CHAPTER 9

SUMMARY, GENERAL DISCUSSION & FUTURE PERSPECTIVES

SUMMARY

Osteoarthritis (OA) is a heterogeneous disorder, which can affect all joints in the body, but is especially prevalent in the knee joint[1]. Eventually, the disease progresses and leads to joint destruction with symptoms of pain and functional impairments. Currently, no disease modifying drugs are available and therapy is focused on symptom relief. OA is becoming a significant medical and financial burden in a world whose population is aging.
Although knee OA is a very prevalent disease, underlying pathological and pain mechanisms remain unknown. Therefore, research investigating underlying mechanisms is of utmost importance. 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 [2-7] as it is a predictor of cartilage destruction [8,9] and a determinant of pain[10,11]. The role of synovitis in knee OA, however, is still largely unknown.
Therefore, in this thesis we investigated the nature of synovial inflammation in knee OA and its possible contribution to the clinical manifestations of knee OA. Results presented in this thesis provide insight into different aspects of synovial inflammation aimed at increasing our understanding of the pathophysiology of OA and aiding to the development of disease modifying drugs in OA.

Part I The nature of synovial inflammation in knee OA

As first step in the analysis of the cellular and molecular nature of synovial inflammation, we performed in **chapter 2** a narrative systematic review to summarize the current knowledge of inflammatory properties, immune cells and their cytokines in synovial tissues of OA patients. Our hypothesis that knowledge was readily available was underscored by the fact that we extracted 100 articles. In this literature overview, we found that synovial inflammation histologically similar to the one observed in rheumatoid arthritis (RA) was commonly described in OA and that the most frequently detected cell types were macrophages, T cells and mast cells, while B cells were almost never found. Cytokines related to T cell or macrophage function were also described in OA synovial tissues, although their cellular source was only scarcely investigated. Overall, the conclusion was that inflammation is in general lower in OA than in RA and this reflects also in the abundance of most infiltrating immune cells. Strikingly, however, the number of mast cells was as high as, or sometimes even higher than in RA synovial tissue. Based on the results of this extensive review a research agenda was made, which served as a foundation for the follow-up studies described in this thesis.

- Analysis of immune cells and their activation state in synovial tissues of knee OA patients in relation to clinical disease characteristics.

- In-depth analysis of mast cells and their activation state in synovial tissues in knee OA patients at various disease stages and their relationship to clinical disease characteristics.
- Investigation of synovitis throughout the knee OA disease course.
- Association of synovitis with clinical parameters as pain, structural damage and progression.

FACS analysis, as described in **chapter 3**, of synovial tissues and of infrapatellar fat pad (IFP), an articular tissue in which immune cells are abundantly present, of patients with knee OA revealed that macrophages and T cells, followed by mast cells, were the most prominent immune cells, and that subpopulations of both T cells and macrophages were in an activated state. Furthermore, we found that CD4+ T cells were associated with pain, offering a cellular basis for the long-known association between synovitis and pain in knee OA.
As we learned from **chapter 2**, mast cells could be important in OA. Therefore, in **chapter 4,** we showed that synovial tissue of OA patients had a significantly higher number of mast cells than synovial tissue of RA patients, although RA synovial tissues displayed more severe inflammation grade by H&E staining compared to OA synovial tissue. The clinical relevance of mast cells in OA synovial tissues was suggested by the association between the number of mast cells and structural damage. No association with pain was observed.

The synovitis scoring method developed by Guermazi et al. [12] enables the scoring of synovitis throughout the whole knee on contrast-enhanced (CE)-MRI. In **chapter 5,** we validated this scoring method by comparing the scores with microscopic and macroscopic features of inflammation in OA synovium. From our results we concluded that the synovitis scoring method by Guermazi et al. is a comprehensive and valid non-invasive method to investigate the degree of synovitis in the whole knee in knee OA patients and we therefore used this synovitis scoring method to investigate the role of synovitis in the clinical burden of knee OA (**chapter 6 and 8**) and the course of synovitis over time (**chapter 8**). Furthermore, our dedicated setup enabled us to compare synovial inflammation in different OA severity stages. We showed that synovial inflammation was more severe in end-stage knee OA patients compared to patients with mild to established knee OA.

