated in clinical trials for the treatment of cancer and cardiovascular diseases.

Although the current clinical trials will facilitate future studies assessing PPARγ agonists as anti-arthritis drugs, caution must be exercised. The molecular mechanisms by which PPARγ agonists modulate gene expression in articular cells need to be better characterized and care must be taken to prevent adverse side effects by ensuring that agonists are specific for PPARγ, avoiding PPARγ-independent mechanisms.

References

Role of PPARγ in osteoarthritis

4. Proteinase-activated receptor-2: An attractive DMOAD target for the treatment of osteoarthritis

Nathalie Amiable, Steeve Kwan Tat and Christelle Boileau

Osteoarthritis Research Unit, University of Montreal Hospital Research Centre (CRCHUM)
Notre-Dame Hospital, Montreal, Quebec, Canada

Abstract. Much research is currently being carried out to expand the knowledge of the pathophysiology of osteoarthritis (OA). The understanding of intimate pathways involved in this disease could lead to the identification of new therapeutic targets that could slow down or stop the disease progression, thereby preventing loss of joint function. Proteinase-activated receptor (PAR)-2, a member of the 7 transmembrane domain G protein coupled receptor family, has recently been described as a potential mediator in OA pathophysiology. This chapter will review and discuss the pertinent information regarding PAR-2 and its involvement in OA. Data have shown evidence supporting a possible therapeutic intervention against PAR-2 to control inflammation and catabolism in OA tissues.

Introduction

Recent studies have shown that the pathophysiological process in osteoarthritis (OA) involves both catabolism and inflammation in every tissue.
of the joint. These pathological changes are believed to be related to a complex network of not only biomechanical but biochemical pathways that implicate the diffusion of catabolic factors and cytokines between the different joint tissues. Molecular cross-talk between the articular tissues is also believed to be an integral part of the pathogenesis of OA. Significant evidence points to an association between inflammation, which is among the most significant changes that take place during the development of OA, and the appearance and progression of the disease. Recent studies have also revealed that, in addition to the cartilage and synovial membrane, biological and morphological disturbances occur in the subchondral bone during the OA process, and these alterations have been suggested to be responsible for early pathological changes in the cartilage.

To date, this disease remains incurable and only symptomatic treatments are available. The most widely used are analgesic (acetaminophen), non-steroidal antiinflammatory, and anti-cyclooxygenase (COX)-2 drugs. Attempts to find a cure have been disappointing, and there is a serious need to identify potential new therapeutic targets that will not only prevent symptoms including pain, joint swelling, and inflammation but also arrest disease progression, thereby preventing loss of joint function.

Considerable evidence indicates that inflammatory factors are crucial in the articular tissue destruction occurring during OA. The most important are the proinflammatory cytokines, nitric oxide, some eicosanoids, and the recently identified proteinase-activated receptor (PAR) family.

Proteinase-activated receptors

Proteinase-activated receptors belong to the superfamily of seven transmembrane domain G-coupled receptors [1-3], and were discovered during the investigation of a functional receptor for thrombin [4, 5]. To date, four members have been identified; namely PAR-1, PAR-2, PAR-3 and PAR-4. These receptors exhibit differential tissue expression as well as selectivity upon activation.

It is their unique mechanism of activation which distinguishes them from other G protein-coupled receptors (GPCRs). In fact, many GPCRs are activated in a reversible manner by hydrophilic ligands, whereas PARs are activated in an irreversible manner by proteases. In situ, the enzymes that activate PARs belong to the serine protease family. The activation of PARs occurs at a specific site on the N-terminal domain of the receptor unmasking a new N-terminal sequence. In turn, this sequence acts as a tethered ligand and auto-activates the receptor via an intramolecular binding of the tethered
PAR-2: A DMOAD target for osteoarthritis

ligand with a conserved region of the second extracellular loop [1, 2] (Figure 1).

In situ, PAR-1, -3 and -4 are activated by thrombin [6, 7], whereas PAR-2 is mainly cleaved by trypsin but also by other serine proteases including trypptase, matrix serine protease-1 and matriptase [3, 8-11]. It has also been reported that plasmin can activate PAR-1 [12] and PAR-4 [6, 13]. Some other enzymes, such as cathepsin G or elastase, inactivate PAR-1 [2] but can activate PAR-2 in certain cell types [14]. PAR-3 activation alone does not induce intracellular signalling; however, it can act as a co-factor for PAR-4 [15]. Interestingly, PARs can also be activated by small specific synthetic peptides. These peptides are derived from the tethered ligands of each receptor [5, 16]. This activation mechanism is faster and much simpler since these agonists bind directly to the extracellular second loop of the receptor without proteolytic cleavage of the N-terminus.

![Figure 1. Mechanism of PAR-1 activation and signalling.](image)

Figure 1. Mechanism of PAR-1 activation and signalling. Thrombin (α-Th) binds to and cleaves the N-terminus exodomain. The new tethered ligand is generated by the proteolytic cleavage and binds intramolecularly to the receptor to trigger transmembrane signalling. A peptide agonist derived from the N-terminus can activate the receptor independently of protease-mediated cleavage of the N-terminus. PAR-1 couples to a variety of G protein α-subunits and activates many signalling cascades and cellular responses. From [17].
PAR signalling

Protein G activation by PARs represents a new concept in GPCRs. Depending on the G protein involved, different intracellular signalling pathways are induced [17] (Figure 1). For example, PAR-2 mediates activation of phospholipase C [18] or protein kinase C (PKC) [19] through $G_{q/11}$ and $G_i$. However, when PAR-2 interacts with $G_{12}$ and $G_{13}$, different signalling cascades are activated including the mitogen activated protein (MAP) kinases Erk1/2, p38, and Jun N-terminal kinase (JNK) as well as nuclear factor kappa B (NFκB) [20-24].

Some of the PARs undergo a desensitization process which has been especially studied for PAR-1. After activation, PAR-1 is rapidly desensitized by the phosphorylation of the C-terminus via G protein coupled receptor kinases (GRKs). However, signal termination can also be induced by the binding of $β$-arrestin to PAR-1, independently of all phosphorylation. With regard to PAR-2, the C-terminus contains several potential sites of phosphorylation, but to date no study has demonstrated the role of GRKs in PAR-2 desensitization. After desensitization, the PARs are internalized and, in the majority of cases, degraded by the lysosomes [17, 25]. For PAR-1, in some cell types, an existing intracellular pool allows the cell surface to be reloaded with new receptors [26]. In other cell types, upon activation by thrombin, a small proportion of cleaved PAR-1 is recycled to the membrane and able to be activated by synthetic peptides [27].

Proteinase-activated receptor-2

Of the four identified receptors, PAR-2 seems to be strongly implicated in numerous inflammatory diseases such as gastrointestinal and pulmonary inflammation, as well as in cardiovascular, renal, and immune diseases. Although PAR-1 is mainly associated with tissue repair, the specific tissue distribution of PAR-2 suggests a possible role of this receptor in the development of these diseases.

PAR-2 is expressed in numerous cell types such as keratinocytes, endothelial cells, T lymphocytes and neutrophils [3, 28-31]. Studies performed in these cell types revealed that PAR-2 activation induces the synthesis of cytokines and prostaglandins, and leads to the activation of MAP kinases and NF-κB signalling pathways, factors highly implicated in inflammatory responses [1]. Moreover, stimulation of endothelial cells by proinflammatory cytokines leads to an upregulation of PAR-2 expression, whereas PAR-1 expression remains unchanged [32]. This PAR-2 expression upregulation is also observed in the brain tissues of human
immunodeficiency virus (HIV) infected patients [33]. PAR-2 is also involved in vascular permeability induction [34], leukocyte infiltration [35], and the relaxation of the smooth muscle cells [36], and has been found to be implicated in inflammatory intestine pathologies such as Crohn’s disease and ulcerative colitis [37]. In recent years, there has been a growing interest in understanding the role of this receptor in nociception [38] and neurogenic inflammation [39]. In this context, a positive correlation between PAR-2 expression and substance P level was demonstrated in the sensitive nerves [39], and hyperalgesia was markedly diminished or absent in PAR-2 knockout mice [40].

**PAR-2 activity in arthritic diseases**

Some of the members of the PAR family have recently been shown to be involved in inflammatory pathways and an important role for PAR-2 in rheumatoid arthritis and, more recently, in OA has been reported. Studies have demonstrated the presence of PARs, particularly PAR-2, in mouse and human joints [41, 42] and in numerous cell types implicated in rheumatoid arthritis and OA including synovial fibroblasts, chondrocytes, macrophages, neutrophils, mast cells, T lymphocytes and dendritic cells [28, 29, 43-46]. Other studies have revealed the presence of tryptase and thrombin in affected joints [47, 48], known activators of PAR-2 and PAR-1 respectively. Together these findings support the potential role of PARs, and particularly PAR-2, in the development of treatments for some inflammatory articular conditions.

To date, a limited number of in vivo studies in animals have confirmed the role of PAR-2 as a major mediator of articular inflammation. For example, the injection of a PAR-2 agonist peptide in the rat hind paw induced an inflammatory reaction characterized by articular swelling and granulocyte infiltration [34]. Likewise, an injection in the mouse knee led to joint perfusion and swelling in wild type mice compared to PAR-2 knockout mice [49]. Busso et al. [50] observed in PAR-2 knockout mice following an induced model of arthritis, a general reduction in the structural alterations caused by the development of the disease, as well as a decrease in the humoral immunity mediator demonstrated by the level of circulating antibodies. These findings are in accordance with that of Fields et al. [43] demonstrating that PAR-2 is implicated in mouse dendritic cell maturation. In fact, the maturation of these cells is critical in the initiation of the immune response during rheumatoid arthritis, thus confirming the role of PAR-2 during the early and late immune responses of this disease. More recently, our group showed that PAR-2 production was significantly inhibited in an induced-OA dog model treated with a plant extract of Brachystemma
calycinum D. don [51], the treatment of which led to decreased matrix metalloproteinase (MMP)-13 production as well as severity of OA cartilage lesions, indicating the possibility that PAR-2 could be an important mediator of some of the protease production involved in cartilage degradation.

The presence of PAR-2 has recently been documented in human cartilage/chondrocytes and synovial membrane/synovial fibroblasts, and its expression levels found significantly increased in OA compared to normal cells [44, 45, 52, 53]. The levels of PAR-2 were upregulated by the proinflammatory cytokine interleukin (IL)-1β in human OA synovial cells [53] and by IL-1β, tumour necrosis factor (TNF)-α, and transforming growth factor (TGF)-β in human OA chondrocytes [45, 52]. The MAP kinase p38 was identified as the major signalling pathway responsible for regulating PAR-2 receptor synthesis in human OA chondrocytes [52]. In turn, in these cells, specific PAR-2 activation induces the MAP kinases Erk1/2 and p38. Moreover, PAR-2 activation in these cells has been associated with an increased production of MMP-1 and MMP-13 as well as with COX-2, which are well-known key mediators of OA catabolism and inflammation [52]. These findings are in accordance with those of Milner et al. [11] showing that the increased expression of MMP-1 and MMP-13 in human OA cartilage occurs following PAR-2 activation by matriptase, a protease belonging to the serine protease family.

Masuko et al. [54] demonstrated that PAR-2 could play an important role in the angiogenic process, a mechanism frequently observed in OA [55], and that activation of this receptor increases the release of VEGF in human OA chondrocytes.

Recently, our group showed that PAR-2 is expressed in human subchondral bone osteoblasts and is specifically upregulated in OA osteoblasts [56]. Interestingly, in these cells, PAR-2 specific activation resulted in the modulation of the OPG (osteoprotegerin)/RANKL (membranous receptor activator of nuclear factor κB ligand) ratio, and more specifically through an upregulation of the level of membranous RANKL without affecting OPG production [56]. OPG and RANKL are factors closely linked to each other and have been described as a key cytokine system involved in the differentiation and function of osteoclasts [57-61]. RANKL, which is localized on osteoblasts, is a key factor responsible for the enhancement of osteoclastogenesis via interaction with its receptor RANK, which is localized on osteoclasts. The binding of RANKL to the extracellular RANK domain leads to the activation of specific signalling pathways involved in the formation and survival of osteoclasts, resulting in bone resorption. In turn, OPG, which is also produced by osteoblasts, acts as a soluble decoy receptor for RANKL. By interacting with RANKL, OPG
inhibits the binding of RANKL to RANK, thereby preventing RANK activation and subsequent osteoclastogenesis and, as a result, inhibits bone resorption. Thus, equilibrium between OPG and RANKL plays a crucial role in the pathophysiology of bone [57-61]. Data revealed that PAR-2 activation increased the level of RANKL without affecting the OPG, consequently decreasing the OPG/RANKL ratio. Moreover, PAR-2 activation also increased the bone resorptive activity. These findings strongly suggest that activation of PAR-2 in human subchondral bone favours a remodelling/resorption process via the OPG/RANKL system [56].

In addition to the subchondral bone cells, the OPG/RANK/RANKL molecular triad has also been observed to be expressed and produced by another articular cell, the chondrocyte [56, 62]. Interestingly, in a recent study, in vitro treatment of human chondrocytes with exogenous OPG resulted in increased levels of two catabolic factors, namely MMP-13 and PAR-2 [56], thus contributing to the catabolism/degradation of the articular cartilage.

Lately, in vivo studies on PAR-2 in an induced model of OA in mice demonstrated that wild type mice developed OA whereas the PAR-2 knockout mice were protected against the development of the disease, and that the inhibition of PAR-2 in wild type mice reduced the progression of OA [63, 64].

