IMAGING TISSUE CHARACTERISTICS
IN OSTEOARTHRITIS AND RHEUMATOID ARTHRITIS

Nick Besselink

The research described in this thesis was financially supported by the Dutch Arthritis Association (ReumaNederland), LSH Impuls subsidy (Health~Holland), Hemics bv, and the department of Rheumatology, University Medical Center Utrecht.

Printing was financially supported by Chipsoft bv, Hemics bv, Pfizer bv, and Sanofi Genzyme.

ISBN: 978-94-6380-011-2.

Printed by: ProefschriftMaken || www.proefschriftmaken.nl
Cover design by: Remco Wetzels.

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BEELDVORMING VAN WEEFSELKARAKTERISTIEKEN
IN ARTROSE EN REUMATOÏDE ARTRITIS

(MET EEN SAMENVATTING IN HET NEDERLANDS)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. H.R.B.M. Kummeling, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op woensdag 31 oktober 2018 des middags te 2.30 uur

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geboren op 3 maart 1989 te Arnhem

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CONTENTS

CHAPTER 1

General introduction

The musculoskeletal system

The musculoskeletal system is a complex system allowing body movement and simultaneously providing shape, support, and stability. It consists of bone, cartilage, muscles, tendons, and ligaments. Bones come together in joints that allow movement to a variable extent. Synovial joints, the most common and mobile joint type, are characterized by a synovial capsule in which bones contact, covered by a layer of hyaline articular cartilage, within a fibrous joint capsule (see chapter 4 – figure 1). Examples of synovial joints are the knee, the metacarpophalangeal (MCP) joints, and intercarpal (IC) joints of the hand and wrist.

Synovial joint

Synovial membrane

The articular capsule consists of an outer fibrous layer made up of dense connective tissue, and is attached to the bone margins of the joint. Inside of the outer fibrous layer is a more cellular capsular layer, the synovial membrane. This membrane consists of an intimal lining and supportive sublining layer. The intimal lining is in direct contact with the joint cavity and contains many macrophage-like and fibroblast-like synoviocytes, the latter producing and maintaining lubricious synovial fluid. The two major lubricating components are lubricin (also called proteoglycan 4; PRG4) and hyaluronic acid (HA).[1]

Cartilage

Articular cartilage is highly specialized connective tissue, providing a low-friction surface for articulation and facilitating transmission of load. Healthy articular cartilage, i.e. hyaline cartilage, consists of an extracellular matrix (ECM) interspersed with chondrocytes (1-5% of the total cartilage volume).[2] The ECM mainly consists of collagen and proteoglycans, and is produced and maintained by these chondrocytes. Proteoglycans found in cartilage consist of a protein core with covalently attached glycosaminoglycan (GAG) chains. The negatively charged GAGs, specifically in the large aggrecan molecules, attract cations, increasing osmotic pressure and drawing water into the matrix. Within the elastic collagen network this provides the essential mechanical properties (resilience and elasticity) of articular cartilage. Cartilage is not vascularized and depends on its surroundings for its essential nutrients, delivered by diffusion. Articular cartilage is aneural and therefore incapable of directly generating pain. Cartilage has a limited regenerative capacity rate, because of the relatively low cell number and with that renewal rate of the abundant matrix. This altogether makes cartilage damage a difficult to treat, musculoskeletal disorder.[3]

Bone

Bone is a richly vascularized, calcified, connective tissue. It provides a framework for the attachment of tendons, stores essential minerals, and contains the bone marrow that

produces blood cells. Bone development, regeneration, and remodelling are continuously adapting in response to tensile, compressive, and shear forces that are generated through movement and loading. Below the layers of hyaline cartilage, is a layer of calcified cartilage that contacts with the subchondral bone at the tidemark. Biochemical factors are expected to cross this tidemark, but nerves from the bone do not, except under severe osteoarthritic conditions.[4]

Joint homeostasis

For the synovial joint to function properly, tissue turnover and interactions must be regulated.[5] The highly vascularized synovial capsule supplies the cartilage with nutrients,[6] by controlling molecular traffic in and out of the joint space through this semipermeable membrane. High-molecular-weight molecules, like HA and lubricin, do not cross the membrane, in contrast to small molecules like cytokines and chemokines.[7] The synovial fluid functions as a medium between the cartilage and synovium (see chapter 4 – figure 1). At the transition zone of subchondral bone to cartilage, biomechanical and biochemical interactions occur, so-called bone-cartilage crosstalk. Motion and weight-bearing lead to healthy crosstalk leading to bone remodelling, and repair of microdamage.[8]

Rheumatoid arthritis and osteoarthritis; pathology and treatment

Rheumatoid arthritis (RA) and osteoarthritis (OA) are diseases involving synovial joints. These diseases lead to joint degeneration, but each have their own specific clinical presentation and pathogenesis.

