CHAPTER 1

INTRODUCTION

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Osteoarthritis (OA) is a heterogeneous disorder primarily resulting in joint destruction with high clinical burden that can affect all joints in the body, but is especially prevalent in the knee joint[1]. Knee OA is characterized by knee pain and stiffness, and is accompanied by cartilage loss and abnormalities in the subchondral bone. For scientific purposes, criteria sets for classification of knee OA were developed though a multicentre study group by Altman et al in 1986. A prerequisite for all criteria sets was knee pain (table 1)[2].

Table 1: American college of Rheumatology (ACR) criteria sets for Knee Osteoarthritis (OA), Modified after Altman et al, 1986 [2]

Clinical and laboratory	Clinical and radiographic	Clinical
Knee pain +	Knee pain +	Knee pain +
At least 5 of 9: ▪ Age > 50 ▪ Stiffness < 30 minutes ▪ Crepitus ▪ Bone tenderness ▪ Bone enlargement ▪ No palpable warmth ▪ ESR < 40 mm/hour ▪ RF < 1:40 ▪ SF OA	At least 1 of 3: ▪ Age > 50 ▪ Stiffness < 30 minutes ▪ Crepitus + Osteophytes	At least 3 of the 6: ▪ Age > 50 ▪ Stiffness < 30 minutes ▪ Crepitus ▪ Bone tenderness ▪ Bone enlargement ▪ No palpable warmth

** ESR: erythrocyte sedimentation rate, RF: rheumatoid factor, SF OA: synovial fluid signs of OA (clear, viscous, or white blood cell count < 2.000/mm^2).*

Epidemiology and risk factors

Not only is OA one of the most common rheumatic disorders, it is also in the top 25 most prevalent diseases in the population worldwide as was reported by the World Health Organization (3.64% of the population in both sexes (2.56% in males and 4.74% in females) [3] [4]. There were approximately 1.189.000 patients (444.000 male and 745.000 women) with OA (prevalence: 53,8 per 1.000 males and 88,5 per 1.000 women) in the Netherlands in 2011 (Source: www.volksgezondheidenzorg.info/onderwerp/artrose). OA is most prevalent in the knee joint (prevalence 227.000 in males and 367.000 in women). The clinical burden of OA is high, which is underscored by the fact that OA is responsible for the largest number of years lived in disability (YLD) in the elderly female Dutch population[5]. Therefore, OA is becoming a significant medical and financial burden in a world whose population is aging.

OA is considered a multifactorial disease, in which factors determine the susceptibility for OA. These factors, together with local biomechanical factors, determine the localization and severity of OA in a certain joint. The most important risk factors are female gender and age.

High body mass index (BMI) also shows an increased risk of developing OA. BMI acts both via systemic and local pathways (table 2)[1].

Table 2: Risk factors for the occurrence and progression of knee Osteoarthritis (OA) – modified after Bijlsma et al, 2011 [1]

		Risk factors for knee OA
Occurrence		
-	Deleterious	age, gender, physical activity, body-mass index, intense sport activities, quadriceps strength, bone density, previous injury, vitamin D, malalignment (including varus and valgus), genetics
-	Protective	Hormone replacement therapy
-	Protective/deleterious	Smoking
Progression		
-	Deleterious	Age, body-mass index (including obesity), vitamin D, malalignment (varus and valgus), chronic joint effusion, synovitis, intense sport activities, subchondral bone oedema on MRI
-	Protective	Hormone replacement therapy

Pathogenesis

OA is characterized by focal lesions of the articular cartilage, combined with a hypertrophic reaction (sclerosis) in the subchondral bone and new bone formation (osteophytes) at the joint margins. Although for a long time OA was considered to be a degenerative process, a combination of complex degenerative and repair processes leads to changes observed in OA. Several factors are involved in these processes, such as mechanical stress, biochemical (such as inflammatory mediators) and genetic factors[1].

Recently, OA has been relabelled as a whole organ disease as not only abnormalities in the cartilage but also in subchondral bone and synovial tissue are involved. Moreover, pathologic abnormalities such as periarticular muscle weakness, lax ligaments, meniscal degeneration and neurosensory system alteration are frequently present in these patients[6].

- **Cartilage abnormalities in OA**

Articular cartilage is composed of extracellular matrix (ECM) which contains collagen (mainly type II fibrils with both collagen IX and XI integrated) and proteoglycans. Chondrocytes are the only cells found in the ECM and are responsible for production, maintenance and destruction of the cartilaginous matrix. Healthy articular chondrocytes maintain a stable phenotype and do not show proliferation and differentiation. In OA, chondrocytes develop terminal differentiation and hypertrophy leading to disruption of the cartilage. Moreover, chondrocytes respond to injuries by producing degrading enzymes and by developing inappropriate repair responses[1,7].