Another system, the ephrin ligands/receptors, was demonstrated to be capable of modulating PAR-2 [65]. In human subchondral bone osteoblasts and chondrocytes, our group recently demonstrated the expression and production in normal and OA conditions of two members of this family, the ephrin-B2 ligand and its receptor EphB4. In vitro findings showed that EphB4 receptor activation by ephrin B2 in human OA subchondral bone and chondrocytes positively impacts the abnormal metabolism of these cells [65, 66], suggesting that enhancing this system could lead to a protective effect on OA structural changes in these tissues.

In the musculoskeletal system, the ephrin-B2 ligand/EphB4 receptor system was first described for its ability to control bone remodelling [67]. Both ephrins and Eph receptors are cell membrane bound proteins and their interaction leads to a bidirectional (osteoblast/osteoclast) Eph/ephrin signalling, which through EphB receptors is considered forward and through ephrin B ligands reverse [67]. Ephrin B2, mainly expressed by osteoclasts but also by osteoblasts, and EphB4 receptors, expressed by both osteoblasts and osteoclasts [67-69], are involved in the control of bone homeostasis, in which EphB4 forward signalling favours osteoblast differentiation, whereas the reverse signalling through the ephrin B2 ligand leads to an inhibitory effect on osteoclast function. The overall outcome of such interaction favours bone formation [67].
In human OA cartilage, ephrin-B2 activation of EphB4 receptors inhibits the most important interleukins and MMPs involved in OA cartilage breakdown as well as PAR-2 [65]. Consequently, future therapeutics aiming at this ephrin system could in turn indirectly target PAR-2’s mechanism of action.

In recent years, an interesting hypothesis has been put forth that certain PARs could also function as receptors for some MMPs. Indeed, PAR-1 has been suggested to act as the MMP-1 receptor [70]. Moreover, MMP-13 was also suggested to produce its effect via a membranous receptor [71, 72]. Although the identity of this putative receptor remains to be determined, it is tempting to suggest that PAR-2 could be such a receptor.

**Activation of PAR-2 in osteoarthritic tissues**

During OA, the articular cells are exposed to numerous inflammatory proteases such as trypsin or mast cell tryptase, known also to activate PAR-2 [1, 2]. However, as these proteases are not considered the major proteases found in OA tissues, PAR-2 receptors are certainly activated by other in situ serine proteases in the articular tissues.

In OA tissues the serine proteases that are upregulated belong mainly to the plasminogen activator (PA)/plasmin system, and the urokinase PA (uPA) plays a major role [73-75]. This enzymatic system has been found to be upregulated in cartilage and subchondral bone. In the latter tissue, data from a study performed using an OA dog model showed that in vivo treatment that reduced subchondral bone remodelling and resorption was associated with a reduction in the level of the uPA [76]. Although highly speculative, one might hypothesize that PAR-2 may have been involved in this process. In fact, in cartilage, this enzymatic system, in addition to its macromolecular degrading action, has been found to be responsible for increasing the activation of proteases including collagenase [73, 77]. The capability of the specific PAR-2 activation to increase the levels of MMP-1 and MMP-13 in cartilage [52] strongly suggests the involvement of this serine protease system in the in situ activation of PAR-2. Moreover, in OA cartilage, PAR-2 activation also upregulated COX-2 production [52] whereas, interestingly, uPA was essential to COX-2-mediated breast cancer invasion cells [78].

It has been suggested that matriptase could also be an endogenous activator of PAR-2 in vivo in human skin [79, 80] and recently in articular tissues [11, 81]. This enzyme was shown to play an important role in tissue matrix degradation in arthritic diseases. More precisely, matriptase gene expression was significantly elevated in OA cartilage and the protein present in chondrocytes. This protease induced MMP-1, MMP-3, and MMP-13 gene
expression in cartilage. Matriptase synovial perfusion activates PAR-2, and matriptase-dependent enhancement of collagenolysis from OA cartilage was blocked by PAR-2 inhibition [11]. This enzyme also induces the release of proinflammatory cytokines in endothelial cells through PAR-2 activation and subsequently contributes to atherosclerosis progression [79].

HtrA serine peptidase 1 (HtrA1) is another protease which could be implicated in PAR-2 activation. In fact, HtrA1 substrate cleavage occurs at a specific site between arginine and serine, the same site at which trypsin cleaves PAR-2 receptors [1, 2]. This enzyme is known to be increased in OA tissues and to play an important role in cartilage degradation. Elevated HtrA1 levels were detected in synovial fluids obtained from rheumatoid and OA patients, with synovial fibroblasts identified as a major source of secreted HtrA1. Treatment of fibronectin with recombinant HtrA1 led to the generation of fibronectin degradation products that could be involved in cartilage catabolism. Interestingly, treatment of synovial fibroblasts with HtrA1 or HtrA1-generated fibronectin fragments resulted in the destruction of extracellular matrix through both direct and indirect mechanisms [82]. HtrA1 was also detected at high levels in OA cartilage [83]. Finally, Chamberland et al. [84] reported that the chondrocyte overexpression of a catalytically active form of HtrA1 induced a marked reduction in proteoglycan content. Likewise, aggrecan degradation fragments were elevated in conditioned media from the chondrocytes overexpressing active HtrA1. These findings suggest HtrA1 as a protease involved in proteoglycan turnover and OA cartilage degradation.

**Targeting PAR-2 for osteoarthritis treatment**

As data from in vitro human studies and in vivo animal studies strongly support the involvement of PAR-2 in OA pathophysiology, future therapeutic targeting of the cartilage degradation and subchondral bone alterations that occur during the development of OA could be achieved via an agent that specifically inhibits PAR-2 activation/synthesis. Indeed, specific inhibition could be attained by direct intervention by blocking its activation site, or indirectly by inhibiting its activating enzymes.

**Direct intervention**

One approach could be to directly antagonize PAR-2 activation by using a specific antagonist peptide, small interfering RNA (siRNA), or specific antibody. These possibilities have demonstrated high potency in inhibiting joint swelling in a mouse model [85]. More particularly, PAR-2 specific
A blocking peptide has already demonstrated its efficacy in inhibiting inflammation in arthritis [85], in renal failure [86], and in cellular and vascular responses [87]. Interestingly, one of the PAR-2 blocking peptides, K-14585, has shown various potencies including the inhibition of cell signalling such as the MAP kinase p38 phosphorylation and PAR-2-mediated p65 NF-κB phosphorylation and NF-κB DNA binding [88], factors implicated in proinflammatory pathways. However, care should be taken from these results since K-14585 could function as a PAR-2 antagonist or agonist [88].

TGF-β could also be of particular interest in neutralizing the PAR-2 receptor. Tsai et al. [53] showed that TGF-β inhibited, through three distinct pathways, the stimulation of PAR-2 expression by IL-1β in human OA synovial cells. Firstly, TGF-β inhibits PAR-2 activity by inhibiting IL-1β-induced p38 signal transduction; secondly, indirectly via MMP-13 inactivation; and thirdly, by inducing CTGF, a factor that represses PAR-2 expression. TGF-β could thus prevent OA progression by its ability to induce CTGF production, maintain extracellular matrix integrity, and downregulate PAR-2 expression.

**Indirect intervention**

PAR-2 activation could also be blocked by inhibiting the enzymes responsible for its activation. Efforts are currently being made to determine the endogenous serine proteases that could cleave PAR-2 at its activation site in order to generate the tethered ligand. As described above, several candidates have been proposed. Hidaka et al. [89] recently demonstrated in vivo that a novel protease inhibitor, gabexate mesilate, decreased the level of PAR-2 activity in rats affected by lung lesions induced by lipopolysaccharide (LPS) injection. Gabexate mesilate is a serine protease inhibitor used therapeutically in the treatment of pancreatitis [90], and showing antiinflammatory activity [91]. The use of such inhibitor should therefore be tested in experimental OA models for its effect on the OA inflammatory process alterations in cartilage, subchondral bone, and synovial membrane.

In addition, as previously discussed, other systems are able to modulate PAR-2 receptor production in human OA tissues. In this context, the system represented by the ephrin-B2 ligand and its specific receptor EphB4 could be an interesting therapeutic target to act indirectly on PAR-2. It has been shown that synthesis of ephrin-B2 remains unchanged in normal and OA subchondral bone osteoblasts, whereas the expression and production of the EphB4 receptors were higher in OA than normal cells [68], suggesting an attempt to restore normal extracellular matrix. Thus, the exogenous stimulation
**Figure 2.** Schematic representation of the role played by proteinase activated receptor (PAR)-2 in cartilage and subchondral bone in osteoarthritis. In osteoarthritic cartilage, PAR-2 can be increased in chondrocytes by IL-1β, TNF-α and TGF-β and activated by specific enzymes of the serine protease family. This induces Erk1/2 and p38 signalling pathways, which in chondrocytes results in the synthesis of catabolic and inflammatory factors (MMP-1, MMP-13, and COX-2) leading to cartilage breakdown. PAR-2 is also expressed in OA subchondral bone osteoblasts. It can be increased by IL-1β, TNF-α and PGE2 and its activation induces the phosphorylation of Erk1/2 and JNK signalling pathways, which in turn induce, on OA subchondral bone osteoblasts, the expression of membranous RANKL, thereby promoting osteoclastogenesis through RANK/RANKL interaction leading to increased bone resorption.

of EphB4 by a soluble ephrin-B2 could be an interesting approach since this system appears not sufficiently stimulated during OA. Consequently, this will induce an inhibition of PAR-2, and therefore the PAR-2-induced bone resorption in OA subchondral bone osteoblasts. Moreover, this strategy could also contribute to reducing angiogenesis, a process regularly observed in OA [55], since PAR-2 exerts proangiogenic effects [92, 93].

**Conclusion**

Much evidence supports the involvement of PAR-2 in the abnormal remodelling process that occurs in the major articular tissues in OA. PAR-2 activation can induce the synthesis of inflammatory mediators, proangiogenic factors and bone proresorptive molecules in OA tissues, providing a critical
link between inflammation and tissue remodelling and destruction (Figure 2). Although the initiating events of OA have yet to be identified, PAR-2 appears to be a good candidate as it mediates many of the pathophysiological pathways including the regulation of proinflammatory cytokines and catabolic factors. Thus, the use of direct or indirect PAR-2 inhibition strategies are pharmacological avenues that need to be further explored.

Moreover, the implication of PAR-1 in the development of OA should also be studied since it has been shown that the inhibition of thrombin, the main activator of this receptor, could reduce the development of arthritis in mice [42] and PAR-1 knockout mice showed reduced arthritis severity [94]. However, although some in vivo studies have shown the role of PAR-2 in OA, these were mostly using mouse models. Further validation should be performed in OA animal models using larger animals to ensure that they accurately reflect the link between PAR-2 and the evolution of OA as seen in humans.

References

PAR-2: A DMOAD target for osteoarthritis

5. Subchondral bone involvement in the pathophysiology of osteoarthritis

Daniel Lajeunesse

Osteoarthritis Research Unit, University of Montreal Hospital Research Centre (CRCHUM)

Notre-Dame Hospital, Montreal, Quebec, Canada

Abstract. A growing body of evidence suggests that the intimate link between the subchondral bone and overlying articular cartilage is not limited to physical contact but that physical and biochemical changes in one drive a response in the other. This chapter will review the fact that this intimate contact is not limited to biomechanical driven signals but extends to biochemical exchanges between the two tissues. Indeed, it is now recognized that a cross-talk must take place between them. Moreover, recent evidence also challenges our views concerning the role of subchondral bone in OA pathophysiology and the nature of changes in this tissue as driving OA progression and/or initiation. Studies on the pathophysiological changes that take place in OA subchondral bone tissue and how they influence articular cartilage will also be reviewed.

Introduction

Osteoarthritis (OA) can be described as a progressive loss of articular cartilage, appositional new subchondral bone formation and sclerosis of the
trabeculae and growth plate, formation of osteophytes, and an imbalance between cartilage loss due to matrix degradation and an attempt to repair this matrix [1]. Synovitis is often observed resulting from a response to the changes in hard tissues within the joint. In situ structural changes in subchondral bone during the course of OA can now be readily observed using imaging techniques. Indeed, increased subchondral bone activity, as judged by enhanced uptake of technetium labelled diphosphonate, can predict cartilage loss [2], whilst the absence of significant subchondral bone activity indicates less progression of cartilage lesions. Risk factors for OA include age, gender, genetic predisposition, mechanical stress and/or joint trauma, and obesity [3-8].

**Osteoarthritis changes in subchondral bone tissue**

Bone tissue integrity is maintained by a distinct equilibrium between mechanisms that either remodel or model bone. Bone remodelling includes the coupling of mechanisms that resorb bone and form new bone on a previously resorbed surface, whereas bone modelling is a mechanism that drives changes in the architecture and volume of bone via direct apposition to existing bone surfaces. In OA, all of these mechanisms may be altered at some point in time. Indeed, joint cartilage degeneration is associated with intensified remodelling of the subchondral bone and increased bone stiffness [9]. In general, OA patients have high body mass index and show a more highly preserved bone mass [10-13] independent of body weight [14].