Rheumatoid arthritis

RA is a common chronic autoimmune disease with arthritis and other features of systemic inflammation. Although its exact cause is unknown, genetic factors do contribute,[9] and multiple environmental factors have been associated with RA as well.[10] For example, smoking accounts for 20-30% of the risk for developing RA.[11] The disease is probably initiated by dendritic cells that present self-antigens to autoreactive T cells, which in turn activate autoreactive B cells. This results in autoantibody production and immune complex deposition in joints. Synovitis (proliferation, hyperplasia, hyper-vascularisation, and infiltration) of predominantly the small joints and bone erosions are characteristic for the disease.[6,12–14]

Early intervention to control synovial tissue inflammation, is important to retard progression of tissue damage in a later disease stage. Adequate treatment during the early phase of the disease is more likely to control the long-term disease course, than similar treatment later in the course of the disease, when autocrine inflammatory and tissue destructive pathways fuel the disease.[15,16] Tight-control and treat-to-target treatment strategies with a variety of disease-modifying antirheumatic drugs (DMARDs), aimed at the individual patient, are

key nowadays. (Drug free) remission and prevention of any joint damage have become attainable goals. The challenge at present is to further optimize treatment, minimizing over-treatment with unnecessary side effects and costs.

Osteoarthritis

OA is the most common chronic condition of the joint. It has a higher prevalence than RA, affecting 1.1 million people in the Netherlands and 240 million people worldwide. Moreover, its prevalence is expected to increase over the next decades, due to aging (longevity), and a rise in prevalence and severity of obesity, already at younger ages.[17] Age, female gender, genetic predisposition, and joint injury are known risk factors for the development of OA, but also lifestyle factors, like overuse of joints, affect the risk of developing OA. In the process of wear-and-tear, OA primarily, but not solely, affects weight-bearing joints, and is increasingly recognised to affect virtually all structures within and around joints. Acute and severe synovitis are not characteristic of OA, but subclinical, low-grade synovitis is frequently observed,[18] early or later in the disease.

Treatment modalities of OA lag behind, compared to those available in RA. There still is no disease-modifying treatment, and the first step in current management is conservative, predominantly focused on patient education, pain relief, and minimizing functional disability. These do not suffice in a substantial number of patients and patients generally live with joint pain for many years. Many patients eventually need to undergo joint surgery; in the Netherlands in 2015 there was an estimated 52.800 new knee OA patients, and 28.798 knee arthroplasties were performed.[19]

The lack of disease-modifying treatment may come from a variety of factors, one possible explanation is that OA actually consists of different phenotypes that might each require different treatment approaches. For example, patients with an inflammatory OA phenotype might benefit from anti-inflammatory therapy, whereas in patients with a mechanical OA phenotype benefit is more likely to come from tissue regenerative therapy.[20,21]

A clinically recognizable subgroup of hand OA, so-called inflammatory or erosive **hand OA** is characterized by inflammatory symptoms and signs. These patients usually experience more rapid disease progression, more pain, functional impairment, and negative clinical, laboratory and ultrasonography (US) outcomes as compared to other types of hand OA.[22] Synovitis in hand joints can become apparent as joint swelling and adds to pain, functional impairment, and progression of joint damage as compared to OA joints without synovitis.[23–25] Early detection of this synovitis and therapeutic intervention might appear to be the key to retard the long-term detrimental effects of erosive hand OA and hand OA in general, and might contribute to the understanding of the pathogenic mechanisms.

OA phenotypes that are initiated and perpetuated by degenerative pathology, intensified by wear-and-tear, are able to drive the disease independently of inflammation. There is special

emphasis on **knee OA** phenotypes due to its predominance as an osteoarthritic disorder, its impact on quality of life, and the accessibility to the variety of tissues in that joint.[26]

Monitoring disease activity and treatment

Disease progression and treatment effects can be monitored through clinical and/or structural outcomes. Although both can be essential in particular contexts, they can dissociate from one another.[27] Invasive diagnostic approaches like biopsies provide location-specific information of inflammation and tissue damage but require opening or puncturing the joint and potentially disturbing or worsening the joint homeostasis. Biochemical markers can provide dynamic insights into a variety of disease mechanisms, but frequently lack specificity for local processes. Clearly, **novel, non-invasive tools for diagnosis, monitoring disease activity and progression, and stratification of synovial joint pathology are needed.**[28,29] Imaging may provide these tools.

Synovitis, presenting itself (sub)clinically, whether in early RA or secondary in OA is one of the initial quantifiable disturbances of joint homeostasis. Synovitis can be assessed through currently available imaging modalities, such as magnetic resonance imaging (MRI) and US. They are sensitive, related to histological signs of inflammation, and more and more readily available in general hospitals. Standardised scoring systems have been developed, or are currently being evaluated, for both.[30–33] However, imaging multiple joints over time is time-consuming, costly, and interpretation is to some extent subjective. Therefore, MRI and US are less suitable for application in tight-control, treat-to-target treatment strategies in daily practice. Various imaging techniques have become available that might be less subject to these shortcomings, i.e. capable of quantifying inflammation in multiple joints, objectively, with short acquisition times.[34,35] Of the presently available techniques, **optical spectral transmission** (OST) has some advantages. It does not require an intravenous agent or mandatory blood screening, like in indocyanine green (ICG) contrast-enhanced fluorescence optical imaging. It automatically generates an objective, quantitative score.[28] An OST measurement takes little time, it requires limited training and can be performed by a nurse. OST could be a promising technique for tight-control and treat-to-target strategies of the smaller joints like those of hands and wrist, and applicable in common clinical practices. However, feasibility, reliability, and validity of the technique, as well as effectiveness and cost-effectiveness of OST in clinical practice need further research.