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

To gain a better understanding of the role of synovitis in the OA disease process, we studied the association between the presence of synovial inflammation and clinical manifestations of OA. An earlier study showed that OA synovitis on CE-MRI is patchy and heterogeneous throughout the knee [13]. However, it was unknown whether synovitis at different sites occurs independently or forms patterns. In **chapter 6,** we used principal component analysis (PCA)

to determined patterns of synovitis on CE-MRI. PCA is a statistical method that determines groups or patterns (named components) based on correlation of features with each other, without including assumptions related to possible mechanisms or anatomical sites. We observed three distinct patterns (Figure 1). Further analyses showed that the pattern that included several patellar sites as well as the site adjacent to the posterior cruciate ligament (PCL) was associated with pain, whereas the other two patterns did not associate with pain, suggesting that pain perception is a localized response. Furthermore, the pattern that included several patellar sites and the site adjacent to the PCL, as well as the pattern that included synovitis at site of a loose body, was associated with radiographic damage in cross-sectional analysis.

As we (in the **chapters 5 and 6**) and others have shown, synovitis might play a role in structural damage in OA. Other studies have also shown that other MRI abnormalities besides synovitis could be implicated in radiological progression in knee OA patients, as well. However, many of these features are known to be correlated to each other. Moreover, the knee consists of two joints: the patellofemoral joint (PFJ) and the tibiofemoral joint, and each has different weight bearing properties. Therefore, in **chapter 7** we investigated patterns of different tissue abnormalities of both joint using PCA in relation to radiographic progression over a 5-year period. Results suggested that, one the one hand, there seems to be a local response to triggers (clustering of MRI features at same anatomical site), while on the other hand, there is also a non-location specific mechanism for formation of osteophytes. With respect to radiographic progression, results suggest that the PFJ and the TFJ are related and that not only existing structural damage enhance further progression, but also processes reflecting increased bone turnover, such as bone marrow lesions, result in progression. Interestingly, effusion was not incorporated in any of the components, although this was probable due to used cut-off value.

Synovitis can only be properly visualized using CE-MRI. Therefore, to understand the development of synovitis throughout the disease course and the effect of change of synovitis over time on OA disease progression and in pain, in **chapter 8** we investigated synovitis change on CE-MRI over a 2-year period. Results showed that changes in mean total synovitis scores were not significant on group level although the synovitis score changed during the disease course in individual patients. Increase of synovitis over time was associated with cartilage deterioration, suggesting a role for synovitis as a target for disease-modifying treatment. Change in synovitis was not associated with change in pain, whereas cartilage deterioration was, suggesting that synovitis is of lesser importance in change of subjective pain or cartilage deterioration serves as a mediator in the association between synovitis and pain.