Initially, OA patients were described as showing increased bone mineral density (BMD) as measured by dual X-ray absorptiometry, suggesting that new bone synthesis exceeds degradation in OA [15]. However, further studies have revealed a decrease in BMD based on thinning and loss of bone trabeculae [16]. Indeed, subchondral bone structure and organization is not uniform among different OA patients or as the disease progresses. The subchondral bone plate, which can be viewed as a cortical bone plate, and the subchondral trabecular bone also demonstrate specific alterations. In subsets of OA patients, the indices of bone resorption indicating loss of trabecular tissue indicated by the increase in cross-linked N-telopeptide of type I collagen (NTX) and C-telopeptide (CTX) [17], suggest a progressive loss of trabecular bone, not specifically of subchondral bone. Moreover, alterations in the microarchitecture of sclerotic bone in OA, described using microcomputed tomography (microCT), indicates that trabeculae had more plate-like structures than rod-like structures [18, 19]; alterations that would likely alter bone stiffness. Therefore, the sclerotic subchondral bone plate experiencing enhanced absorption of load-bearing stress could initiate a
Subchondral bone involvement in the pathophysiology of osteoarthritis

decrease in bone structure in trabeculae due to a reduction in load transfer to this tissue, leading to osteoporosis-like changes [20]. Since bone stiffness and BMD are not uniform in OA subchondral bone tissue [21, 22], these variations in the bone plate probably cause more damage to cartilage than any other parameters under normal conditions [23]. Modifications in OA subchondral bone stiffness and quality can result from microdamages, such as microcracks and submicroscopic cracks [24], and are a key condition for OA progression, as an accumulation of microdamages in this tissue was shown to be directly associated with OA [25]. In addition, although the subchondral bone in OA patients was considered to have a better bone mass, the observation that this tissue shows signs of calluses due to numerous microfractures [26, 27] indicates the opposite. Repetitive microfractures followed by incomplete healing in OA subchondral bone could promote stiffness of the bone, which would no longer be an effective shock absorber, and lead to a more generalized bone alteration, thus to increased apparent BMD or volume. Moreover, the observed association between osteophytes and femoral BMD suggests that the pathophysiology of OA could be linked to an alteration in bone formation [28]. The mechanisms could be linked to an abnormal response to growth signal due to altered levels of local growth factors or abnormal post-receptor signalling. Of note, insulin-like growth factor (IGF)-I, IGF-II and transforming growth factor β (TGF-β) levels were all shown to be elevated in iliac crest bone biopsies of patients with OA [29]. As this site is distant from weight-bearing joints, this finding suggests a generalized bone metabolic dysfunction in OA. The same group also reported elevated serum osteocalcin levels in women with hand OA, and elevated osteocalcin in cortical bone explants [30]. Since osteocalcin is an osteoblast marker, this would also suggest abnormal osteoblast function in OA. More recently, other investigators reported abnormal expression of phenotypic markers in OA subchondral bone osteoblasts [31-35]. Elevated endogenous TGF-β1 levels have also been described in these cells and are linked to their abnormal mineralization capacity [36, 37].

Magnetic resonance imaging (MRI) revealed the presence in OA patients of edema-like lesions, also called bone marrow lesions (BMLs). These lesions in addition to bone attrition are strong indicators of bone turnover as well as structural deterioration in knee OA [7, 8, 38]. BMLs predict an increase in knee cartilage loss in patients with or without knee pain and are informative of bone quality and indirectly of BMD. Indeed, BMLs are indicative of sclerotic bone that is poorly mineralized.

Such indication of undermineralization of OA bone was derived from different groups using different approaches. Some used levels of phenotypic markers of osteoblasts and of type I collagen content to determine that bone
explants from OA patients showed elevated levels of $\alpha_1$ chains compared to $\alpha_2$ chains, which appeared to be driven by elevated endogenous TGF-\(\beta1\) levels in these cells and reduced calcium content [32, 37, 39, 40]. Others reported that the organic content of OA bone was elevated whereas the inorganic portion was reduced even more so than in osteoporosis patients [41, 42]. Moreover, data showing that the volume fraction of trabecular subchondral bone was increased but that the bone tissue modulus was reduced at sites of cartilage defects associated with OA [43] indicate an increased rate of bone remodelling with incomplete mineralization.

Considering that OA bone tissue is undermineralized as in osteoporosis, the initial concept that the two diseases were mutually exclusive should be reconsidered. However, the mechanisms leading to the two pathologies may be different. The recent report by Bellido et al. supports the concept that alterations in trabecular bone such as those observed in osteoporosis may in fact promote OA. Indeed, in a rabbit model of osteoporosis induced by ovariectomy and glucocorticoids (OP) and in which OA was surgically induced at 7 weeks post-ovariectomy (OPOA), cartilage damage observed in OPOA rabbits was exacerbated compared to OA rabbits, suggesting that an increased remodelling of bone can promote cartilage damage [44].

Now, could OA be considered a systemic bone disorder? A number of data would support this notion. For example, the alteration in the remodelling of OA subchondral bone is a key event in the disease. We now know that in OA, phases of resorption are as important as appositional new bone formation and sclerosis [45, 46]. Imaging studies revealed that differences in the shape of the femoral head actually precede manifestations of clinical OA [47]. Moreover, accumulating data from MRI clearly show that BMLs are risk factors for OA progression [8, 38]. Data from in vitro studies also indicate that subchondral bone tissue isolated from OA patients is not uniform [33, 48, 49], which is reminiscent of the observation that OA patients show increases in BMD, yet analysis of their bone tissue shows reduced bone mineral content and increased osteoid volume as well as alterations in subchondral bone microstructure. Indeed, a low mineralization pattern can be observed in explants of the femoral heads of OA patients at autopsy compared to normal individuals [39, 41, 50]. In animal models of spontaneous OA, increased osteoid volume is often more severe than cartilage changes [51] as opposed to OA-induced models that initially show subchondral bone resorption followed by accretion. Moreover, in spontaneous OA animal models, the severity of cartilage fibrillation and loss generally exceeds subchondral bone changes only in advanced OA [51].
Changes in bone cells

Role of mesenchymal stem cells

Bone tissue integrity and microarchitecture may be affected in OA patients, suggesting that cellular activities may also be affected. Therefore, the initiation and/or progression of the OA process may be linked to abnormal formation and biosynthetic activity of cells derived from mesenchymal stem cells (MSCs) [52]. MSCs can give rise to osteoblasts, chondrocytes, myoblasts, adipocytes and tendon cells [53]; cells that are all affected in OA.

Inasmuch as MSCs could be involved in the OA process, this involvement could be based on alterations in cell proliferation or differentiation, or both. Indeed, MSC numbers, proliferation rate, population-doubling time and the capacity to differentiate into multilineage cells may be altered in OA [54-56]. However, conflicting results have been reported. A study showed that alterations in in vitro proliferation of bone marrow MSCs from OA patients were not significantly different from normal MSCs or their number of osteogenic precursors with age [57]. This is in sharp contrast to observations of osteoporosis patients. In contrast, Murphy et al. [56] indicated reduced proliferation rates of MSCs obtained from OA patients compared with healthy controls. However, the osteogenic activity of MSCs of patients with advanced OA was enhanced while their chondrogenic and adipogenic activity was reduced.

In OA individuals, differentiation into target cells may also be altered in vivo leading to abnormal tissue homeostasis [58]. This appears to be due to alterations in the response of MSCs to cytokines and growth factors [59, 60]. Importantly, this suggests that cells not presently residing in the affected tissue may actually play an important role in the future behaviour and homeostasis of bone tissue. Of note, the potential of MSCs isolated from synovial fluid to differentiate into multilineage cells is more elevated in OA than in rheumatoid arthritis patients [61], indicating that, although related, these two diseases may have profound diverging etiology and progression. The impaired chondrogenic and adipogenic capacity of OA MSCs [56], in addition to a possible reduction in myocytic capacity since muscle strength is reduced below normal age-related loss [62] possibly due to muscle cell dysfunction [63], suggests that OA MSCs either remain in an undifferentiated pool or differentiate into a limited number of lineage cells such as the osteogenic cells. Such a mechanism would lead to impairment observed in the OA joint tissues except for bone tissue. As it is becoming increasingly recognized that MSCs play an important immunoregulatory role in bone
tissue [64, 65], it is tempting to speculate that this role may be altered in OA individuals.

**Role of subchondral bone osteoblasts**

Alterations in osteoblast markers have been reported in situ [30] in OA patients and later confirmed in vitro [31, 66], which implies that the in vivo alterations are due to abnormal cellular metabolism, not to alterations in systemic regulation. However, this would not rule out that local autocrine/paracrine regulation may be important in the alterations observed in OA subchondral bone osteoblasts. In vitro, OA subchondral bone osteoblasts grow at a faster rate than normal cells [31], which is in accordance with their previously reported increased rate of proliferation [67]. It is also of note that OA osteoblasts show reduced apoptosis [67, 68]. Abnormal development of OA subchondral bone osteoblasts would also concur with the numerous reports on their abnormal phenotype [31, 34, 36]. In addition, the alteration in the key differentiation parameter of osteoblasts, namely mineralization, as mentioned above, also agrees well with the reported undermineralization of OA subchondral bone tissue [39]. Moreover, abnormal responses to parathyroid hormone (PTH), prostaglandin E2 (PGE2), IGF-1 and TGF-β1 have all been observed in OA subchondral bone osteoblasts, suggesting abnormal development of these cells. Yet, we still do not have any clues as to why the response to growth factors, hormones or eicosanoids is altered in these cells. An abnormal development possibly due to altered response to growth factors would agree with the recent hypothesis proposed by Aspden [69] that OA would be a pathological growth problem, not a problem of tissue decay, with excessive and poorly regulated growth of musculoskeletal tissues. In this context, cells would reach and/or revert to an abnormal developmental phenotype with a loss of proper function such that tissue integrity could not be attained.

**Control of subchondral bone tissue homeostasis**

The control of skeletal patterning, tissue remodelling and cell development involves a number of signalling molecules including TGF-β, bone morphogenetic proteins and Wingless proteins (Wnts). The potential role of TGF-β has been alluded to previously. In contrast, our knowledge of the Wnt pathway is limited. Zhu et al. [70, 71] recently demonstrated a key role of either an increase or a decrease in the Wnt β-catennin pathway in mouse cartilage and in the development of cartilage loss that could be associated with OA-like features.
Wnts are a conserved family of growth factors involved in numerous processes [72, 73]. Wnt3a and Wnt7b are among the most potent Wnt agonist ligands in bone tissue [73, 74]. As Wnt ligands act as stem cell growth factors [75], they could potentiate the recruitment of MSCs to a particular differentiation pathway such as the osteogenic pathway. Wnts can have positive or negative effects on osteogenic cells depending on their state of differentiation and the cellular context. Wnts signal via the canonical β-catenin and at least two non-canonical pathways, the diacylglycerol/protein kinase C (PKC) and the planar cell polarity (PCP) pathways [72, 76, 77]. The canonical pathway is considered the most important for osteoblast differentiation [76, 77]. Wnt ligands bind to the receptors Frizzled (Fz) in a promiscuous way, meaning different Wnts bind to different Fzs and vice versa [74, 77]. Following binding of Wnts, Fzs cooperate with a single-pass transmembrane molecule of the low density lipoprotein (LDL)-receptor-related protein (LRP)5, and 6 in vertebrates, and both receptors are required to initiate a Wnt signal. Five families of extracellular Wnt antagonists have been identified: secreted Fz-related proteins (sFRP), Wnt inhibitory factor 1 (Wif1), Cerberus, Wise and Dickkopfs (DKK). Upon Wnt ligand binding to Fz/LRP, the canonical signalling pathway is activated. Fz interacts with the cytoplasmic protein Dishevelled (Dsh) that becomes phosphorylated. The coreceptor LRP5/6 interacts with Axin via five phosphorylated PPP(S/T)P repeats of the cytoplasmic tail of LRP in response to Wnt binding. Glycogen synthase kinase 3 (GSK3) phosphorylates the PPP(S/T)P repeats that regulate the docking of Axin, while casein kinase 1-γ (CK1γ) phosphorylates multiple motifs close to the GSK3 sites. Once bound to their respective membrane receptors, Dsh and Axin cooperate to activate β-catenin. The stability of β-catenin is regulated via the destruction complex composed of the scaffold protein Axin, the tumour suppressor protein adenomatous polyposis coli (APC), and CK1α, δ or γ and GSK3α or β, which phosphorylate β-catenin in the absence of Wnt ligands. The kinase activity of the complex is inhibited when Wnt ligands activate the Fz/LRP coreceptors, increasing non-phosphorylated β-catenin in the cytosol, which then translocates to the nucleus to bind to the N-terminus of lymphocyte-specific enhancer element/T cell-specific transcription factors (LEF/TCF) and activate the transcription of Wnt target genes [78, 79]. Heterotrimeric PP2A, a protein phosphatase that inhibits serine kinases, is required to elevate β-catenin levels in response to Wnt. PP2A can bind to both Axin and APC, suggesting that it also dephosphorylates GSK3 substrates [80].

In vitro results with OA subchondral bone osteoblasts suggest that the canonical Wnt/β-catenin pathway is reduced compared to normal osteoblasts.
Yet, endogenous PGE$_2$, via an unknown pathway, partly rescues it in the high endogenous PGE$_2$-producing OA osteoblast group [81]. PGE$_2$ plays a similar role in colon cancer cells via the PGE$_2$ receptor2 (EP2) coupled to phosphoinositide 3-kinase and protein kinase Akt activation leading to an alteration in GSK-3$\beta$ dependent $\beta$-catenin phosphorylation [82]. It is also of note that non-steroidal antiinflammatory drugs (NSAIDs) such as aspirin have been shown to reduce the Wnt/$\beta$-catenin signalling pathway via stabilization of phospho $\beta$-catenin [83]. Moreover, DKK1 and DKK2 can both inhibit Wnt3a-induced osteoprotegerin production in osteoprogenitor cells [84] and, interestingly, our group previously reported that osteoprotegerin production was reduced in low endogenous PGE$_2$-producing OA subchondral bone osteoblasts [49]. These findings indicate that a functional intracellular machinery for the Wnt signalling pathway is present in OA subchondral bone osteoblasts as in normal cells. However, further studies will be needed to unravel which pathways are altered in OA that lead to the final reduction in Wnt signalling.