The need for monitoring joint-sparing treatment in OA

There are no therapeutic treat-to-target treatment strategies for OA, because effective disease modifying osteoarthritis drugs (DMOADs) are lacking.[36] However, disease modification by means of surgical treatment is possible, with interventions such as high tibial osteotomy (HTO) and knee joint distraction (KJD). In these joint-sparing treatments the main

focus lies on pain relief, function improvement, and **cartilage tissue regeneration**. Disease modification is therefore evaluated by pain relief combined with the change in cartilaginous tissue (characteristics). Currently available imaging techniques still have limited resolution at the level of tissue damage and repair. Therefore, they perform best in the large joints, like the knee. Due to the limited penetration depth of US, US imaging is less suitable for these larger joints. For instance, power Doppler in a hip joint is not sensitive.[37] MRI has provided us with several advantages over the current gold standard of conventional radiography. MRI machines do not emit ionizing radiation. The technique is suited for soft tissue imaging and is capable of generating isotropic 3-D images. Administering a contrast agent, like gadolinium, enhances and improves the quality of MRI images. Apart from quantitative assessments (like of thickness and volume) also qualitative changes can be studied. However, these more sophisticated MRI approaches too need further evaluation before implementation in clinical practice can be considered.

Aims and outline of this thesis

This thesis consists of two parts that together address the potential role of imaging techniques in monitoring essential tissue characteristics in early onset of disturbed joint homeostasis, and after disease-modifying treatment. The first part will deal with the role of synovitis in OA. The role of imaging synovitis in small hand joints in (the disease-modifying treatment of) RA and hand OA will also be covered. The second part will deal with imaging cartilage tissue regeneration, in the disease-modifying treatment of knee OA.

Part I

As stated by K. Haugen and colleagues,[28] "*To optimise medical treatment in rheumatic diseases, it is important to correctly assess the presence of inflammation, and optical imaging modalities may be useful supplements.*" Accordingly, **chapter 2** discusses OST for the assessment of joint inflammation in hand and wrist joints of patients with early RA. **Chapter 3** describes the set-up of a multicentre, double-blind, randomized, clinical trial, studying whether HandScan (OST) guided treat-to-target treatment strategy is at least as effective and more cost-effective than conventionally guided treat-to-target strategy.

Chapter 4 describes the role of the synovium and capsule in OA, including contributing and perpetuating factors in synovitis, pathways promoting synovitis, clinical impact, and possible therapeutic approaches. The relevance of synovitis in (erosive) hand OA led to the explorative study in **chapter 5**, investigating whether optical spectral transmission can assess synovitis as detected by US, in hand OA.

Part II

Chapter 6 discusses radiographic signs of sustained cartilage tissue repair in addition to clinical benefit two years after two different surgical joint-sparing treatments for knee OA. Knee distraction (KJD) is a more recently introduced joint-sparing treatment,[38–42] with sustained beneficial effects at long-term follow-up in an open prospective study.[43] High tibial osteotomy (HTO) is a more established joint sparing treatment for medial compartment knee OA. Two-year follow-up of two randomised controlled trials (RCTs) comparing KJD with HTO and KJD with total knee arthroplasty (TKA) are described. **Chapter 7** compares the change in knee alignment that is brought about by HTO and KJD. The efficacy of HTO relies on a permanent shift of the mechanical axis of the leg, decreasing the load on the medial compartment, providing a chance for cartilage tissue repair. However, previous research into the effects of KJD on the treatment of medial compartment OA suggested that KJD might also improve alignment, by inducing cartilage tissue repair in the medial compartment. **Chapter 8** describes the cartilage regenerative effects of KJD and HTO, as previously observed on radiographs, at the level of cartilage quality, as reflected by GAG content. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) is evaluated two years after KJD and HTO. The relation between dGEMRIC outcomes and radiographic outcomes is addressed as well.

Chapter 9 summarizes all chapters. Imaging of inflammation in small joints and imaging of cartilage tissue repair in the knee is put into broader perspective. The potential pros and cons for future application of imaging modalities in research and clinical practice are discussed.

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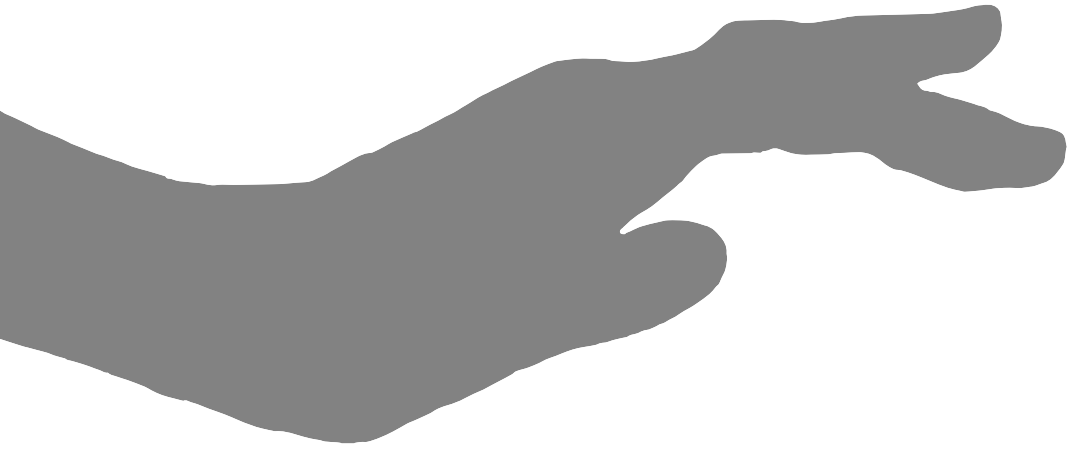
PART I

Monitoring inflammation in hand osteoarthritis and rheumatoid arthritis



CHAPTER 2

Optical spectral transmission to assess inflammation in hand and wrist joints of rheumatoid arthritis patients

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Rheumatology (Oxford) 2018;57:865-72

Abstract

Objective

To develop an optical spectral transmission (OST) model to measure joint inflammation, and thus disease activity. Moreover, to evaluate (patho-)physiological findings that could lead to misclassification of inflammation.