- **Subchondral bone abnormalities in OA**

The subchondral bone is a global term that includes the subchondral bone plate, the underlying trabecular bone and bone marrow. An important feature of OA pathophysiology

is subchondral bone remodelling, which is characterized by increased subchondral bone thickness (sclerosis), formation of new bone at the joint margins (osteophytes) and subchondral bone cysts development[7,8]. Another feature in the subchondral bone that received a lot of attention is bone marrow lesions (BML), which can be visualized on MRI. The nature of BMLs is not fully elucidated, but seems to reflect local regions of sclerosis with increased bone volume fraction and increased trabecular thickness. However, OA subchondral bone also contains lower trabecular spacing. These features are thought to represent trabecular remodelling or compression[9,10]. Both osteoblasts as well as increased expression of insulin-like growth factor 1 (IGF-1) and transforming growth factor - β (TGF-β) by subchondral bone are thought to play a role in the abnormal bone remodelling in OA[11 7,8].

- **Synovial tissue abnormalities in OA**

For a long time, OA was considered a non-inflammatory condition. More recently, however, it became evident that synovial inflammation could play an important role in the pathophysiology of OA [3,12-16] as it is a predictor of cartilage destruction [17,18] and a determinant of pain[19,20]. Although the biological processes underlying the appearance of synovial inflammation are poorly understood, it has been suggested that cartilage breakdown products could lead to activation of immune cells and production of pro- and anti-inflammatory mediators, which in turn could stimulate further cartilage breakdown, creating a negative feedback loop in OA (Figure 1) [16].

Several immune cells have been found in OA synovial tissue, such as macrophages, T cells, mast cells, B cells and others. Furthermore, several cytokines such as tumor necrosis factor- α (TNF-α), interleukin 1- β (IL1-β) and others have been implicated in OA. The role of inflammation, immune cells and cytokines in OA, however, is less clear and pathophysiologic mechanisms are still hypothetical as these are not linked to clinical parameters. Likewise, it is unclear how synovial inflammation evolves during the disease course, since studies investigating this topic were conflicting [12,14,15,21]. Therefore, it still seems unclear whether synovitis causes pathological changes or whether OA causes synovitis.

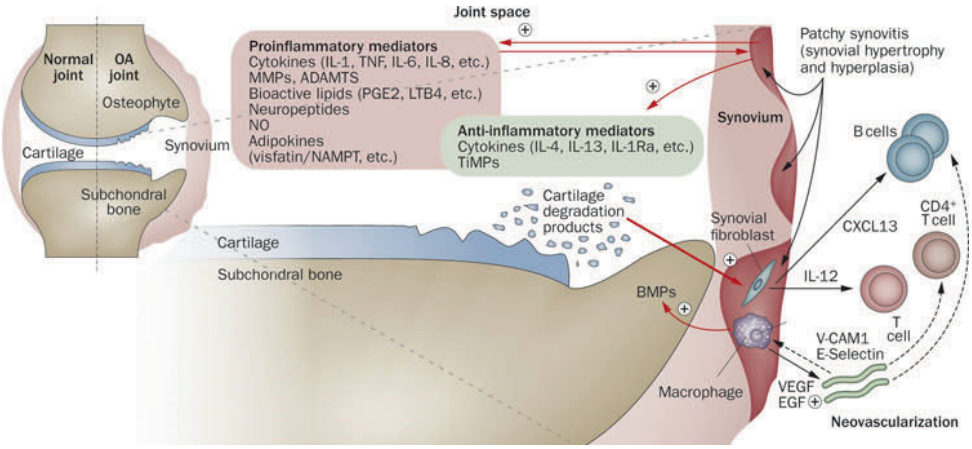

Figure 1. Involvement of synovial tissue in Osteoarthritis (OA) pathophysiology, from Sellam & Berenbaum, 2010. [16]
** ADAMTS: a disintegrin and metalloproteinase with thrombospondin motifs, BMP: bone morphogenetic protein, CCL2: CC motif chemokine ligand 2, CXCL13: C-X-C motif chemokine 13 ligand 13, EGF: endothelial growth factor, GM-CSF: granulocyte-macrophage colony stimulating factor, IL: interleukin, IL-1Ra: IL-1 receptor antagonist, LIF: leukaemia inhibitory factor, LTB4: leukotriene B4, MMP: matrix metalloproteinase, NAMPT: nicotinamide phosphoribosyl transferase (also called visfatin), NO: nitric oxide, NGF: nerve growth factor, PGE2: prostaglandin E2, TIMP: tissue inhibitor of metalloproteinase, TNF: tumor necrosis factor, vCAM-1: vascular cell adhesion molecule 1, vEGF: vascular endothelial growth factor.*

- **The role of infrapatellar fat pad in OA**

The infrapatellar fat pad (IFP), also known as Hoffa's fat pad, is an intracapsular structure that fills the anterior knee compartment. Due to its location, adjacent to the synovial tissue, and due to its inflammatory nature, it is conceivable that the infrapatellar fat pad has a role in pathophysiology of OA, although its role in OA is largely unknown[22,23].