**Subchondral bone-derived putative factors driving cartilage alterations**

The concept of a possible cross-talk between the subchondral bone and articular cartilage was hampered by the absence of a route of communication between the two tissues. To permit such an exchange, identification of a vasculature invasion or microcracks had to be obtained. Whilst the subchondral bone is richly vascularized, it was long held that the hyaline cartilage is not. Articular cartilage is keyed into the subchondral bone plate. Situated beneath this thin end plate zone, the trabecular subchondral bone contains fatty bone marrow and trabecular bone. Arterial terminal branches, probably end arteries, are present in the subchondral bone plate and end in sinusoids of uneven calibre and of irregular distribution. A transverse sinus is formed of these sinusoids that terminate in venous radicles. Compressive or shearing forces can directly affect this venous plexus. Tiny vessels penetrate the subchondral bone plate and can invade the deeper zone of the calcified cartilage up to the tidemark. This vascular perfusion slowly declines in humans until approximately age 70 when it reaches a plateau [85]. This perfusion also declines individually within joints and in load-bearing areas. The blood flow in the subchondral bone is 3 to 10 times higher than in trabecular bone [86], which allows for approximately 50% of the glucose, oxygen, and water requirements of articular cartilage [87, 88]. The age-related decline in perfusion possibly leads to decreased nutrition in the deep layers of articular cartilage. The variation in this vascular supply with age...
influences the thickness of the subchondral bone end plate, along with body weight, location, function, and genetics. However, it is generally much thicker in the central weight-bearing area. Moreover, the normal subchondral bone tissue protects articular cartilage against damage caused by excessive load because although bone is harder than articular cartilage, it is also a better shock absorber. Indeed, articular cartilage deforms less over a stiff and dense bone structure whereas variations in stiffness or in density will deform it more. Locally, the observation of the elevated levels of hepatocyte growth factor (HGF) in OA subchondral bone tissue [89] suggests its effect on the neovascularization of this tissue as on other tissues [90-92]. Vascular penetration, a characteristic of OA, occurs through the tidemark, with vessels invading the more superficial, non-calcified articular cartilage [26, 93]. However, it is uncertain whether this neovascularization brings more oxygen to the cartilage layers because new vessels at the osteochondral junction are consistently associated with new bone formation in the form of osseous cuffs to the fibro-vascular channels. Consequently, as ossification advances through the deep layers of articular cartilage, the original osteochondral junction is obliterated [88, 94]. It is likely that this process leads to significant variations in oxygen tension and modifies the adaptive responses of the chondrocyte, triggering either repair or degeneration processes.

The recent histochemical studies demonstrating that the deep layer of hyaline cartilage is also vascularized imply that the hyaline cartilage can be nutritionally supplied via the subchondral bone in addition to the synovial fluid. Any microvascular damages affecting the venous circulation in the bony tissue could therefore cause alterations in chondrocyte function [95]; however, whether these damages are secondary to bone changes or directly cause bone changes in OA has not yet been investigated. A correlation between OA and cardiovascular disease risk factors exists [96-98], whereas the abnormal vascularization of OA tissues could initiate cartilage tissue damage [93]. The hypothesis that OA could be an atheromatous vascular disease as proposed by Conaghan et al. [99] is supported by the observation that leptin, which is also increased in OA patients in both serum and synovial fluid [100-103], increases arterial wall thickness, decreases vessel distensibility, and elevates C reactive protein levels [104], thereby contributing to abnormal vascular function in OA.

Despite the previous belief that the tidemark between articular cartilage and subchondral bone was impermeable, we now know that a cross-talk between cartilage and subchondral bone is possible and is considered an integral part of the disease process [105, 106]. Although not formulated in these terms, the hypothesis of a possible role for subchondral bone in the initiation and progression of cartilage degeneration, where increases in bone
mass and thickness might modify biomechanical properties that favour the appearance/progression of structural changes in the articular cartilage, was first proposed by Radin and Rose [107]. Nowadays, the progressive structural changes in the subchondral bone as OA progresses are considered part of the disease process. In turn, these changes include biochemical pathways involved in the homeostasis of both tissues and could therefore contribute to cartilage degradation [29, 31, 35, 36, 39, 108, 109].

Factors secreted by osteoblasts can directly modify chondrocyte differentiation [34, 35, 89, 108, 110]. Hence, locally produced cytokines/growth factors/eicosanoids could diffuse from subchondral bone tissue through the bone-cartilage interface and stimulate cartilage breakdown. Biological signals could be diffused through channels and fissures between cartilage and bone [87, 111, 112]. Microcracks have also been reported in the calcified layer of aging articular cartilage [26, 112], which could allow the transfer of humoral information from the subchondral bone region to the basal layer of cartilage. A direct assessment of chemical diffusion between the subchondral bone plate and articular cartilage was performed by Pan et al. [113] by measuring in situ sodium fluorescein diffusion between the two tissues in mice using an imaging method based on fluorescence loss induced by photobleaching. These results suggest that the subchondral bone and articular cartilage form a functional unit with both mechanical and biochemical interactions. They also suggest that biomechanical factors could influence and/or promote the diffusion of biochemical signals between the two tissues. Indeed, such a hypothesis was recently tested directly in vitro [114]. Isolated osteoblasts from porcine mandibular condyles grown in vitro were subjected to high-magnitude cyclic tensile stress and this modified their capacity to generate factors that in turn disrupted chondrocytes in co-culture systems. These chondrocytes showed altered type II and type X collagen, aggrecan, and cartilage oligomeric matrix protein production, and increased matrix metalloproteinase (MMP)-1, MMP-3 and MMP-13 genes, reminiscent of alterations also observed in OA chondrocytes. Therefore, these results indicate that mechanical constraints also influence the features of isolated osteoblasts and can modify their intrinsic capacity to produce factors that influence articular chondrocytes. Such an observation would also concur with the finding that OA osteoblasts obtained from sclerotic and non-sclerotic areas of tibial plateaus show different features [31, 34-36, 115] and affect chondrocytes differently in co-cultures [34, 35, 108]. Hence, the progressive alterations in bony tissue and articular chondrocytes in OA could therefore be explained by both biomechanical and biological factors. As an initial concept to explain OA pathology was based on the effect of biomechanical factors, these data offer an explanation as to how disturbed mechanical factors and
malalignment could impact osteoblasts from the subchondral bone plate and modify their capacity to alter neighbouring chondrocytes from the articular cartilage.

Conclusion

Recent research has contributed to furthering our knowledge that OA can no longer be considered a disease of a single tissue but is rather a whole joint organ failure. As multiple tissues are involved, we must rethink the causes of the disease and broaden our research scope to include affected tissues and their mesenchymal stem cells. In particular, since OA incidence and progression are linked with bone tissue changes including increased bone resorption and bone remodelling, we must consider mesenchymal stem cell recruitment and differentiation as part of the process. Lastly, if such a systemic regulation of stem cells and tissues exists, a cross-talk between the articular cartilage and the subchondral bone permitting chemical exchanges between the two tissues is also plausible and supported by recent data. However, we still need to acquire a better understanding of these changes and the biochemical signals between bone and articular cartilage. This in turn will help us to devise better therapeutics aimed at treating not only the consequences but the causes of OA.

References

34. Sanchez, C., Deberg, M.A., Piccardi, N., Msika, P., Reginster, J.Y., and Henrotin, Y.E. 005, Osteoarthritis Cartilage, 13, 979.
41. Li, B., and Aspden, R.M. 1997, J Bone Miner Res, 12, 641.
Subchondral bone involvement in the pathophysiology of osteoarthritis

60. Luyten, F.P. 2004, Curr Opin Rheumatol, 16, 599.
77. Clevers, H. 2006, Cell, 127, 469.
84. Fujita, K., and Janz, S. 2007, Mol Cancer, 6, 71.
Subchondral bone involvement in the pathophysiology of osteoarthritis

6. New comprehensive methods for the biomechanical analysis of knee osteoarthritis

Jacques de Guise, Neila Mezghani, Rachid Aissaoui and Nicola Hagemeister
Imaging and Orthopaedics Research Laboratory, University of Montreal Hospital Research Centre (CRCHUM) and École de Technologie Supérieure (ETS), Montreal, Quebec, Canada

Abstract. Although the pathogenesis of osteoarthritis (OA) results from a complex interplay of factors, there is a predominant role of mechanical features in the development and progression of knee OA. Biomechanical gait analysis providing quantitative information on the knee joint structure and motion is therefore important as it can offer new insights into evaluation of the OA knee joint during functional activities. This chapter will review the biomechanical data acquisition of OA patients during gait. The systems used to perform data acquisition in terms of motion, force, muscle activation and inertial capture will also be described, as well as the main results from the literature on how these biomechanical parameters are modified during OA. Finally, some techniques allowing for the classification of the findings from biochemical analyses of knee OA, which can be of great help in the disease diagnosis and treatment, will be presented.

Introduction

Osteoarthritis (OA) is the most common type of musculoskeletal disorder, and the knee is the most affected joint [1]. Although the
pathogenesis of OA is complex, systemic factors as well as local mechanical factors are of prime importance [2, 3]. Systemic factors include age, sex and racial characteristics. Mechanical factors, although of key importance in the etiopathogenesis of OA, act in association with the systemic factors. Among the mechanical factors, muscle weakness, joint injury, and obesity [4] are leading factors that account for the development and progression of the disease. Knee OA is also strongly linked to weakness of the quadriceps muscles [1, 5].

When treating OA, clinicians aim to reduce pain and improve function. When this fails, surgery remains the last option. However, to counter this fate and as stated by Hunter [3], we need to “change this paradigm to intervene when structural changes may be reversible.” This suggests that the focus should be on modifiable risk factors and to reassess, among other options, physiotherapeutic approaches. However, such approach needs to be properly considered using appropriate biomechanical assessment techniques.

In view of the major role of mechanical factors in the development and progression of knee OA, biomechanical gait analysis measuring mechanical loading during walking is important as it provides quantitative information about the structure and motion of the knee joint, which can offer new insights into its evaluation during functional activities. As a result, knee joint disease can be better identified, facilitating diagnosis and treatment.

Figure 1. Gait analysis recording equipment setup (Imaging and Orthopaedics Laboratory, University of Montreal Hospital Centre, Montreal, Quebec, Canada).
Biomechanical data acquisition

This section reviews the biomechanical data acquisition equipment and methods. Figure 1 represents a typical gait analysis setup. In brief, a subject walks on a force platform that records the ground reaction forces (GRF). Generally, active or passive markers are fixed onto the human body segments and viewed by a motion capture system that records their three-dimensional (3D) trajectories. Kinematic data such as 3D knee joint angles are estimated from these trajectories. These data combined with GRF and inverse dynamic models are then used to calculate joint moments in all three anatomical planes. Additionally, surface electrodes positioned on the subject collect electrical activity for specific muscular groups.

Motion capture

Motion capture systems are generally composed of optoelectronic cameras which track 3D coordinates from active or passive markers that are placed over standardized anatomical landmarks to identify body segments. The markers can be either active (CODA, Charnwood Dynamics, Marseille, France; Optotrak, Northern Digital Inc., Waterloo, ON, Canada) or passive (also called reflective) (Vicon, Los Angeles, CA, USA; Motion Analysis, Santa Rosa, CA, USA) and are positioned in locations that represent the action of the underlying joint [6]. Passive systems send out infrared light signals and detect the reflection from the markers using multiple video cameras (minimum 3 but 6-8 cameras are often recommended). Active markers are light emitting diodes (LED) that are powered and cabled, and each LED sends a pulse sequence. The pulses are recorded by three non-collinear cameras (Optotrak, Northern Digital Inc.; VisualEye, Phoenix Technologies Inc., Burnaby, BC, Canada) mounted on a fixed base.

Both passive and active marker systems require markers to be attached to the subjects. There are two approaches for the positioning of markers on the limbs [7]. One approach is to place markers directly onto the skin, usually over a bony anatomical landmark. The other is to fix a set of at least three markers to each limb segment (rigid body), either directly or placed on a rigid structure. Both of these approaches allow representation of the motion of the body segment, but are subject to skin movement artefacts when movements out of the sagittal plane have to be assessed. Several non-invasive knee attachment systems have been validated for gait applications, and have shown to reduce skin motion artefacts [8, 9]. Figure 2 illustrates an example of such an attachment system (KneeKG, Emovi, Laval, QC, Canada).
Figure 2. Attachment system developed and validated by the Imaging and Orthopaedics Laboratory, University of Montreal Hospital Centre, Montreal, Quebec, Canada. Semi-circular rigid ring is affixed between medial and lateral femoral condyle and tracks the 3D displacement of the femoral segment, whereas a semi-rigid plastic sheet is fixed along the longitudinal tibial axis and tracks the tibial segment [8, 10, 11].

Force platforms

The general designation given to the forces that cause movement is kinetics. The latter includes both internal and external forces. External forces come from the ground or from external loads. As body weight drops onto and moves across the supporting foot, vertical, anterior-posterior and medial-lateral GRF are generated on the ground that can be measured with appropriate instrumentation [6]. The measurement of the GRF is performed using a force platform, which a patient walks across. It consists of a non-deformable steel plate with transducers at its corners to convert an applied load into electrical output signals. The two most widely used force platforms are the piezoelectric based transducers (e.g. from Kistler Instruments,
Amherst, NY, USA) and the strain gauge-based platform (e.g., from AMTI Force and Motion, Watertown, MA, USA; Bertec Corporation, Columbus, OH, USA).

The force platforms are generally integrated into a walkway or an instrumented treadmill. The force platform positioned into a walkway below floor level provides a natural walking environment, but needs repetitive measurements in order to ensure the proper recording of a full stride. The treadmill is more convenient for gait analysis studies in a small area and allows control of several test conditions such as treadmill slope and speed. However, Riley et al. [10] reported that generally there is a subtle difference (i.e. an underestimate of two degrees) in the sagittal joint angular displacement between the treadmill and over ground gait data, but concluded that the magnitudes of these differences are all within the range of repeatability of measured kinematic parameters. Moreover, walking on the treadmill can initially be an unfamiliar experience, which in turn may influence the parameters being measured. Therefore, measurements have to be done after an adaptation time [11]. The GRF are frequently used in conjunction with numerical models and accurate joint centre location to compute the related rotator moments, centres of pressure and joint torques (moments at the joint).