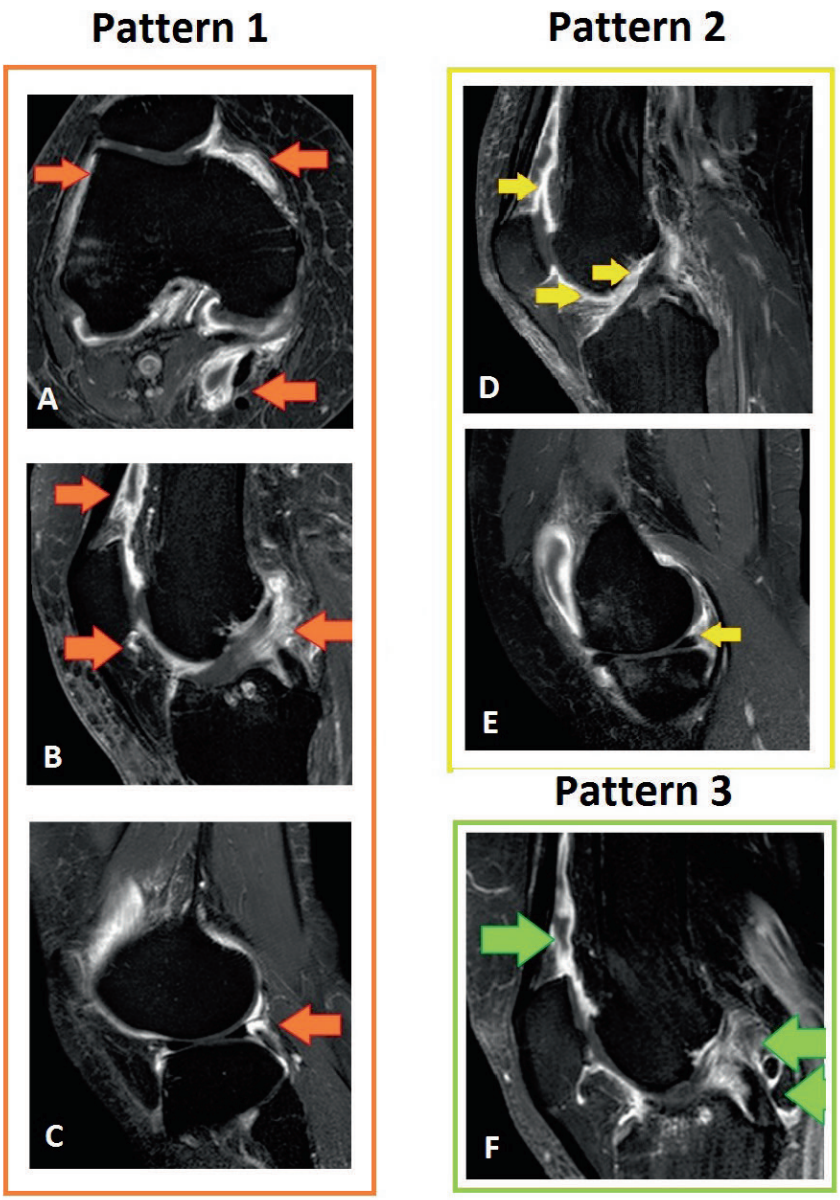

Figure 1. Gadolinium(Gd)-chelate enhanced T1-weighted images showing thee synovitis patterns observed in knee osteoarthritis (OA) patients. Pattern 1 (A-C), A. Axial image showing synovitis at the medial parapatellar site, lateral parapatellar site and Baker cyst, B. Sagittal image with synovitis at the suprapatellar site, infrapatellar site and site adjacent to the posterior cruciate ligament (PCL), C. Sagittal image showing synovitis at the lateral meniscal site. Pattern 2 (D and E), D Sagittal image showing synovitis at the suprapatellar site, intercondylar site and site adjacent to anterior cruciate ligament (ACL), E. Sagittal image showing synovitis at the medial meniscal site. Pattern 3 (F), F. Sagittal image showing synovitis at the suprapatellar site, at the site adjacent to PCL and surrounding loose body (green arrowhead).

GENERAL DISCUSSION

Immune cells in OA

Our aim was to investigate which cells are present in the inflamed synovium of OA patients and which could potentially play a role in OA. In **chapter 3** we detected roughly the same cells as described in our systematic review (**chapter 2**). Interestingly, we found that several immune cells have been activated in the inflamed synovium: T-cells in **chapter 3**, but also macrophages and mast cells in **chapters 3 and 4.** These observations suggest that these cells could potentially play a role in the disease.

Results from **chapter 3** show that in the absence of further activation, T cells mainly produce IL-6 and IL-4 ex vivo, suggesting that they might have been activated in vivo. The possible role of this subset of T cells in disease progression remains to be investigated.

Intriguingly, T cells are able to produce a variety of cytokines (including IFNγ and TNFa), upon stimulation, suggesting that IFNγ producing T cells exist in the inflamed OA synovium, but are not activated and therefore other cells might be responsible for producing this cytokine in OA. This is in line with previous investigations, in which IFNγ could also not be detected in CD3-positive cells in OA synovium, by means of immunohistochemistry[14,15].