Methods

Forty-six RA patients were included in this cross-sectional study, where ultrasonography (US) scores, duplicate OST measurements, and DAS28 were acquired. With US as reference standard, diagnostic performance of OST in detecting inflammation at joint level was evaluated using receiver operating characteristic (ROC) curve analyses. At patient level, correlations with US were analysed for DAS28 and OST, and at joint level, for OST, tender (TJC) and swollen joint counts (SJC). Joint pathology potentially influencing misclassification by OST (erosions, osteophytes, tendon(sheath) inflammation, (ab)normal vasculature, and chondrocalcinosis) was evaluated for significance in a multivariate nominal logistic-regression model.

Results

Diagnostic performance of OST was good for MCP (AUC-ROC:0.88), PIP (AUC-ROC:0.83) and wrist (AUC-ROC:0.74) joints and for all joints together (AUC-ROC:0.85). At patient level, DAS28 correlated very poorly (ρ=0.06) and OST moderately (ρ=0.54) with US. At joint level, US correlation with OST was strong (ρ=0.64), with SJC weak (ρ=0.30) and with TJC very weak (ρ=-0.02). Misclassification of inflammation by OST was relatively rare (17%). Dorsal erosions (OR:4.0), osteophytes (OR:2.1), and extensor tendinitis (OR:4.6) increased the risk of underestimating inflammation of MCP and PIP joints, osteophytes (OR:3.0) also increased the risk of overestimating inflammation.

Conclusions

OST is a sensitive, specific, and objective technique to assess joints inflammation of hands and wrists of RA patients, even though bone and tendons pathology increases the risk of misclassification.

Introduction

Rheumatoid arthritis (RA) currently is treated according to treat-to-target strategies[1,2] aiming for low disease activity and preferably remission.[3] Current clinical disease activity measures like the 28-joint Disease Activity Score (DAS28) have questionable reproducibility and lack sensitivity for low disease activity states;[4] MRI and ultrasound (US) are sensitive, but scanning multiple joints is time-consuming, and costly. Recently, it was concluded that new imaging methods for assessing joint inflammation in RA are necessary to accurately assess changes in disease activity over time, and that it is important to evaluate feasibility, reliability, and validity of the new modalities to ascertain high quality outcome measures.[5]

A new modality is optical spectral transmission (OST); it measures the blood-specific absorption of light transmitted through tissue. This absorption is decreased in case of joint inflammation and angiogenesis. Previous research using OST has shown it is a good candidate for assessing joint inflammation of hand joints. OST is stronger than swollen (SJC) or tender joint count (TJC) associated with inflammation assessed by US.[6] When evaluating subclinical inflammation in MCP2-5 and the wrists with MRI in patients in remission (DAS28<2.8), a high correlation on the individual joint level with OST was found (RAMRIS; r=0.52, p=0.005).[6] However, high sensitivity and specificity could not always be replicated and OST performed better in PIP and MCP joints than in wrists.[6,7] To improve diagnostic performance of OST, especially in the wrist, a new light source has been implemented, with software adjusting light intensity based on an initial optical transmission map of the hand, tailoring light transmission to the individual patient's hands.
OST measures light transmitted through tissue, which is reduced in presence of joint inflammation, e.g. related to RA. Additionally, tissue changes, such as osteophytes or erosions, might also influence light transmission, leading to over- or underestimation of joint inflammation.

The aim of this study was to develop an OST model to measure joint inflammation, and thus disease activity in patients with Rheumatoid Arthritis. Moreover, (patho-)physiological factors that could lead to misclassification of inflammation were evaluated.

Methods

Subjects
Fifty patients with RA according to ACR/EULAR 2010 criteria were recruited at the outpatient clinic of the department of Rheumatology & Clinical Immunology at the University Medical Center Utrecht (UMC Utrecht), between May and September 2016. Of 4 patients, a reliable OST value at the patient level could not be established because of movement or incorrect positioning of hands during the scan, leaving data of 46 patients for analysis. To be eligible

for inclusion, patients with RA had to have at least one swollen finger or wrist joint, be between 18 and 90 years, have a recent (≤ 2 weeks) ESR value, and to be able to give informed consent. Exclusion criteria were obvious deformations of the hand, hand or wrist joint prostheses, other inflammatory diseases that could explain pain or swelling in the hand joints (e.g. psoriatic arthritis, gout), intra-articular treatment with glucocorticoid injection in the hand or wrist joints within 3 months, trauma or surgery of the hand and wrist joints within 6 months prior to the study, light sensitivity (i.e. Erythropoietic protoporphyria), and photodynamic therapy.

Assessments were US evaluation, OST measurements, and DAS28, all systematically performed according to established guidelines by separate experienced examiners, blinded for other study outcomes. All measurements were performed within 1.5 hours at the UMC Utrecht. The study complied with the Declaration of Helsinki. The study was approved by the Ethics Committee of the UMC Utrecht (*NL 50848.041.15*). All patients provided written informed consent before enrolment.