Electromyography

Electromyography (EMG) is the analysis of the electrical activity of the contracting muscles. It is useful for detecting muscles that are or are not working, the on/off timing of the muscle activity, and the intensity or amplitude of the muscle electrical signals. EMG data acquisition is generally achieved using surface electrodes; however, the EMG recorded using skin surface sensors may not be very specific due to the interference from adjacent muscles. It can also be performed using fine wires inserted into the muscle of interest by hypodermic needles. The latter approach, although providing valuable local information regarding the targeted muscle activity, is invasive, uncomfortable, and can be painful.

Inertial systems

Inertial systems are composed of accelerometers, gyroscopes and magnetometers. Accelerometers are sensitive to the linear acceleration of the body, gyroscopes to the angular velocity of the body segment, and magnetometers to the direction of magnetic pole. The technology called MEMS (Micro Electro Mechanical Systems) helps to reduce the size and weight of these sensors in order to facilitate their use in human motion
studies. In general, a fusion algorithm that combines all triaxial sensor information enables the determination of the relative orientations of the body limb segments [12, 13]. However, although being low cost and portable for data monitoring, the current MEMS gyroscope technology is prone to drift problems, i.e. the angular velocity of the rigid-body is continuously drifting at a very low rate and this is mainly due to the terrestrial movement. These phenomena could cause errors in the estimation of the rigid-body orientation for long monitoring periods.

**Biomechanical characterization of knee OA**

**Spatiotemporal parameters**

The gait cycle is defined as the period from the heel contact of one foot to the next heel contact of the same foot. It is common to start the cycle (0% of the period) with the first contact so that the end of the cycle (100% of the period) will be the initial contact of the next cycle. The gait cycle can be divided into two parts: the stance phase and the swing phase. The stance phase is the period of time during which one or two feet are in contact with the ground, and lasts approximately 60% of the gait cycle. The swing phase is the period when one foot is not in contact with the ground and lasts approximately 40% of the gait cycle. The stance phase can moreover be divided into three sub-phases, namely the ipsilateral double support, the ipsilateral single support and the final contralateral double support periods. The swing phase is also divided into three sub-phases: the initial swing, the mid-swing, and the terminal swing (Figure 3) [6].

![Figure 3. The gait cycle.](image_url)
The general gait parameters, also known as spatiotemporal parameters, are the cycle time (or cadence), the stride length, and the walking speed (or walking velocity). The cadence may be measured by counting the number of individual steps taken during some known interval of time. Similarly, the walking speed can be measured by timing the subject during some known walking distance. The stride length can be determined directly by counting the number of strides during a walk of known distance, or indirectly by multiplying the speed by the cycle time. Spatiotemporal parameters are generally considered important in knee OA assessment.

**Walking speed**

Several studies have investigated whether patients with knee OA adopt a particular strategy regarding walking speed. Some studies determined that elderly patients had particularly lower walking speeds than the healthy control subjects of the same age group [14-20]. Furthermore, the gait speed tends to decrease with increasing Kellgren-Lawrence (KL) disease severity grade; i.e., patient groups with moderate OA (KL 3) and severe OA (KL 4) exhibited a significantly lower self-selected walking speed than the control group [17, 18]. Liikavainio et al. [21] investigated the effect of speed on the GRF at level walking for a group of elderly men with OA (KL 1-4) and a healthy matched control group. The most important findings of the former study [21] were that the differences in gait kinetics were minor in level walking at each predetermined gait speed in patients with knee OA compared with healthy age- and sex-matched control subjects. Moreover, no differences were reported in self-selected walking speeds between knee OA patients and healthy subjects [22-24].

**Stride length and cadence**

At a self-selected walking speed, knee OA subjects’ cadence and stride length are shorter compared to asymptomatic subjects [14-16, 25, 26], and the stride time and double support period increase accordingly. The overall stance phase is longer for knee OA subjects [17, 18, 27]. At a constant fixed walking speed, moderate OA patients walked with stride characteristics similar to the control subjects [27, 28]. This implies that the changes in stride characteristics result partially from a reduced gait speed as part of the adaptive mechanism of knee OA patients [21]. A further analysis, which considers gender in the analysis of spatiotemporal parameters, shows that although the normalized gait speed, normalized step length, and cadence did not differ between males and females, the males had a significantly shorter
stance and double support phase and a longer swing and single support phase than females [29].

**Kinetic data**

**Vertical loading rate**

Several studies have compared the GRF vertical component curves in knee OA patients and healthy subjects, leading to conflicting results as to the vertical loading rate (VLR), i.e. the slope of the GRF vertical component curve (Figure 4). Indeed, some studies reported that the VLR is higher in individuals with knee OA than in healthy control subjects [22, 30]. The increase, evaluated at 50%, occurs in conjunction with an increased intersegmental axial loading rate at all joints of the lower extremity [22]. However, others reported no difference between the VLR of hip or knee OA patients and those of healthy subjects during treadmill walking at a low speed [26]. Such differences between studies can be explained by the fact that the

![Figure 4](image_url)

**Figure 4.** Biomechanical data. Left: vertical force. Right: medio-lateral force (upper panel) and antero-posterior force (lower panel). Vertical units are expressed in percentage of body weight.
gait protocols differ in terms of walking speed. Yet, other studies showed lower knee OA loading rate and vertical peak parameters [15, 31]; at a self-selected speed, significant reductions in the GRF vertical component variables have been noted when comparing subjects with moderate OA (KL 2–3) and severe OA (KL 4) to a control group [20].

**Moments**

The external adduction moment at the knee is the resulting moment creating a knee adduction during the stance phase of gait [25]. It represents the load distribution in the tibiofemoral compartment. Its effect is due to the fact that, in normal people, the line of action of the resulting GRF is oriented medially from the knee centre [32]. Miyazaki et al. [33] have shown in knee OA patients with a follow-up of 6 years that the external adduction moment was significantly correlated with a decrease in intraarticular space ($r = 0.62; p<0.0001$). They also showed that if the adduction moment increased by 1%, the risk of intraarticular space reduction increased 6-fold. The adduction moment could then be used as an indicator of disease progression. However, caution must be exercised when interpreting the increase of the adductor moment as an increase in OA severity since the former is related to the walking speed due to inverse dynamic modeling approach.

**Impulsive loading and accelerometric data**

Impulsive forces in the knee joint have been proposed to be a co-factor in the development and progression of knee OA. During normal walking, impulsive forces are created in the foot-ground interface at heel strike. These forces travel up the lower limb as a shock wave, also known as the heel strike transient [34]. Impulsive loading and shock absorption by the skeleton during gait have been studied using skin (SMA) and bone (BMA) mounted surface accelerometers. In an in vitro accelerometric study (i.e. BMA), Chu et al. [35] reported a reduction of 5% in load attenuation capacity in a degenerative knee compared to a healthy one. Hoshino and Wallace [36] investigated the impact absorbing properties of the knee joint during longitudinal impulsive loads and found a significant decrease in the absorbing capacity of a degenerative knee using BMA techniques. Radin et al. [37], using accelerometers fixed on the lateral side of the shank and the thigh (i.e SMA), showed a significant difference in longitudinal tibial and femoral accelerations between painful and asymptomatic knees at initial foot contact. However, SMA induces important artefacts during locomotion activities in comparison with BMA. In a pioneered exploratory investigation, Lafortune et al. [38] quantified the
difference between the SMA and BMA techniques during a running task. They reported a substantial increase in magnitude of acceleration for SMA measurements compared to BMA at the tibial level. Although BMA techniques reduce skin movement artefacts, they are highly invasive for clinical use. Recently, Turcot et al. [39] developed an experimental numerical method of fixation of an accelerometer onto an exoskeleton which enables the transformation of the SMA measurements into estimated BMA measurements. This technique used inertial systems combined with a calibration technique as well as frontal X-ray of the knee to assess the linear acceleration of the internal tibial plateau as well as the internal femoral mid-condyle. Using this method, they reported [40] a statistically significant increase in linear acceleration of about 182%, 55%, and 163% in medial lateral internal tibial, medial lateral internal femoral, and anterior posterior internal femoral acceleration, respectively, for an elderly OA group (KL 3, 4) when compared to a matched healthy elderly group. This method has recently been used in therapeutic intervention, and its reliability and robustness was assessed in a repeatability study. Hence, in a controlled trial, data showed that therapeutic interventions (such as strengthening and proprioceptive exercises) had a beneficial effect of reducing the increase in femoral acceleration in the anterior posterior direction for the group with higher grade OA (KL 3, 4) [41]. This study demonstrated that the estimation of knee acceleration parameters is sensitive to changes in knee OA gait after rehabilitation. It also indicates that a three month treatment which combines strengthening and proprioceptive exercises could have beneficial effects on knee OA pain reduction by increasing anterior posterior knee stability and minimizing joint loading transmission during gait which reduces pain during walking [42].

**Kinematic data**

Kinematics of patients suffering from knee OA has been studied mostly in the sagittal plane [14, 15, 17, 25, 29, 43-45]. Investigators compared specific parameters of the gait cycle, such as knee angle at heel strike, maximum knee angle during loading, minimum knee angle at the end of the single support phase, maximum knee angle during the swing phase and range of motion during the gait cycle. The authors generally agree that OA patients walk with a reduced maximum flexion angle during the swing phase [14, 15, 17, 44] and reduced range of motion during the whole gait cycle [17, 25, 30].

Results on kinematics in the frontal and transverse plane are scarce. Tibial rotation has been studied only in a static position with ultrasound
imagery [46] at 20° of knee flexion. Nagao et al. [46] reported that OA patients tended to reduce internal rotation and that this effect increased with disease severity. Results obtained during a movement from 20° to 5° (similar to the unipodal phase during gait) led to the conclusion that “…osteoarthritis knee joint then moved more like a simple hinge joint” denoting that the screw-home mechanism was no longer present in these knees.

Some authors studied frontal plane kinematics. Indeed, varus/valgus malalignment, in addition to external adduction moment, has been recognized to predict OA progression [1, 47]. Varus/valgus malalignment is measured on a pangonogram (hip-knee-ankle film) as the angle between the longitudinal axis of the femur (aligning the centre of the femoral head with the centre of the knee) and the longitudinal axis of the tibia (aligning the centre of the knee with the centre of the ankle). Sharma et al. [48] studied knee OA progression and showed that a varus malalignment was associated with a 4-fold increase in the risk of joint space narrowing and correlated with pain. This study also showed that a malalignment greater than 5° was related to a 3-fold increase in risk of functional impairment.

In 2004, Chang et al. [49] defined the varus thrust as the “visualized dynamic bowing-out of the knee laterally, i.e. the abrupt first appearance of varus (or the abrupt worsening of existing varus) while the limb is bearing weight during ambulation, with return to a less varus alignment during the non-weight-bearing (swing) phase of gait.” These authors [49] performed a qualitative assessment of the varus thrust using a videotaping technique during level walking and data showed that varus thrust was present in 17% of cases. In a recent 3D kinematic study on OA patients with varying degrees of knee OA using an external attachment system (KneeKG, Emovi) allowing precise and repeatable measurement of knee kinematics in all three anatomical planes, an excellent correlation between mechanical knee alignment and abd/adduction movement during gait was found [50]. Moreover, this study also showed that frontal plane kinematics evolved with severity of the pathology towards adduction [51].

**Electromyography**

The electrical signal associated with the contraction of a muscle is called an electromyogram (EMG). Voluntary muscular activity results in an EMG that increases in magnitude with the tension [52]. Periarticular muscle weakness at the knee complex may also cause OA [53]. Although weakness in the quadriceps muscle is common in patients with knee OA [5], it has generally been considered a consequence of the pain that occurs with loading of the affected joint, leading the patient to minimize load bearing, in turn
leading to disuse atrophy of the muscle [54]. However, the extent to which the muscle weakness in OA subjects may be associated with pain or indirectly with the effect of chronic pain disuse and muscle atrophy is not clear [55].

Given the role of muscles in influencing knee joint load and knee instability, an understanding of deficits in muscle function associated with knee OA becomes important. Three aspects of muscle deficits have been considered: muscular strength, muscle-activation patterns, and proprioception. In general, quadriceps strength is measured by the torque developed at the knee level and reported in Nm. However, strength also varies with body size and it should be reported in a normalized form as a percentage of body weight. Patients with knee OA are 20% to 40% weaker in relative quadriceps strength than healthy controls [56]. Muscle strength deficits are generally associated either with muscle fibre atrophy or inhibition of the ability to activate the muscle. Ikeda et al. [57] reported a 12% decrease in the cross-sectional area of the quadriceps in women with signs of OA compared to controls. Using surface EMG data from vastus lateralis and medialis, lateral and medial hamstring and gastrocnemius muscles, Hubley-Kozey et al. [58] analyzed three groups consisting of asymptomatic subjects and moderate and severe OA patients during gait analysis. They found a significant effect of pathology on four muscle co-activation patterns between these groups. They concluded that muscle co-activity provides additional information related to OA severity.