Macrophages are the most abundant immune cells in OA (**chapter 2** and **chapter 3**), however their role in OA remains largely unclear. Therefore, in **chapter 3** we investigated for the first time, different subsets of activated macrophages ("classically activated" (M1) and "alternatively activated" (M2)) in OA synovium. Interestingly, macrophages isolated from OA synovium produce mainly M1 cytokines and very few M2 cytokines (**chapter 3**), suggesting a predominantly pro-inflammatory response. Moreover, production of these cytokines in the absence of additional activation indicates that these cells might have an activated state in the synovium. Future studies investigating the localization of these activated cells and their potential association with clinical disease characteristics will offer more insight into the mechanisms involved in the activation of these cells and in their potential involvement in disease pathogenesis.

With respect to the activation of mast cells in **chapter 4,** we observed degranulation of mast cells in OA synovium. However, only a small percentage of mast cells was degranulated and this was not different at different stages of the disease, nor between RA and OA. Furthermore, although degranulation of mast cells was not associated with pain or radiographic damage, the number of mast cells did show an association with structural damage. This observation suggests that mechanisms other than degranulation could be of importance in OA.

Observations from this thesis suggest that several synovial immune cells could be of clinical importance in OA; T cells could be of importance in pain perception (**chapter 3**) and mast cells seem to have a role in structural damage (**chapter 4)**. However, the numbers of patients investigated were small and therefore findings should be replicated in larger

cohorts. Furthermore, it remains unknown which immune cells are associated with cartilage destruction and it is currently unclear which cells infiltrate or become activated in the early stages of the disease course and how these immune cells act throughout the disease course.

Inflammation in knee OA not only quantitatively but also qualitatively different from RA

In both **chapters 2 and 6,** differences in the nature of synovial inflammation between OA and RA were addressed. Although for a long time it was assumed that the degree of synovial inflammation is less severe in OA compared with RA, there was a general assumption that the nature of inflammation (e.g. composition of immune cells) was the same. RA, however, is a systemic autoimmune disease, leading to prominent inflammation and joint destruction, whereas OA is not considered to be an auto-immune disease. Therefore, it seems unlikely that the nature of synovial inflammation, such as ratios of immune cells, would be exactly the same in the two different diseases. We hypothesized that inflammation in OA is not only quantitatively different, but also qualitatively different compared to synovial inflammation in RA. In this thesis we found evidence for this hypothesis, since B cells are virtually absent in OA **(chapter 2 and 3)** and since numbers of mast cells are the same **(chapter 2)** or even higher **(chapter 2 and 4)** in synovial tissues of OA patients compared to synovial tissues of RA patients. Another observation that supports the hypothesis that synovial inflammation in OA is different from synovial inflammation in RA is that on CE-MRI synovitis in OA is heterogeneous [13] and patterns of synovitis seems to occur **(chapter 4),** suggesting that synovitis in knee OA is a localized, rather than a general systemic response observed in RA (personal observation). However, since no valid scoring system exists for CE-MRI in RA it is currently unknown whether a homogeneous nature exists. Based on lack of B cells, higher number of mast cells and the patchy nature one could speculate that inflammation in OA seems to have a more innate immunity profile, whereas RA is more profoundly an adaptive immunity disease.

Mast cells in OA and other diseases

Mast cells are thought to play an important role in the pathophysiology of RA and are known to have destructive effects on the knee joint though cytokine and chemokine release, direct cell to cell contact or by tryptase release (degranulation)[16]. Unfortunately, the role of mast cells in OA remains largely unknown as only a small number of studies focused on mast cells in OA (**chapter 2**). Observations in this thesis suggest that mast cells could be of importance in OA, as we found that the numbers of mast cells are as high or even higher in OA synovial tissues compared to RA synovial tissues (**chapter 2 and 4**) and mast cells seem to have a role in structural damage (**chapter 4**). Interestingly, observations from **chapter 4** resemble results from a recent study comparing mast cells in synovial tissues of spondylarthropathy (SpA) patients with synovial tissues of RA patients. This study described that mast cells in

synovial tissues of patients with SpA were more numerous compared to synovial tissues of RA patients, despite a less degree of inflammation. [17] Although inflammation in SpA and OA are thought to be different, some similarities are seen. For instance, in SpA formation of bony enlargements called syndesmofytes are observed, which are comparable although not similar to osteophytes observed in OA. In SpA the only disease modifying drug with proven efficacy is sulfasalazine, which has been shown to inhibit both degranulation and TNF secretion by mast cells [18,19] and this drug has also been found to have an protective role on chondrocytes in an experimental study[20]. Therefore, targeting mast cells in OA (for instance via sulfasalazine) could be potentially beneficial in OA patients. However, first further research is needed to elucidate the role of mast cells in OA.