Ultrasonography

US was performed by one experienced examiner (PvdM, physician assistant rheumatology) using a MyLab 60 system (Esaote, Genua, Italy) with an 18-6 MHz linear array transducer. Patient and probe positioning were according to EULAR guidelines.[8] Joint regions assessed were the wrists, and in the hands; metacarpophalangeal (MCP) 1-5, proximal interphalangeal (PIP) 2-5, interphalangeal 1 (IP1). Assessed were synovitis of joints, and in addition, as factors potentially leading to misclassification by OST, tendinitis/tenosynovitis, dorsal vascularity at the MCP and PIP joint region, osteophytes, erosions, and chondrocalcinosis. Synovitis of joints was assessed at all joint regions, both the dorsal and volar aspect, except at the wrist, where radio- and intercarpal (RC and IC) joints were only scored dorsally. Greyscale US (GSUS) findings, combining joint effusion and synovial thickening,[9] and power Doppler (PDUS) findings were scored according to Outcome Measurements in Rheumatology Clinical Trials (OMERACT)[10,11] on a semiquantitive scale (0-3). Tendinitis/tenosynovitis findings of extensors and flexors at the joint regions were scored by longitudinal (LT) scan, if GSUS > 0, for the presence or absence of PDUS. The hand's superficial venous system is located mainly at the dorsal side of the hand and has a highly variable pattern. Dorsal vascularity at the MCP and PIP joint regions could be confused with tendinitis/tenosynovitis of the extensor tendon, but could be differentiated by recognition of interposed normal connective tissue.[12] This vascularity was scored as present or absent. Osteophytes, as cortical protrusions, were scored at all dorsal joint regions, as present or absent. Erosions were defined as discontinuities of the joint bone surface visible in two perpendicular planes; the dorsal and volar joint regions were separately scanned and scored for erosions as present or absent.[13] Chondrocalcinosis, defined as focal hyperechoic deposition at fibrocartilage complex (TFCC) level, was scored dorsally in LT view, at the extensor carpi ulnaris tendon superficial to the

ulnar-carpal joint, as present or absent.

Since grade 1 GSUS synovitis has been found in healthy subjects and is of limited prognostic value in RA,[14,15] US inflammation was defined as GSUS synovitis >1 or PDUS synovitis >0 or GSUS/PDUS tenosynovitis>0. For individual subjects, the number of joints with inflammation was counted (US joint count). Also, the sum of GSUS synovitis, GSUS tenosynovitis, PDUS synovitis and PDUS tenosynovitis scores were calculated (US joint index).

Optical spectral transmission measurements

OST measurements were performed with the HandScan (*Hemics BV., Eindhoven, The Netherlands*) operated by a rheumatology nurse. Both hands were inserted through cylindrical openings that contain pressure cuffs. Scanning laser light (wavelengths of 660 and 808 nm) illuminated the (P)IP, MCP and wrist joints of both hands and reference areas, all from the palmar side. Light transmitted through the joints and reference areas was recorded continuously at the dorsal side by a CMOS camera at a rate of four frames per second, alternatingly for the 660 nm wavelength and the 808 nm wavelength. A complete measurement consisted of four phases and was performed within 100 seconds: first, inflation of the cuff to 5 mm Hg (10 s); second, inflation of the cuff to 50 mm Hg; third, maintaining 50 mm Hg (60 s) and finally deflation of the cuff to 5 mm Hg (30 s), see supplementary figure 1. Regions of interest (ROI) were traced automatically for all joints regions (joint ROI) and automatically for a region distally to each joint (reference ROI), based on the pictures taken by the CMOS camera. Reference ROIs allowed for correction for systemic effects unrelated to inflammation, such as body temperature and use of vasoactive medication, see supplementary figure 2. OST measurement was performed twice, before and after US, with approximately 20 minutes of rest after US examination (to enable normalization of blood flood), to evaluate the test-retest reliability of OST.

Clinical assessment

Subjects filled out a global assessment of disease activity regarding the past two days on the visual analogue scale (VAS; 0 – 100). Swollen (SJC) and tender joint counts (TJC) was performed in 28 joints by trained research nurses in all subjects. With recent ESR values available, disease activity (DAS28-ESR) was calculated.

Statistical analysis

Development and diagnostic performance of OST

To convert light transmission data into joint inflammation data of individual joints, an algorithm had to be developed. This process consisted of image analysis, model development and model validation (for a more detailed description see *supplementary material – Development and validation of the OST model*). This model provided an OST joint index for each joint (P)IP1–5, MCP1–5 and the wrists of both hands; OST index range: 0-3), and a total

OST index, being the weighted average of all joints involved times 22 (maximum number of joints, OST index range: 0-66). For the development of the model, duplicate measurements were used. For all other analyses, duplicate OST indices were averaged, given the excellent results of test-retest reliability of OST, evaluated by Pearson's correlation coefficient, ICC, means, and visualized using Bland-Altman plots, at both joint (individual joint OST) and patient level (total OST) (see details in *supplementary material – Test-retest reliability of OST*). Diagnostic performance of OST and of TJC and SJC was compared with US as gold standard (scoring joint inflammation as absent or present), by receiver operating characteristic (ROC) curve analyses with 95% confidence interval (95%-CI) estimation. For OST, this was done for the following joint/joint groups: (P)IP1–5, MCP1–5, wrist joint, and separately for all joints together. For the assessments averaged, there were 1003 joints for analysis: 11 joints, bilateral assessments: 22 joints per patient, 46 patients, and in total 9 individual joint of 5 patients excluded, because of tattoo's, or rings that could not be removed. To evaluate diagnostic performance with that of previously published OST research,6 differences in area-under-the-curve (AUC) of ROC curves were tested for statistical significance using a Z test for equality.