Knee joint proprioception is essential in the coordinated activity of surrounding muscles. It is generally measured by the sense of joint position. Deficits in proprioception have been found in knee OA subjects. However, the link between proprioception, physical function, and pain is still not clear [56]. There is some evidence that quadriceps weakness precedes the onset of knee OA and hence could increase the risk of disease development, particularly in women. Thus, quadriceps strengthening exercises may play a role in the earlier prevention of OA development in women. Turcot et al. [40] also showed that a three month treatment combining strengthening and proprioceptive exercises could be beneficial in knee OA pain reduction by increasing anterior posterior knee stability and stabilizing joint loading transmission during gait. In fact, the authors [40] found that subjects with moderate OA (KL 1 or 2) increased their isometric quadriceps hamstring ratio by 16% whereas in severe OA (KL 3 or 4) subjects, the latter ratio was limited to an 11% increase. Care must be taken, however, because there is limited evidence that stronger muscles protect against OA progression in persons who have established disease [56].
Asymptomatic and osteoarthritic knee biomechanical data classification

Biomechanical data classification methods aim to distinguish between the asymptomatic and pathological OA knee groups, using pattern classification. There are two major steps in pattern classification: feature extraction and category assignment. The goal of feature extraction is to transform the original data (i.e. spatio-temporal, GRF, kinematics) obtained from gait analysis to a vector quantity which represents individual subject behaviour. The aim of category assignment is to classify each feature which represents an individual subject into one of the two groups (asymptomatic or OA) based on the similarity of the feature with the corresponding group. For many studies, feature extraction represents an important issue, because it is crucial that the gait pattern representation bears as much relevant information as possible to allow proper classification [59-66]. In general, two types of features can be distinguished in gait pattern representation: a local feature which characterizes a gait pattern by parameters measured at specific instants of time on the biomechanical data curve, and a global feature, which, in contrast, corresponds to a global parameter computed throughout the whole data curve. Both local and global features have been used for the classification of asymptomatic and OA knee biomechanical data. For instance, pathological and healthy gait patterns obtained from force platforms were discriminated by a neural network model using a feature vector which contains ten parameters extracted from the vertical GRF [59]. The accuracy of the classification was about 80% [59]. In several other studies, the discrimination of knee OA from normal subjects used the Dempster-Shafer theory (DST) of evidence [60, 61, 63]. The DST transforms the input characteristics into a set of three belief values: a level of belief that a subject has OA knee function, a level of belief that a subject has normal knee function, and an associated level of uncertainty. The subjects’ classification uses a combination of the evidence from all the features. The DST studies have investigated various characteristic features: the cadence, the magnitude of the peak vertical GRF, and the knee range of motion in the sagittal, frontal, and transverse planes. The accuracy of the DST method was equal to 96.7% [60]. In a hybrid version of the latter study, principal component analysis (PCA) was used as a data reduction tool in conjunction with the DST to improve the level of accuracy to reach a value of 97% [63]. Although the use of local features in earlier studies helped to assign individuals to an asymptomatic or OA group, this method fails to detect different levels of severity of OA. Recently, Mezghani et al. [65] developed a global feature representation of the individual associated with a nearest neighbour...
classification method to help distinguish asymptomatic from OA knee gait pattern. The global feature contains 100 parameters that represent the coefficient of the wavelet decomposition of the vertical, anterior-posterior as well as the medial-lateral GRF. Using this global feature, the authors [65] reported an accuracy level of about 90%. Although this value, 90% accuracy, seems lower than that found in a previous report [60], it helps to distinguish between the severity of OA. In fact, the OA severity was further divided into two categories, resulting in a hierarchical classification [65]. The OA patients were grouped into two OA severity categories according to the KL scale: KL grades 1, 2 for the first category, and grades 3, 4 for the second one. The latter method enables the classification of OA graded 1, 2 and OA graded 3, 4 with an accuracy of 77%. This result is in agreement with a recent study by Sen Köktas et al. [66] who reported an accuracy level of 80% with OA patients divided into four categories of severity including asymptomatic. A global representation has also been used for feature characterization of knee OA accelerometric tibia signals using classical Fourier decomposition [64]. Although the accuracy was not reported in this study, the vertical acceleration component, which describes the instability and the alteration in the transmission of shock during walking, was found to be a potent discriminant of knee OA patients from asymptomatic subjects.

The above studies demonstrated the validity of both the features and the classifiers for automatic classification of asymptomatic and OA knee gait patterns as well as for analysis of OA severity. The high classification accuracies, sensitivities, and specificities demonstrate that the developed classification methods can be used to support orthopaedic surgeons when making clinical diagnoses of OA.

**Impact of the improved biomechanical knowledge of osteoarthritis on clinical outcome**

Biomechanical knowledge has grown exponentially in the last decade. Many highly sophisticated techniques such as 3D magnetic resonance imaging have been developed that allow a very precise assessment of knee kinematics, contact zones of the cartilage, and quantification of cartilage loss (see Chapter 8 – *Quantitative magnetic resonance imaging in the evaluation of structural changes in knee osteoarthritis patients*). All these techniques bring valuable information about the pathogenesis of OA to the scientific community.

Clinical assessment allows diagnosis and symptom assessment, whereas biomechanical assessment provides insight into knee function. Research has shown a relationship between altered biomechanics and degenerative changes
New comprehensive methods for the biomechanical analysis of knee osteoarthritis

in the knee, which in turn raises questions about the protective role of the joint structures during functional tasks. Nevertheless, it is important to acknowledge that different types of data can be acquired in gait analysis and that each type of data provides different information on biomechanics or limb function. For example, spatiotemporal parameters may indicate how patients adapt their gait pattern to pain and disease. Moments and vertical force measurements help to interpret this adaptation in terms of movement strategies. Kinematic data, in turn, seem to be appropriate for monitoring gait adaptation over time and to identify abnormal movements that could be corrected by proper physiotherapy. However, EMG data are necessary to identify the ‘guilty’ muscles [7].

The time has now come to integrate biomechanical with clinical assessment. It is recognized that this could be valuable for the objective assessment of treatment options (either conservative or surgical). Biomechanical assessment also allows an understanding of the underlying factors causing the progression of the disease. The modifying factors are those that are targeted by biomechanical measurements, even before the disease has caused irreversible damage to the joint. Thus, interventions can be developed that address this specific question: Is it possible to change joint biomechanics before it is too late and, if so, how should it be done?

One remaining problem is the applicability of current biomechanical techniques to the clinical context. In modern society, reduction in health costs is an ongoing concern. Therefore, there is a strong need to develop technologies in addition to computed tomography scans and magnetic resonance imaging that are capable of providing precise, complementary, and value-added information. The use of low cost gait analysis is very promising. However, some researchers still point out the difficulty of interpreting such an overwhelming amount of information as that provided by gait analysis. This could be overcome by the use of computer-aided systems to assist with recognizing patterns within very complex sets of data. Techniques originating from the pattern recognition field, such as neural networks, fuzzy logic, and PCA, could be used to reduce data dimensionality and facilitate the classification of massive data sets in terms of movement patterns.

In the coming years, the research agenda in this field could be three-fold: firstly, to assess knee biomechanics pre- and post-treatment (physiotherapy and surgery) and compare different treatment options; secondly, to integrate biomechanical assessment within clinical guidelines and clinical practice; and finally, to improve automatization of multidimensional and complex data analysis in order to assist clinical interpretation of results.
References

7. Biomarkers in osteoarthritis

Lukas M. Wildi¹ and Giorgio Tamborrini²

¹Osteoarthritis Research Unit, University of Montreal Hospital Research Centre (CRCHUM)
Notre-Dame Hospital, Montreal, Quebec, Canada
²Department of Rheumatology, University Hospital Zürich, Zürich, Switzerland

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
Biomarkers in osteoarthritis

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,
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)
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 hyper-intense 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 2.** Structural changes in osteoarthritis depicted by ultrasound.

<table>
<thead>
<tr>
<th>Joint structure</th>
<th>Typical changes in osteoarthritis</th>
<th>BIPED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>Osteophytes</td>
<td>BD</td>
</tr>
<tr>
<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>
<tr>
<td></td>
<td>Loss of homogeneity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loss of anechogenicity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Irregularities of the anterior and posterior margins</td>
<td></td>
</tr>
<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>
</tr>
<tr>
<td></td>
<td>Synovial effusion</td>
<td></td>
</tr>
</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].
Biomarkers in osteoarthritis

**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>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hip: P</td>
</tr>
<tr>
<td>CTX-I</td>
<td>Serum and urine</td>
<td>Type I collagen degradation</td>
<td>Knee: BDP</td>
<td>Hip: -</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>Serum</td>
<td>Anabolic bone turnover</td>
<td>Knee: BPED</td>
<td>Hip: -</td>
</tr>
<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>
<td></td>
<td></td>
<td></td>
<td>Hip: B</td>
</tr>
<tr>
<td>CTX-II</td>
<td>Urine</td>
<td>Type II collagen degradation</td>
<td>Knee: BPED</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hip: BPD</td>
</tr>
<tr>
<td>Coll 2-I and Coll 2-I NO₃</td>
<td>Serum and urine</td>
<td>Type II collagen degradation</td>
<td>Knee: DBP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hip: D</td>
</tr>
<tr>
<td>CS846</td>
<td>Serum</td>
<td>Cartilage aggrecan synthesis/turndover</td>
<td>Knee: P</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hip: -</td>
</tr>
<tr>
<td>Keratan sulfate</td>
<td>Serum</td>
<td>Aggrecan degradation</td>
<td>Knee: BPED</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hip: -</td>
</tr>
<tr>
<td>PIILANP</td>
<td>Serum</td>
<td>Type II collagen synthesis</td>
<td>Knee: BPD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hip: -</td>
</tr>
<tr>
<td>PIICP</td>
<td>Serum</td>
<td>Type II collagen synthesis</td>
<td>Knee: D</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hip: B</td>
</tr>
<tr>
<td>TIINE</td>
<td>Urine</td>
<td>Type II collagen neoepitope</td>
<td>Knee: BP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hip: none</td>
</tr>
<tr>
<td>Multiple tissues</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></td>
<td></td>
<td></td>
<td></td>
<td>Hip: -</td>
</tr>
<tr>
<td>COMP</td>
<td>Serum</td>
<td>Cartilage degeneration</td>
<td>Knee: BPD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hip: BPD</td>
</tr>
<tr>
<td>HA</td>
<td>Serum</td>
<td>Increased HA turnover</td>
<td>Knee: BPED</td>
<td></td>
</tr>
<tr>
<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>Knee: BE</td>
<td></td>
</tr>
<tr>
<td></td>
<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>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hip: -</td>
</tr>
<tr>
<td>Synovium</td>
<td>Gle-gal-PYR</td>
<td>Urine</td>
<td>Collagen fibril degradation in synovium</td>
<td>Knee: BD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hip: -</td>
</tr>
</tbody>
</table>

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 neopeptiopes of the ¼ 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 neoeptiope 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/PIICP [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 neoeptiope 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 neoeptiope 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
Biomarkers in osteoarthritis

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 PIICP) diffuse into the synovial fluid and subsequently into serum. PIIINP and PIICP 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
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

Biomarkers in osteoarthritis


Biomarkers in osteoarthritis

80. Alexandersen, P., Karsdal, M.A., Byrjalsen, I., and Christiansen, C. 2011, Climacteric, 14, 236.
8. Quantitative magnetic resonance imaging in the evaluation of structural changes in knee osteoarthritis patients

Jean-Pierre Raynauld
Osteoarthritis Research Unit, University of Montreal Hospital Research Centre (CRCHUM)
Notre-Dame Hospital, Montreal, Quebec, Canada

Abstract. The quantitative evaluation of knee osteoarthritis (OA) cartilage damage and other joint structural changes and their progression over time has become a reality with the use of magnetic resonance imaging (MRI). Although anatomical changes could be seen by this and other radiological means, quantification of the cartilage alterations has been a real challenge for many years. Quantitative MRI (qMRI) assessment of cartilage volume/thickness with fat-suppressed gradient echo sequences and digital post-processing techniques offers high accuracy and adequate precision for studies in OA patients, and robust acquisition protocols for multicentre trials are now available. qMRI provides reliable data on cartilage volume throughout all the compartments and topographical areas of the knee in addition to relevant information on other knee tissue structures. By using MRI, studies have revealed strong correlations between cartilage volume loss and meniscal damage and subchondral bone edema, which are
now considered important risk factors for OA progression in addition to some clinical factors. These advances in MRI technology have enabled the evaluation of knee joint damage and OA progression over time in both cross-sectional and longitudinal studies. Moreover, qMRI can now be used in human clinical trials to evaluate treatment response to disease modifying OA drugs. Therefore, using qMRI as a tool in OA trials offers the further benefits of substantial reduction in number of patients needed for a study and reduced overall study length resulting in improvement of patient retention, compared to using standardized radiographs. Of great interest, there is now fully automated qMRI assessment for some of the knee OA structures including cartilage volume, osteophytes and synovial effusion.

Introduction

Assessment of structural damage of the articular cartilage is important for monitoring the progression of osteoarthritis (OA) and evaluating therapeutic response. For many years, clinical studies of drug interventions on symptomatic knee OA have focused mainly on clinical parameters such as pain and joint function, using self-administered questionnaires, but without assessing the effect of treatment on joint structural changes caused by the disease or the role of treatment in preventing cartilage degradation. However, although attempts have been made to evaluate cartilage damage and its progression in OA using radiographs or arthroscopy, these means showed major limitations.

Firstly, serial radiographs of affected joints were used for documenting the progression of OA over time [1] but the sensitivity to changes in the articular structure needed improvements in the standardization and interpretation of radiographs. Although such improvements have produced good measurements of joint space width (JSW) and the progression of joint space narrowing (JSN) [2, 3], the sensitivity to change requires a minimum follow-up of 2 to 3 years and a large number of patients (at least 1,500 for a two-arm study) in order to establish the effect of a pharmacological intervention on OA progression. Moreover, JSW is dependent on the integrity of the surrounding tissues, especially the meniscus and the subchondral bone, preventing it from acquiring information solely on the cartilage changes. For example, enucleation of the medial meniscus of the knee, which may occur during longitudinal studies, can significantly affect the JSW value and this measurement’s reliability [4]. In turn, this could also impair its use in the assessment of cartilage degradation over time. Furthermore, JSN progression provides only one measurement point, which considerably restricts the statistical power of this technique, in addition to giving no indication of the cartilage volume but only a measurement of the space between the condyle
and the tibia bones. The other means, arthroscopy, although shown to reliably and sensitively assess cartilage changes at one year [5], enables only the cartilage surface to be evaluated. This method is also semi-quantitative and, above all, invasive. Large studies using this tool are, therefore, difficult to conduct.