Clinical aspects of knee OA

Knee OA is characterized by pain, stiffness and reduced function, and consequently, by decreased mobility and participation. Knee OA can involve two joints in the knee; the tibiofemoral joint, encompassing both medial and lateral tibiofemoral compartment, and the patellofemoral joint. Pain experienced during walking on level ground is usually a symptom observed in patients with tibiofemoral OA, while pain during climbing or ascending stairs is more often observed in patients with patellofemoral OA, although OA is usually found in both joints simultaneously creating a possible overlap of symptoms. Physical examination of the knee reveals bony enlargements (osteophytes), crepitus, bone tenderness, restricted joint movement and effusion. In clinical practise plain radiographs of the knee can be used to confirm the diagnosis, by showing structural abnormalities such as osteophytes and joint space narrowing. Joint space narrowing reflects a loss of articular cartilage (Figure 2). However, the role of radiographs in research of the knee is much debated as OA is now

thought of as a whole organ disease including subchondral bone and synovial tissue, which cannot be visualized on radiographs. Therefore, MRI plays an upcoming role in OA as it enables visualisation of all structures of importance in the knee[1,24].

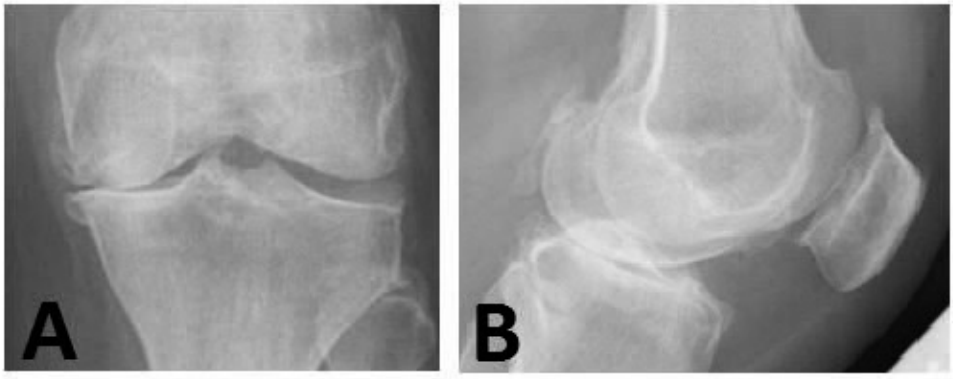

Figure 2. Radiological views of knee osteoarthritis (OA). (A) Medial tibiofemoral knee OA on a weight-bearing fixed flexion posteroanterior view. (B) Patellofemoral OA on a lateral view.

Natural history OA

Knee OA is a chronic disorder but its natural history can vary greatly. OA generally develops progressively over several years although may remain relatively stable for prolonged periods[25]. In patients at risk, local mechanical factors such as malalignment, muscle weakness or alterations in the structural integrity of the joint environment (for instance abnormalities of the meniscus or ligaments or articular abnormalities caused by previous trauma) facilitate the progression of the disease, especially in knee OA[26]. The correlation between clinical outcome and radiographic course is relatively poor at the individual level: whereas symptoms can improve, the radiographic picture rarely does[27]. Progression of OA can result in disability, pain and joint destruction, requiring a total joint replacement[28].

Therapy

Unfortunately, no disease modifying therapies exist for OA and current therapies rely on symptom relief such as pain medication, NSAIDs and physical therapy. In end-stage knee OA, an arthroplasty is the only effective therapy.

AIM OF THESIS

Synovial inflammation is present in knee OA and seems to be of importance in pain perception and disease course. However, its role in the pathophysiology of OA is largely unclear. Therefore, in this thesis we aimed to investigate:

1. The nature of synovial inflammation in knee OA
2. The role of synovitis in the clinical burden of knee OA

Insight in synovial inflammation could ultimately lead to better understanding of the pathophysiology of OA and therefore might provide clues for developing disease modifying drugs in OA.