Correlation of OST with US and physical examination

In the ROC analyses for all joint values together (n=1003), for each discrimination threshold of the curve, Cohen's Kappa values were calculated;[16] the cut-off for OST inflammation (present or absent) was based on the discrimination threshold with the highest Kappa value. At patient level, Spearman's rank correlation coefficients of US were computed with DAS28 and total OST (46 patients, one OST per patient). DAS28, being a composite score, comprises of more factors than physical examination alone, potentially decreasing correlation with US, moreover DAS28 also encompasses joints other than the hand and wrist, therefore at joint level, the sum of inflamed joints as determined by OST and US are compared to the TJC and SJC of the hand and wrist joints only (MCP 1-5, (P)IP 1-5 and wrist, bilaterally; a total of 22 joints per patient).

Influence of (patho-)physiological findings on misclassification of inflammation by OST

To determine which (patho-)physiological factors caused misclassification by OST, 2 by 3 tables were created where the presence or absence of a factor was tabulated against classification (3 classes). These 3 classifications were a) false negative: joint inflammation at US, but not at OST; b) false positive: joint inflammation at OST, but not at US; c) true positive and true negative combined: joints with both imaging modalities scored as inflamed or not inflamed. Pearson Chi-Square tests were used to determine whether a factor was related to the distribution of classification. If the Chi-Square p-value was <0.1, the variable was selected for further analysis. This was a multivariate nominal logistic regression with the classification (a, b versus c as reference) as dependent nominal variable, and the selected variables as independent variables. Variables with no statistically significant independent

contribution to misclassification (whether false negative, false positive or both) were removed one-by-one from the model starting with the least significant one, to arrive at the final model.

The multiple regression analyses to develop the OST model were performed using Hemics in-house software (*InFlame RA-160205, November 3, 2016*), all other analyses by SPSS (*IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.*). All tests were two-sided; p-values < 0.05 were considered statistically significant.

Results

Patient demographics are reported in Table 1; 71% of the patients was rheumatoid factor positive, and 71% aCCP positive. Patients had a moderate disease activity: mean (SD) DAS28: 3.9 (1.20). All subjects tolerated both OST measurements without any adverse events.

Development and diagnostic performance of OST

An OST model was developed for assessing inflammation in the hand and wrist joint of RA patients. Details on development and validation of this model are discussed in the *supplementary material – Development and validation of the OST model*. OST performed well at joint level, separately for the MCP 1-5 (AUC-ROC: 0.88, 95%-CI: 0.84 to 0.92), (P)

Table 1: Patient demographics and clinical, US and OST data.

	n=46
Age in years	60 (13)
Female (%)	33 (72%)
RF positivity (%)	29 (71%)
aCCP positivity (%)	29 (71%)
Swollen joint count (0-28)	4 (IQR: 2 - 6, range: 0 - 18)
Tender joint count (0-28)	6 (IQR: 2 - 10, range: 0 - 23)
VAS	45 (IQR: 28 - 73, range: 0 - 94)
ESR	8 (IQR: 3 - 15, range: 0 - 47)
DAS28-ESR	3.9 (1.20)
Swollen joint count (0-22)	4 (IQR: 2 - 6, range: 0 - 18)
Tender joint count (0-22)	5 (IQR: 2 - 9.3, range: 0 - 20)
US inflamed joints (0-22)	2 (IQR: 0 - 5, range: 0 - 22)
OST inflamed joints (0-22)	4.5 (IQR: 1.5 - 6.6, range: 0 - 18)

Numbers are presented as mean (SD) or median (IQR, range) unless mentioned otherwise. Examined joints: shoulders, elbows, wrists, MCP and (P)IP joints of hands, and knees. US inflammation was defined as GSUS synovitis >1 or PDUS synovitis >0. RF, rheumatoid factor; ACPA, anti-citrullinated protein antibody; ESR, erythrocyte sedimentation rate; DAS, Disease Activity Score; US, ultrasound; OST, optical spectral transmission.

IP 1-5 (AUC-ROC: 0.83, 95%-CI: 0.77 to 0.88), and wrist (AUC-ROC: 0.74, 95%-CI: 0.63 to 0.84) joints and for all joints together (AUC-ROC: 0.85, 95%-CI: 0.83 to 0.88), see figure 1. Compared to the use of an LED light source in previous research, implementation of a laser light source in the current HandScan resulted in an increased performance for MCP (ΔAUC-ROC: 0.10, 95%-CI: 0.03 to 0.17, p<0.01), PIP (ΔAUC-ROC: 0.04, 95%-CI: -0.05 to 0.13, p=0.35), wrist (ΔAUC-ROC: 0.11, 95%-CI: -0.04 to 0.26, p=0.15) and all joints (ΔAUC-ROC: 0.05, 95%-CI: 0.00 to 0.09, p= 0.06).[6]

Correlation of OST with US and physical examination

Optimal cut-off of OST, at Kappa of 0.47 (95%-CI: 0.40 to 0.54) for all joints together, yielded a sensitivity of 0.60 (95%-CI: 0.53 to 0.67) and a specificity of 0.89 (95%-CI: 0.86 to 0.91). SJC, at a Kappa of 0.30 (95%-CI: 0.24 to 0.37), yielded a sensitivity of 0.46 (95%-CI: 0.39 to 0.52) and a specificity of 0.86 (95%-CI: 0.84 to 0.88), whereas TJC, at a Kappa of 0.24 (95%-CI: 0.18 to 0.30), yielded a sensitivity of 0.50 (95%-CI: 0.44 to 0.57) and a specificity of 0.78 (95%-CI: 0.76 to 0.81).