Role of imaging in knee OA

- **MR abnormalities in knee OA**

As OA is considered to be a whole organ disease and therefore MRI is widely used in OA research to investigate OA processes that might be of importance in pathophysiology of OA. However, the question arises whether MRI is too sensitive in detecting abnormalities and whether these abnormalities are of clinical relevance. This is underscored by a population based study by Guermazi et al. that found an overall prevalence of any MR abnormalities to be 89% in patients without radiographic OA (KL 0) over 50 years, which is extremely high. Interestingly, the prevalence of MR abnormality was high in both patients with painful knees (91%) and without painful knees (88%) [21].

- **Synovitis and contrast enhancement in knee OA**

The importance of contrast enhancement in evaluating synovitis on MRI is well recognized in distinguishing synovitis from effusion [11,13,22,23] and is illustrated by Figure 2 It is extremely difficult to assess synovitis on non-CE-MR images. Therefore, synovial inflammation was scored on CE-MR images in most of our studies. Due to possible side effects (mostly concerning the kidney) and associated costs, contrast enhancement has not been widely used in large OA cohorts [24]. However, the contrast agent used in our study Gd-DOTA (Dotarem,Guerbet) produces very little side effects.

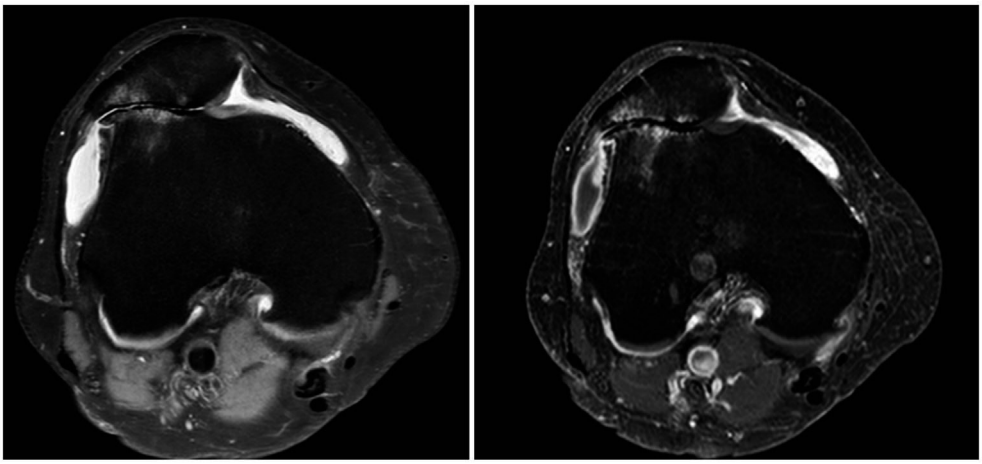

Figure 2. Axial proton density weighted (PDW) image (left) and Gadolinium (Gd)-chelate enhanced T1-weighted image (right) of same OA patients showing more severe synovitis on the medial site (arrow) of the knee on Gd-chelate enhanced image, whereas on the PDW image it is suggested that synovitis is more pronounced at the lateral side of the knee (left image) (adopted from NTvR,2013(4),45-47).

Synovitis throughout the disease course in knee OA

Only a few studies investigated synovial inflammation in synovial tissues in different severity stages of knee OA and the results were conflicting, as some authors found synovitis and cytokine expression more pronounced in patients with "late" OA undergoing arthroplasty[3,25,26] whereas others reported the opposite [6,27] or did not find differences[28]. However, to fully understand the role of inflammation in the disease course of OA, we believe it is of importance to understand whether synovial inflammation precedes structural damage or whether it is a response triggered by the OA process. Further investigation of synovial cells at different disease stages of OA could provide insight into this question, as it could indicate which immune cells infiltrate the synovium in early stages of the disease and at which stage immune cells become activated.