Magnetic resonance imaging (MRI) allows precise visualization and assessment of joint structures such as cartilage, bone, synovium, ligaments and menisci and their pathological changes. MRI acquisitions are non-invasive and non-radiant, providing a clear advantage over arthroscopy and fluoroscopy.

**Clinical practice for MRI acquisition of the knee**

The use of a 1.5 Tesla (T) or 3.0 T magnet is nowadays mandatory for quantitative evaluation of cartilage volume. The MRI acquisition of the knee is performed with a knee coil, also called an extremity coil because most are compatible with ankle acquisition. This coil entirely surrounds the knee, providing a more homogeneous signal and better image quality (Figure 1).

![Human knee cartilage in sagittal view acquired with a 1.5 Tesla magnet using a fast imaging at steady state precession (FISP) acquisition with fat suppression. This acquisition sequence produces maps with the highest cartilage contrast. Cartilage interfaces are delineated.](image)

**Figure 1.** Human knee cartilage in sagittal view acquired with a 1.5 Tesla magnet using a fast imaging at steady state precession (FISP) acquisition with fat suppression. This acquisition sequence produces maps with the highest cartilage contrast. Cartilage interfaces are delineated.
The patient lies on the table in supine position and is inserted feet first in the magnet. The coil is not centered in the magnet but is shifted to the side of the pathologic knee for better patient comfort. Sagittal slices of about 1-1.5 mm are at present used for cartilage volume quantification. Throughout any longitudinal study, the field of view is constant to preserve the pixel resolution and is fixed between 140-160 mm. To allow the best quality, a $512 \times 512$ matrix is used for a final $0.3125 \times 0.3125$ image resolution [6]. A fast-imaging acquisition technique preserving short repetition time is also used to ensure an acceptable acquisition time. If the protocol is correctly set up, the main cause of artefact is the movement of the patient, which can result in the sequence being rejected during quality control assessment. Proper immobilization of the knee in the coil is a major key to prevention of patient movement.

Advances in MRI technology have led to significant improvement in spatial resolution and contrast, enabling researchers to evaluate anatomical damage of all the joint structures across both cross-sectional and longitudinal planes. For cartilage volumetry, gradient echo sequences are preferred to spin echo and are configured in T1 weighted acquisitions. The most commonly used gradient echo sequences are the FLASH (fast low angle shot), spoiled GRASS (gradient recalled acquisition at steady state), SPGR (spoiled gradient recalled), FISP (fast imaging at steady state precession), or DESS (double echo at steady state). Moreover, 3D sequences are preferred to 2D sequences for the spatial continuity of the signal providing better coherence between the slices and reducing variability at reading time. To reduce partial volume artefact due to the shape of the joint, sagittal acquisitions are most commonly used when cartilage volumetry is performed on the global knee. However, for studies focusing only on the tibiofemoral portion of the joint (excluding the posterior condyle), coronal acquisitions are suitable. Additionally, fat suppression is required to provide a sufficient dynamic range to the image contrast to delineate the cartilage, but also to eliminate chemical-shift artefacts, which arise at the cartilage-bone interface. This is accomplished either by spectral fat-saturation (FS) using a prepulse tuned to the resonant frequency of fat or by frequency selective water excitation (WE). Acquisition times are generally shorter for selective WE protocols than for those using FS, as the latter requires an additional pulse at the beginning of the sequence.

**Knee cartilage volume quantification**

Although structural cartilage changes can be seen with the above acquisitions, quantification of these changes has been the real challenge for many years. Initial attempts to quantitatively measure cartilage volume were
performed only in healthy subjects [7] or in animal models [8]. The recent improvements in image analysis have led to the reliable quantitative measurement of cartilage volume and thickness in both normal and disease conditions such as in OA. Cartilage volume quantification of the complete knee joint (femur and tibia) is now used for determining changes in this tissue over time (Figure 2) [9]. Research teams are now using the abovementioned specific MRI acquisitions combined with computer software to obtain valuable information on cartilage volume in normal and OA subjects [10-13]. Moreover, standard cartilage views can be anatomically segmented, allowing for the evaluation of cartilage volume and thickness in anatomical subregions as well as specific focal defects [9].

The reliability and precision of quantitative MRI (qMRI) assessments of any given radiological centre are first established with the use of phantoms

![Figure 2](image.png)

**Figure 2.** Representation of grey-coded images of human osteoarthritic knee cartilage volume. Cartilage thickness was defined as the Euclidian distance between the bone-cartilage interface defined by the baseline image and the cartilage-surrounding tissue interface. Each thickness value was measured. A typical data set provides approximately 60,000 reading measurement points for the femur and 40,000 for the tibia. The volume is defined as volume between the bone-cartilage interface offset-map and its corresponding cartilage-synovium offset-map. Change in knee cartilage volume is obtained by subtracting follow-up cartilage volume from baseline volume. Maps showing the difference between baseline and one-year acquisition are displayed for femur and tibia and decreased thickness was seen mostly in the medial condyle and tibia.
mimicking human tissue interfaces. Several acquisitions of these over short periods of time are used to assess the precision of both image acquisition and data extraction. These phantoms are also useful to assess any drift of the MR signal over a long time period combined with periodical machine maintenance. The principal issue is the assessment of distortion of the MRI equipment.

There have been demonstrations of the precision and reliability of the MRI technology for the assessment of change in cartilage volume of the knee over time in OA patients. For example, Eckstein et al. [14] published a study on precision errors in healthy volunteers under short term imaging conditions (acquisitions taken one after the other with joint repositioning), long term imaging conditions (acquisitions taken approximately over 9 months, but post-processed immediately one after the other), and re-segmentation (post-processing) of the same data sets spaced over 12 months. They found that long term precision errors (1.9 to 3.9 coefficient of variation [CV%]) were not significantly larger than short term acquisition errors (2 to 3.6 CV%). In addition, no systematic drift was observed, suggesting that scanner conditions had remained stable throughout this period. However, in this study semi-automated re-segmentation errors were somewhat higher over time. Further, excellent inter- and intra-reader reliability of such semi-automated MRI technology to quantify cartilage volume/thickness in patients with knee OA has been demonstrated by our group [13]. The objectives were to assess measurement reliability by determining the differences between readings of the same image made by the same reader two weeks apart (test-retest reliability), determining the differences between the readings of the same image by different readers (between-reader agreement), and determining the differences between the cartilage volume readings obtained from two MRIs of the same knee acquired a few hours apart (patient positioning reliability). MRI examinations of the knees of normal subjects, patients with different stages of symptomatic knee OA, and a subset of duplicate images were independently and blindly quantified by three readers using the imaging system. Between-reader agreement of measurements was excellent, as shown by intra-class correlation (ICC) coefficients ranging from 0.958 to 0.997 for global cartilage, 0.974 to 0.998 for the compartments, and 0.943 to 0.999 for the femur. Test-retest reliability of within-reader data was also excellent, as was patient positioning reliability, with Pearson correlation coefficients ranging from 0.978 to 0.999 and from 0.978 to 0.999, respectively.

**Cross-sectional quantitative cartilage volume measurement**

Estimates of cartilage thinning during normal aging (in the absence of OA) were derived from cross-sectional data obtained from healthy elderly
subjects without history of knee joint symptoms, trauma, or surgery (50 to 78 yrs; 11 men, 12 women) relative to a cohort of young, healthy subjects that met the same criteria (20 to 30 years; 49 men, 46 women) [15]. The authors reported an estimated 0.3% to 0.5% reduction in cartilage thickness per annum for all knee compartments. In the patella, women displayed a higher estimated loss than men, but no gender difference was found for the other compartments of the knee. Burgkart et al. [11] determined cartilage volume in OA patients prior to total knee replacement and estimated the loss by comparison with a group of healthy volunteers. They reported a difference of approximately 1300 mm$^3$ in the medial tibia in patients with varus OA, and differences of approximately 1800 mm$^3$ in the lateral tibia in patients with valgus or bi-compartmental OA. These values were found to exceed the precision error in the tibia of healthy volunteers and OA patients by a factor of >20:1. Recently, however, extensive age- and gender-specific reference data on normal volunteers have been published [15, 16], providing T- and Z-scores for the OA population, as currently used in the diagnosis of osteoporosis. One problem with this approach, however, is the relatively large inter-subject variability of cartilage volume in healthy individuals. Because of a weak correlation between cartilage volume and body height and weight but a much greater one with bone size [17], it has been suggested that cartilage volume should be normalized to the original bone interface area (before the onset of disease) to achieve better discrimination between OA patients and healthy subjects.

Optimization of cross-sectional analysis is particularly important for patient inclusion in longitudinal trials. Small cartilage volume alone does not appear to be a suitable selection criterion, because this would include subjects with small bone size rather than those with reduced cartilage thickness. This is particularly relevant as cartilage thickness and joint size have been shown to be not highly correlated [17].

**Longitudinal quantitative cartilage volume measurements**

Data on changes in cartilage volume from longitudinal studies have recently become available. Wluka et al. [12] quantified the changes in cartilage volume in the medial and lateral tibia of individuals with symptomatic and radiographic evidence of knee OA over a period of approximately two years. The mean loss of tibial articular cartilage was 5.3% per year. Age and body mass index (BMI) were also found to be weakly associated with cartilage (tibia) volume loss. The authors found no significant difference in the amount of relative (%) cartilage loss between women and men, and only a relatively low correlation between changes in the medial and
lateral tibia. Further analysis of subjects from the same cohort [18] revealed that the rate of relative (%) cartilage loss in the patella was significantly higher in women (5.3%) compared with men (3.5%). Interestingly, the authors found no significant association between change in the patella and both the medial and lateral tibia, suggesting different OA pathogenetic mechanisms. However, they reported that subjects with higher baseline pain scores displayed greater cartilage volume loss than those with lower pain scores, as did those with high BMI.

Another study by our group examined the progression of cartilage volume loss in patients with symptomatic knee OA over two years [19]. Knee OA progression (cartilage volume loss expressed as % of loss compared to the baseline value for each patient) computed at all the follow-up points was statistically significant: a mean of 3.8% of global cartilage loss (femur and tibia) and 4.3% for the medial compartment (medial femoral condyle and tibial plateau) at 6 months; 3.6% and 4.2% loss at 12 months; and 6.1% and 7.6% loss at 24 months. Using discriminatory function analysis, two groups were identified: slow and fast progressors. The risk factors identified to be associated with the fast progressors were female gender, high BMI, reduced range of movement of the study knee, greater knee circumference, and higher knee pain and stiffness scores as assessed by the Western Ontario and McMasters Universities Osteoarthritis Index (WOMAC) questionnaire. A second study [20] using a larger number of OA patients further identified 3 different populations according to cartilage volume loss: slow (with 2.3% global cartilage volume loss at 24 months), intermediate (7.2%), and fast (13.2%) progressors.

Influence of other knee structure changes on OA cartilage volume loss

Another advantage of MRI compared to conventional imaging technologies, is its ability to globally assess all major joint structures, including the cartilage (Figure 2), meniscus, bone marrow alterations (Figure 3), synovial (membrane and effusion), and ligaments.

Indeed, cartilage volume loss can be dependent on other structural damage such as meniscal damage or joint malalignment. The menisci transmit 50% to 90% of load over the knee joint, depending on knee flexion angle and femoral translation and rotation. The meniscus also contributes to knee joint proprioception and probably also to joint stability [21]. Cicuttini et al. [22] in a study comparing patients who had undergone surgical meniscectomy with controls and an average of 28 months follow-up, showed
that cartilage volume loss over time as assessed by qMRI was greater in patients who underwent partial meniscectomy. This result suggests the strong role of the meniscal apparatus in protecting cartilage, especially in older subjects or those suffering from obesity or joint instability. Biswal et al. [23] also looked at the risk factors for progressive cartilage loss in knee OA patients using MRI. Baseline and follow-up MRIs of the knee (mean 1.8 years apart) were done and cartilage volume loss was graded semi-quantitatively in the anterior, central, and posterior regions of the medial and lateral knee compartments. Data showed that meniscal and anterior cruciate ligament tears were associated with a more rapid cartilage loss. These authors also demonstrated that the central portion of the medial compartment had a more rapid progression of cartilage loss than the anterior or posterior areas. These data are a clear indication that cartilage loss in OA is not evenly distributed in the knee.

Another MRI study done by Berthiaume et al. [24] evaluating the impact of meniscal damage on cartilage volume loss assessed by MRI showed a strong and highly statistically significant association between the global cartilage (femur and tibia) volume loss and the presence of a severe medial meniscal extrusion. An even greater association was found between the medial meniscal extrusion and the loss of cartilage in the medial compartment.

The importance of other structural changes such as bone marrow hypersignal (Figure 3c) in assessing knee OA was first demonstrated by Felson et al. [25]. In this study, patients with knee OA had baseline assessments including MRI and fluoroscopically positioned radiography and were followed for 30 months. Progression was defined as a decrease over follow-up in medial or lateral joint space based on a semi-quantitative

**Figure 3.** Human knee cartilage in sagittal view acquired with a 1.5 Tesla magnet using fast imaging at steady state precession (FISP) acquisition with fat suppression. **a)** Meniscal tear (arrow), **b)** meniscal extrusion (arrow), **c)** bone marrow lesion (BML) (arrow).
grading. Knees with medial bone marrow lesions (BML) showed a higher incidence of medial progression versus knees without lesions (odds ratio for progression, 6.5 [95% CI, 3.0 to 14.0]). These findings, which are in agreement with our group’s study [20], provide additional arguments to support the relationship between the cartilage volume loss and other anatomical knee changes. The latter study [20] demonstrated that the strongest predictors of cartilage volume loss in knee OA patients were the presence of severe meniscal extrusions, severe medial tear, and medial and/or lateral bone hypersignal along with clinical variables such as high BMI, weight, and age.