GEMSTOAN STUDY

In the current thesis patients have been included, which participated in the geMstoan study. The geMstoan study (GEneration of Models, Mechanism & Markers for STratification of OsteoArthritis patieNts), an observational study in established and end-stage knee OA patients to find new biomarkers for OA progression. Between 2008 and 2013, patients with symptomatic radiographic primary knee OA, following the American College of Rheumatology (ACR) classification criteria [2] and who were attending the rheumatology or orthopaedic department of the LUMC or orthopaedic department of the Diaconessenhuis, Leiden, were included. The geMstoan study comprises two groups of patients: one group of patients with end-stage disease that were planned to receive an arthroplasty and another group with mild to established OA that had no indication for an arthroplasty. Patients with mild to established disease received an arthroscopy and were followed for 2 years. Synovial tissues and infra patellar fat pad were obtained during arthroscopy or arthroplasty which enabled us to investigate the nature of synovitis. Furthermore, as baseline and 2 year follow-up radiographs and MR images were made in every patient it was possible to investigate in detail MRI abnormalities and especially. This study has been approved by the ethics committee of the Leiden University Medical Center (LUMC). All patients provided written informed consent.

OUTLINE THESIS

Part I The nature of synovial inflammation in knee OA

There is increasing evidence that inflammation is present in synovial tissue of OA patients. However the role of synovial inflammation, including immune cells and their cytokines,

are not fully understood. Since OA synovial tissues are frequently used as control tissue in histological studies in rheumatoid arthritis (RA), a considerable amount of knowledge is readily available in scientific literature. Therefore, we capitalized on earlier histological studies using OA synovial tissue and performed an extensive narrative review (**chapter 2**), summarizing all current knowledge of synovial inflammation. In this review, we focused on two aspects of inflammation: the degree of inflammation as can be evaluated by H&E staining and secondly, on specific immune cells and their cytokines as these might provide important insights in the underlying mechanisms of synovitis and pain in osteoarthritis.

Although it is shown that synovitis is associated with pain, we learned from **chapter 2** that it is unclear which immune cells and their mediators play a role in pain experience. Therefore, in **chapter 3** we extensively characterized immune cells in both synovial tissue and infrapatellar fat pad of end-stage knee OA patients by flow cytometry analysis and investigated relation of immune cells with pain. To further investigate the role of immune cells in OA different subtypes and activation states of immune cells and their intracellular cytokines were investigated. Furthermore, to investigate underlying mechanisms in pain, associations between immune cells and pain were investigated.

As observations from **chapter 2** suggested that mast cells could play an important role in OA, in **chapter 4** number and granulation state was investigated by immunofluorescence stainings in both mild to established and in end-stage knee OA patients. The association of mast cells with clinical parameters was also investigated. Furthermore, we compared our findings to RA synovial tissue samples.

Although histological assessment of synovial biopsies is currently the golden standard for evaluating synovial inflammation in knee OA, acquisition of synovial biopsies is technically difficult and patient unfriendly as it involves an invasive procedure like arthroscopy. Therefore, a non-invasive method, such as contrast-enhanced (CE) MR imaging constitutes an attractive alternative for visualizing synovial tissue inflammation [29-31]. As the anatomical distribution of synovitis on CE-MRI is patchy and heterogeneous [32], a MRI scoring method should encompass a sufficient number of compartments. A recently developed method by Guermazi et al. is a semi-quantitative method that scores synovitis on CE-MRI at 11 different sites throughout the knee [33] and constitutes a comprehensible and practical method for assessing synovitis in the whole joint. In **chapter 5** we used this scoring system to investigate whether the degree of synovitis on CE-MRI correlates with microscopic and macroscopic features of inflammation in knee OA patients. Furthermore, we aimed to investigate whether the degree of inflammation differs in patients with different stages of knee OA.

Part II The role of synovitis in the clinical burden of OA

Although synovitis is known to be heterogeneous and therefore current scorings systems should investigated synovitis at different anatomical sites, it was not known whether

synovitis at different sites occurs independently or whether they form patterns. Therefore, in **chapter 6** we aimed to investigate whether patterns of inflammation exist in an unbiased manner in knee OA and whether they associate with pain.
Several MRI features are known to be related to radiographic progression. However, these MRI features are known to be highly correlated. Therefore, to increase our insight into the interaction between MRI abnormalities in the different joint tissues and the interaction between the patellofemoral joint (PFJ) and tibiofemoral joint (TFJ) in relation to radiological progression, we aimed to investigate patterns of different tissue abnormalities as assessed with MRI of both the PFJ and TFJ in an unbiased manner in **Chapter 7**.
Furthermore, to understand the role of synovitis in the disease course of OA and its relation to disease progression and pain, in **chapter 8** we investigated changes in synovitis over a 2-year period in knee OA patients.
Finally, in **chapter 9,** we provide an overview of the thesis and discuss our findings.

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