Synovitis in knee OA is not a homogeneous process but affects some anatomical sites in the knee more frequently than others (**chapter 6**). This suggests that synovitis seems to be a response to local triggers rather than a generalized inflammation response. We hypothesized that synovitis could either be triggered by cartilage breakdown products, trauma of structures of the knee or enhanced loading properties.

We found that synovial inflammation was most severe in end-stage knee OA in histological samples as well as assessed on CE-MRI (**chapter 5, chapter 6**) However, when synovitis was investigated over 2-year period, synovitis was found to have a fluctuating nature and did not significantly increase over time (**chapter 8**). These results suggest that degree of inflammation

does not follow a linear increase over time, but tend to worsen in patients with end-stage knee OA. Suggesting that synovitis is a process marker and perhaps a catalyser rather than a causative agent of cartilage deterioration. Which seems in contrast with previous literature suggesting that synovitis precedes cartilage damage [6]. Differences could in part be explained by the fact that there is no consensus on the definition of early OA.

The role of synovitis in structural damage in OA

An important focus in OA research is the development and progression of OA structural damage. Therefore we investigated especially the relationship between synovitis and radiographic damage in cross-sectional analysis (**chapter 4-6**) or between synovitis and structural OA progression in longitudinal analysis (**chapter 7 and 8**). As we learned from **chapter 5 and 6**, synovitis was associated with radiographic damage, although effusion/synovitis seemed to be of lesser importance in radiographic progression when we took also other joint abnormalities in account in a principal component analysis (**chapter 7**). However, the lack of association between effusion/synovitis and radiographic progression found in **chapter 7** could be explained by the fact that no contrast enhancement was used and by the fact that effusion/synovitis did load on components that were associated with progression. Interestingly, in **chapter 8** we found that synovitis change was associated with cartilage deterioration over time. However, it remains unknown whether synovitis acts as an amplifier in the OA process or precedes structural damage. The results in the current thesis suggest that synovitis is a part of the pathophysiological mechanism that leads to structural damage in knee OA and therefore suggests a role for synovitis as a target for disease-modifying treatment.

Malalignment in knee OA

Malalignment of the knee (e.g. varus or valgus deformity) has been suggested in various OA processes. In the current thesis association of patterns of MR abnormalities with radiographic progression in **chapter 7** could in part be explained by varus malalignment, which is often observed in OA. Varus alignment has been shown to be a risk factor for the development of BMLs in the medial TFJ [29-31] and leads to progression of OA in the medial compartment[31,32]. Furthermore, in patients with a varus malalignment the q-angle of the patella increases resulting in an increasing medial patellar force and increased load on the medial compartment of the patella and subsequently in PFJ progression[33-35]. Valgus malalignment has been shown to be a risk factor for development and progression of the lateral knee compartment[36]. In conclusion, malalignment could prove to be of importance in understanding the relationship between joints in the knee and progression in knee OA. It seems conceivable that malalignment would alter the weight baring properties in the knee leading to progression of structural damage and consequently to increased synovitis perhaps also explaining pattern of synovial inflammation observed in **chapter 6**.

Synovitis as determinant of pain

Previous literature has suggested that synovitis is an important determinant of pain. Therefore, a large part of this thesis was aimed to investigate the association between pain and synovitis (**chapter 3-6 and chapter 8**). Interestingly, synovitis at especially the parapatellar sites was more prone to pain experience than synovitis at other sites (**chapter 6**). Earlier studies on the innervation and pain sensation of the knee showed that the patellar region is richly innervated and that anterior synovial tissue in the vicinity of the patella is very sensitive to pain stimulation[37,38]. These findings suggest the location specific role of synovitis on subjective pain and could provide an opportunity for local treatment of synovitis and pain.

Although, we did find an association with pain (**chapter 3, 5 and 6**) overall the effect sizes were not high. In **chapter 8** synovitis change was only associated with change of pain in an unadjusted model, however cartilage deterioration was associated with change in pain. This could suggest that cartilage deterioration is a mediator in the association with pain. In conclusion synovitis therefore seems to contribute, but seems not to be the only factor in underlying biological mechanism in experiencing pain in knee OA patients. Another explanation for the low effect sizes lies in the fact that subjective pain and especially chronic pain is not only determined by biological processes, but is determined by several biological, psychological and social processes interacting creating a biopsychosocial pain model [39-41].