At patient level, total OST scores were compared to US joint count and DAS28 using Spearman correlation. DAS28 correlated very poorly (ρ=0.06, 95%-CI: -0.26 to 0.36, p= 0.71) with US, while OST correlated moderately with US (ρ=0.54, 95%-CI: 0.28 to 0.73, p<0.01). For the sum of affected joints per patient, US correlation with OST was strong (ρ=0.64, 95%-

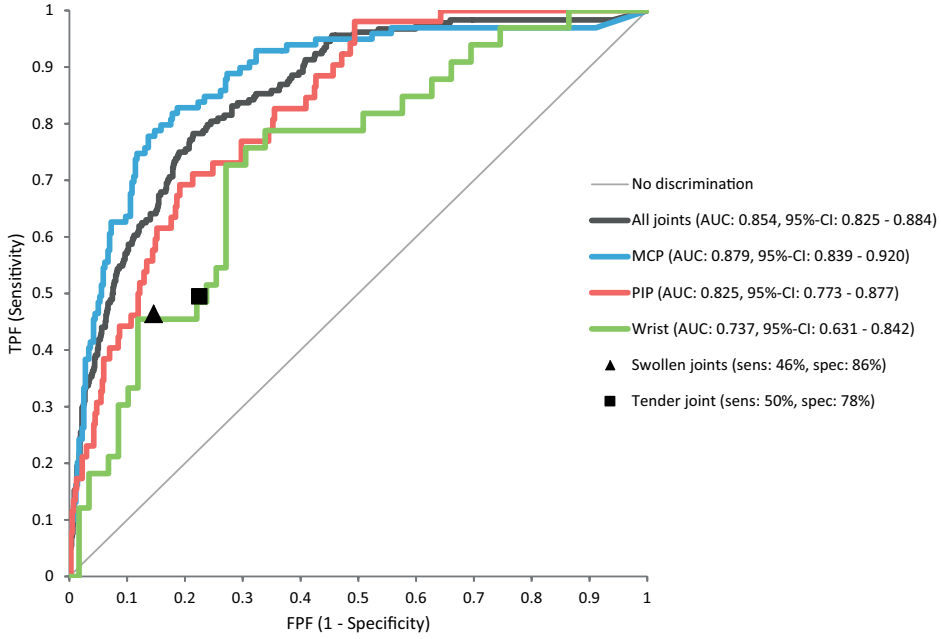


Figure 1: Receiver Operating Characteristic (ROC) curve analysis. Area under the curves (AUC) for optical spectral transmission (OST) versus ultrasonography (US) in all joints, and separately versus US of MCP, PIP, and wrist joints. Sensitivity and specificity of tender and swollen joint assessments (physical DAS28 components) with US as reference.

CI: -0.43 to 0.78, p<0.01) with SJC was weak (ρ=0.30, 95%-CI: 0.11 to 0.46, p<0.01), and with TJC was very weak (ρ=-0.02, 95%-CI: -0.21 to 0.17, p= 0.84).

Influence of (patho-)physiological findings on misclassification of inflammation by OST

A total of 1003 joints was evaluated by both OST and US. Inflammation in 167 (17%) joints was misclassified by OST, using US as reference standard. Out of these misclassified joints, inflammation in 94 joints (9%) was overestimated, and inflammation in 73 joints (7%) was underestimated. In table 2, the frequency of classification per (patho)-physiological variable is presented. The prevalence of the different (patho-)physiological findings in this population varied, as did the side of the hand they most often occurred. Erosions were rare, observed more frequently on the dorsal than volar side (resp. 3% vs 1%). Tendon inflammation was more frequent in flexors than extensors (resp. 5% vs 2%). Presence of a vascular pattern adjacent to the joint was seen in 16% of the joint regions. Osteophytes were observed in 21% of the joint regions. Chondrocalcinosis at TFCC was only observed in 2% of the wrist joints. All (patho-)physiological findings were associated with misclassification in a minority

Table 2: Presence of classification per (patho)-physiological variable, n (%).