A new non-invasive synovial thickness scoring system using MRI was also recently developed [26], which accurately and reliably assessed the severity of synovitis in knee OA patients. The MRI sequences developed to evaluate the synovial tissue provide optimal visualization of both the medial and lateral compartments of the OA knee, where the most clinically relevant structural changes take place. This pivotal work was the first to enable a correlation between the severity of the synovial membrane inflammation and the loss of articular cartilage both using MRI. Moreover, it has clarified the likely role played by meniscal extrusion in the induction of synovitis.

Together, these data demonstrated that meniscal tear and extrusion are among the most significant risk factors associated with the progression of knee OA.

**Clinical trials using qMRI as an outcome tool to evaluate anatomical knee structure**

An important recently published two-year randomized multicentre trial using qMRI and X-rays explored the effects of a lipoxygenase and cyclooxygenase (LOX/COX) inhibitor for DMOAD properties in knee OA patients [27]. Quantitative MRI was used to assess changes in cartilage volume and X-rays (Lyon-Schuss) were used to measure changes in the mean and minimum JSW in the medial compartment. MRI data demonstrated that cartilage volume loss in the global and lateral knee compartment was significantly less in the LOX/COX group compared to the control (the non-steroidal anti-inflammatory drug Naproxen) group at 12 and 24 months, thus having a protective effect in knee OA patients. Patients with medial meniscal extrusion had greater loss of cartilage volume and the LOX/COX markedly reduced the cartilage volume loss at 12 and 24 months, demonstrating the importance of identifying meniscal structure lesions as an important co-factor of disease progression. In this
study, although the LOX/COX compound showed less reduction in the JSW than control, this did not reach significance. These findings clearly demonstrate the important limitation of standard radiographs compared to qMRI in investigating DMOAD effects. Moreover, both drugs were equally effective at reducing OA symptoms. These two outcomes are probably independent of each other, as pain is usually assessed in a relatively short time span while a longer follow-up is needed to appreciate the benefit of joint structural protection as assessed by MRI. Perhaps symptom evaluation should be redefined when evaluated together with joint structure. The American College of Rheumatology criteria for primary OA of the knee [28] are currently based on clinical and/or radiological findings. Since the cartilage is not vascularized or innervated, the pain experienced in OA is likely to originate from bone, synovial, capsule or ligament alterations. The “pure” anatomical cartilage volume loss over time, if chosen to define primary OA, may not be reflected at first by changes in symptoms, which considerably precede the radiological changes and may be accelerated by unsuspected concomitant meniscal damage. This is reflected by studies showing that change in pain level was weakly associated with cartilage volume loss at two years. It is therefore possible that redefining the symptom evaluation according to a longer period would show a stronger relationship between these two variables.

To address the question of the benefit of a DMOAD, a “hard” outcome such as preventing the occurrence of total knee replacement (TKR) could be targeted. In this line of thought, a recent study was performed to identify predictive factors for TKR in the abovementioned study [27] using the according-to-protocol (ATP) OA knee cohort [29]. The incidence of TKR was assessed blindly to the treatment following telephone interviews, and TKRs done in the time frame of 4-7 years following enrolment in the original study were used. Data revealed more TKRs within the control (Naproxen) group (61%) than in the LOX/COX group (39%). Furthermore, baseline score of BMLs in the medial compartment, medial JSW, presence of severe medial meniscal tear, medial meniscal extrusion, and C-reactive protein level were strong predictors of TKR. Changes at the end of the study (24 months) also yielded strong predictors: change in cartilage volume of the medial compartment and global knee, and WOMAC pain and function scores. Multivariate analysis further revealed that baseline severe medial meniscal tear and presence of a medial BML were the strongest independent long term predictors of TKR. In brief, this study shows that in the context of OA clinical trials, clinical data and structural changes identified by MRI allow prediction of a “hard” outcome such as TKR [29].
Comparing MRI with standardized knee radiograph measurements

Studies that have directly compared quantitative changes in cartilage volume using MRI to measurements of JSN in radiographs have produced conflicting results. A cross-sectional study by Cicuttini et al. [16] comparing cartilage volume in the tibia measured by MRI to radiologic grade (osteophytes and JSN) revealed that JSN, as graded from 0 (no disease) to 3 (most severe disease), was inversely correlated with the tibia cartilage volume as assessed by MRI. Such inverse relationship was even stronger while adjusting for age, sex and BMI. Gandy et al. demonstrated [30], in a study of knee OA over a three-year period, narrowing of JSW in weight-bearing extended radiographs of -0.21 mm while no significant change in cartilage volume was found by MRI in any of the knee compartments. They argued that radiography may be more sensitive than analysis of total cartilage plates by MRI because in radiographs, measurements are obtained in the central portion of the joint surface, where most of the changes may occur. However, it should also be kept in mind that the cohort was relatively small (n=11 patients) and that, in contrast to most other studies, the authors used a 1.0 T rather than 1.5 T magnet for their study, with relatively high associated precision errors. Conversely, our group [19] described no significant change in weight-bearing semiflexed radiographs in OA patients over two years, but reported a highly significant change in cartilage volume from MRI in both the medial and lateral tibiofemoral compartments. These findings were further reinforced from a larger cohort with knee OA [20].

MRI appears, therefore, to be significantly more sensitive in detecting volume change in the articular cartilage. It provides direct assessment of cartilage thickness and volume progression, while the JSW is an indirect measurement, which could be subject to a number of artefacts related to factors such as positioning, image acquisition, and changes in joint structure other than cartilage.

Fully automated qMRI assessment of knee OA structure

MRI-based quantitative knee joint structure assessments are and will be increasingly used for evaluation of the efficacy of a DMOAD. To date, only manual or semi-automated qMRI assessment methods have shown enough stability to produce cohort-scaled results. As a next generation tool, fully automated joint structure volume assessment solutions will enhance stability and reproducibility of MRI reading. In recent years, our group has developed
such fully automated segmentation systems for knee OA bone contours [31], as well as quantitative volume evaluation of cartilage [32] and synovial fluid [33].

The developed fully automated MRI method for bone contours relies on easy-to-gather input information from a single MRI acquisition [31]. A validation protocol performed over a large knee OA cohort comparing the developed automated method [31] to a validated semi-automated segmentation technology [9], provided excellent results with the evaluation criteria commonly used in this field. Data revealed that the average surface distance standard deviation was less than half a pixel. Similarly, the test-retest evaluation showed excellent reproducibility with an average of less than half a pixel resolution and maximum value of less than a pixel for the femur. The comparison between cartilage volumes using fully automated versus semi-automated bone surface shows no separability of the volume distributions between these two systems for both the femur and the tibia. In addition, for the cartilage volume, the Pearson correlation coefficients were excellent for both the femur and the tibia (r=0.99) as was the Dice similarity coefficient. In brief, this technology provides stable results and is robust to the variable MR image quality as reflected by the validation analyses performed on knee OA patients. Importantly, this technology also permits, for the first time automatically, the detection and quantitative evaluation of knee osteophyte volume.

For automated cartilage segmentation, a pivotal study assessing knee OA progression and validation experiments was recently reported [32]. The fully automated cartilage volume quantification demonstrated excellent correlations with semi-automated segmented cartilage volume, not only for the global cartilage but also for subregions of the knee. Correlation of cartilage volume and loss between two visits (12 months apart) also revealed excellent accuracy of automated cartilage segmentation of the pathologic knees. Test–retest validation showed a very low error measurement level, suggesting that the developed automated system is reliable and provides precise assessment of human OA knee cartilage volume. Since cartilage degradation is the hallmark of OA and its volume loss is related to the progression of the disease, such a fully automated method combined with the automated MRI method for bone contours would be useful not only for diagnosis, but also for clinical trials with patient follow-up.

Knee joint effusion is a common finding in OA patients and may be related to the activity of the disease. Therefore, non-invasive fully automated quantification of joint effusion volume in the knee would be a valuable tool for diagnostic, follow-up, and clinical studies. Recently, such an automated system for joint effusion volume quantification has been reported [33]. This
system was validated by external means, i.e. calibrated phantoms, manual MRI quantification, and direct aspiration. Data revealed excellent CV with a small (cylinder, 1.4%) and a large (sphere, 0.8%) calibrated phantom, and excellent correlations ($r=0.98$) between the automated and manual quantification of the OA knee joint effusion volume, as well as with direct aspiration ($r=0.88$).

The obvious advantage of these automated methods is the possibility of intensive and autonomous computation, enabling images from a large cohort of patients to be analyzed in a shorter time and, more importantly, increased reading stability. These methods may prevent major problems encountered with the current manual and semi-automated segmentation methods related to contrast, intensity, and gamma tuning for the image display, which have an important influence on the final segmentation contours. Moreover, they will also prevent intra- and inter-observer variations, the subjectivity of human intervention and errors due to fatigue, especially for large clinical trials. However, although such automated quantitative MRI evaluations are highly promising, the responsiveness to change under therapy must be further tested in a longitudinal study in view of its future application.

**MRI and identification of macromolecules in articular cartilage**

The concentration of glycosaminoglycan (GAG) in articular cartilage is also known to be an important determinant of the mechanical properties of this tissue. Concentrations of GAG have been explored by using delayed gadolinium-enhanced MRI of cartilage (dGEMRIC). In a recent study [34], tibias of patients undergoing total knee arthroplasty were imaged by dGEMRIC and the load response to focal indentation was measured as an index of cartilage stiffness at different test locations for each tibia. Overall, a high correlation was found between the dGEMRIC index (T1Gd) and local cartilage stiffness (Pearson correlation coefficients $r=0.90$). In brief, the results from this study demonstrate the importance of MRI in yielding spatial localization of GAG concentration in the evaluation of the mechanical properties of cartilage and suggest the possibility that this evaluation may be further improved by adding other MRI parameters that are sensitive to collagen, since the quality of the cartilage is dependent on the structural organization of the collagen network.

Moreover, Regatte et al. [35] reported the assessment of cartilage degeneration through GAG with the same efficiency as the dGEMRIC approach, but with a completely non-invasive technology. This technology
used a spin-lock pulse sequence allowing evaluation by T1p parameter, comparable with the T1 or T1Gd.

Another MRI acquisition technique, T2-mapping, was further shown to detect changes in cartilage water content. Liess et al. [36] demonstrated on healthy volunteers that reducing the water content of the patellar cartilage by repetitive knee bending can be quantified using a transverse relaxation time (T2) MRI sequence. Hence, the detection of small physiological changes in water content may help in the early diagnosis of OA.

T2 imaging was also suggested to be relevant for collagen variation assessment as discussed in an overview by Mosher et al. [37], which shows the relationship between cartilage T2 imaging and the cartilage water content, proteoglycan concentration, collagen concentration, or tissue anisotropy. Alternatively, the MR diffusion tensor imaging (DTI), could also be useful for detecting early changes in collagen fiber alignment, as DTI allows determination of the degree of diffusion anisotropy and the direction of local diffusion in tissues. Thus, by using DTI technology, Filidoro et al. [38], on a 9.4 T magnet field, were able to identify the orientation of collagen fibers in the patella.

Conclusion

The quantitative assessment of cartilage thickness and volume and other joint structure changes in OA is primarily to objectively evaluate the disease course as well as DMOAD treatment that may slow down OA tissue degradation. The problems faced a decade ago by clinical research that MRI technology must be based on readily available acquisition parameters easily reproducible in most available apparatus are now history. Data have shown such technology to be exportable to other centres with comparable MRI facilities, and can thus be used in multicentre clinical trials. Moreover, the fully automated quantification of joint structure opens the door to intensive and autonomous computation, enabling images from large scale studies to be reliably analyzed in a shorter time frame with high accuracy.

Because of the condition of the patients and the symptoms they experience, image acquisition should be performed in a time-wise fashion without compromising image quality. This is particularly critical for the quantification of disease progression over time. The future of OA research pertaining to prevention or repair of structural damage can be compared, to some extent, to the evolution experienced in the field of osteoporosis in the last few decades. In the beginning a significant bone loss was necessary to diagnose osteoporosis on plain radiographs. With the advent of
osteodensitometry, relatively small changes in bone mass can be detected and early diagnosis can be established. This outcome tool opened the door to clinical research on new therapies to slow or prevent bone mass loss. Everyone knows the impact of these medications on the outcome of osteoporosis today. Similarly, quantification of cartilage loss and the other joint structure changes seen in OA over time will improve the monitoring of OA and help to develop and test new interventions to prevent the progression of this extremely prevalent disease.

In conclusion, data have proven that MRI yields clear visualization of knee structure affected by OA and the superiority of the quantification of the damage by MRI over the standard imaging using radiographs has been demonstrated. The evaluation of knee OA using qMRI must be done in the context of whole organ assessment including meniscal damage, BML, and synovial membrane and effusion alterations. The recently developed fully automated qMRI will enhance the speed and reliability of the evaluation of the progression of joint structural damage. MRI technology should therefore be included in DMOAD clinical trials, used for early OA detection and treatment follow-up, and further employed for appraisal of the correlation between rapid knee structural changes as detected by qMRI and either short-term symptom changes (e.g. knee pain and function) or the prediction of long term hard outcomes, such as the occurrence of joint replacement.

References

Quantitative MRI evaluation of knee osteoarthritis patients