FUTURE PERSPECTIVES

We started this thesis in **chapter 2** with a research agenda for the investigation of synovitis. We conclude this thesis by introducing topics for a new research agenda for future investigations, aiming to fill the gaps of current knowledge concerning the role of synovitis in knee OA that will lead to development of disease modifying drugs.

- Consensus in defining OA severity stage.

To further understand whether synovitis precedes knee OA or whether synovitis is a process marker in OA, it is imperative to have a clear definition of OA. Especially, consensus should be reached on defining different severity stages in knee OA. Currently intensive work is performed to reach a consensus for the definition of “early osteoarthritis”.

- Understanding synovitis throughout the disease course of knee OA

To understand the role of inflammation in the disease course of OA, more longitudinal data of larger groups over longer periods of time are necessary. Furthermore, to fully understand the role of immune cells throughout the disease course it would be interesting to investigate synovial biopsies at several time points throughout the disease course. This would provide insight into the kinetic of cell infiltration and activation during the disease, which could lead to a better understanding of the cellular mechanisms involved in disease progression.

- Further investigation of the role of CD4+ T cells in pain

To further investigate the role of T cells in pain perception it would be interesting to see whether T cells accumulate in the vicinity of nerve endings. Interestingly, in **chapter 3** we found an association of CD4+ T cells with pain. Furthermore, we found that histological inflammation is correlated with inflammation seen on MRI (**chapter 5)**. Therefore, it would be interesting to see whether T cells accumulate at the anatomical sites that formed a pattern that was associated with pain in **chapter 6.**

- Further investigation of the role of mast cells as possible target for therapy in patients with OA

The data presented in this thesis suggest a role for mast cells in the disease process in OA. Future studies could focus on a better characterization of mast cells in OA, for example through large-scale mRNA sequencing, which could provide insight into the possible effector molecules expressed by mast cells, as well as the possible mechanism of activation of mast cells in OA. Furthermore, investigating soluble mediators released upon mast cell activation and their effect on bone/cartilage could contribute to our understanding of the role of mast cells in the pathogenesis of knee OA. Finally, the possibility of using disease modifying drugs that target mast cells should be further explored in pre-clinical and clinical studies of OA.

- Synovitis on CE-MRI in other inflammatory diseases

Although CE-MRI compares well with histological signs of inflammation in knee OA patients, it is unknown whether CE-MRI could be of use in the differential diagnosis of inflammatory diseases in research or clinical practice. Therefore, it would be interesting to see whether the synovitis scoring system by Guermazi et al. could be applied in RA or other inflammatory arthritis diseases.

- Alternatives for visualization of synovitis

Although CE-MRI constitutes a non-invasive method to investigate synovitis in geMstoan study a large proportion of patients could not undergo MRI due to contra-indications for MRI. As OA is a disease of the aging population this is more of a problem in OA compared to other inflammatory arthritis diseases such as RA or SpA. Therefore, other non-invasive methods should be explored to investigate synovial inflammation in knee OA patients. A promising alternative is ultrasound of the knee. Ultrasound is not only is able to visualize synovitis but also has the ability to visualize osteophytes and cartilage damage in multiple planes. Only recently an ultrasound scoring method has been developed, that compares well with radiographic features of OA [42]. It would be interesting to see how well this score compares with abnormalities as seen on MRI, especially with synovitis on contrast enhanced MRI. When ultrasound proofs to be a valid alternative for contrast enhanced MRI it will offer an opportunity to follow synovitis in clinical practice for experienced users of ultrasound.

- To understand the role of biomechanics in knee OA

Especially in the knee OA biomechanics seem to be of importance in developing structural damage and could explain why structural abnormalities in the PFJ and TFJ are related. Investigation of association of patterns of MRI abnormalities with malalignment of the knee could provide more insight. Furthermore, varus or valgus malalignment could also explain the different patterns of synovitis and therefore it would be interesting to whether patterns of synovitis could be explained by either varus or valgus abnormalities.

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