		TP & TN	FN	FP	Total
Erosions at dorsal scanning	*Absent*	757 (85)	59 (6.7)	70 (7.9)	886 (100)
	Present	16 (64)	7 (28)	2 (8.0)	25 (100)
Erosions at volar scanning	*Absent*	769 (85)	63 (7.0)	72 (8.0)	904 (100)
	Present	4 (57)	3 (43)	0 (0)	7 (100)
Osteophytes	*Absent*	632 (88)	43 (6.0)	43 (5.9)	718 (100)
	Present	141 (73)	23 (12)	29 (15)	193 (100)
Flexor tendinitis	*Absent*	730 (85)	60 (7.0)	71 (8.2)	861 (100)
	Present	43 (86)	6 (12)	1 (2.0)	50 (100)
Extensor tendinitis	*Absent*	761 (85)	62 (7.0)	69 (7.7)	892 (100)
	Present	12 (63)	4 (21)	3 (16)	19 (100)
Vascular pattern	*Absent*	657 (86)	55 (7.2)	56 (7.3)	768 (100)
	Present	116 (81)	11 (7.7)	16 (11)	143 (100)
Chondrocalcinosis	*Absent*	62 (69)	7 (7.8)	21 (23)	90 (100)
	Present	1 (50)	0 (0)	1 (50)	2 (100)

TP: true positive classification, TN: true negative classification, FN: false negative classification, FP: false positive classification. Presence of (patho)-physiological findings of a misclassified joint could be coincidence, does not necessarily indicate a causal relation. Prevalences of misclassifications in the presence of a (patho)-physiological finding for dorsal erosion, volar erosions, osteophytes, flexor tendinitis, extensor tendinitis, vascular pattern adjacent to the extensor tendon and chondrocalcinosis are 36%, 43%, 27%, 14%, 37%, 19%, 50%. of the respective cases.

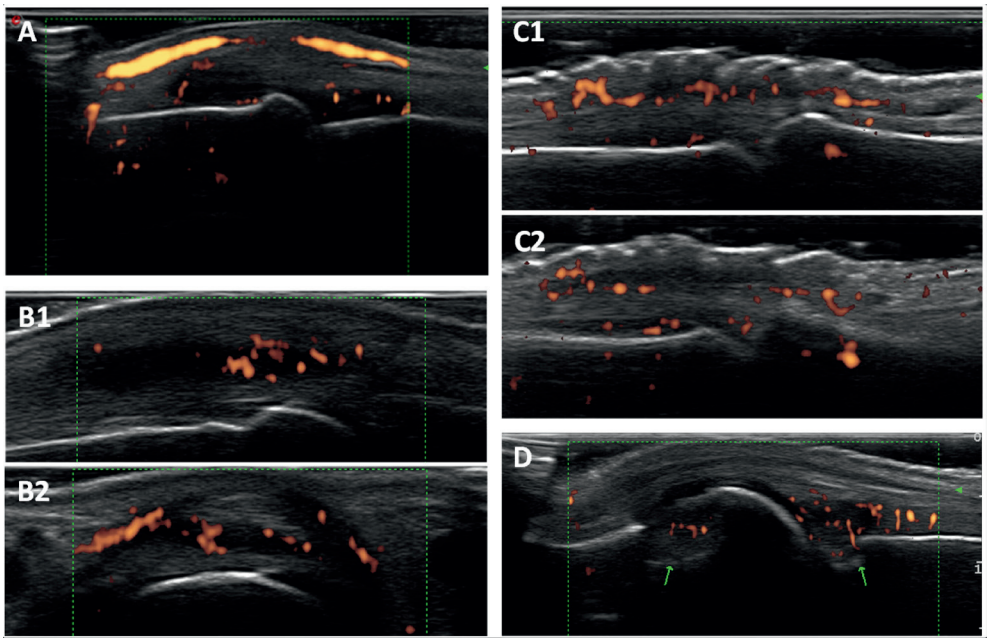


Figure 2: Examples of ultrasound (US) assessment of joint inflammation, focusing on (patho-)physiology potentially misclassifying inflammation by OST. Power Doppler was performed only if the gray scale synovitis score was positive, or in case of an obvious hypo- or anechoic lining adjacent to the extensor tendon. All joints visualized in longitudinal view, oriented with distal end on the proximal side. (A) Vascular pattern adjacent to extensor tendon, and synovitis of MCP 1; true positive classification. (B) Extensor tendinitis without synovitis of MCP 3; true negative classification. Two images from the same joint, first longitudinal scan (B1) inconclusive for tendinitis, therefore an additional transverse scan (B2) was made for objective scoring of extensor tendinitis. (C) Extensor tendinitis (specifically peritendinous vascularization) and synovitis of PIP 2; false negative classification. Two images from the same joint taken in slightly different longitudinal planes to focus on extensor tendinitis (C1) and synovitis of the joint (C2). (D) Active erosive synovitis (green arrows) and synovitis of MCP 1; false negative classification.

of cases (Table 2). No false positive classification was seen in joints with volar erosions. Chondrocalcinosis was only assessed at the wrist (TFCC) and only present twice. Chi-square tests were statistically significant for all variables except for chondrocalcinosis, abnormal vasculature, and flexor tendinitis. With chondrocalcinosis excluded, remaining (pathological) findings relate to MCPs and PIPs only. Figure 2 shows examples of US assessments of joints with (patho-)physiology: a vascular pattern adjacent to the extensor tendon, extensor tendinitis and erosive changes. Cases 2A and 2B were classified correctly by OST, whereas 2C and 2D were misclassified.

In the final multivariate logistic regression model, dorsal erosions, extensor tenosynovitis, and osteophytes remained (see table 3). Inflammation of a MCP or PIP joint, as determined by OST, had a higher risk on a false negative classification if dorsal erosions, volar erosions, osteophytes, or extensor tendinitis were present, and a higher risk of false positive classification in the presence of osteophytes.

Table 3: False classification by OST versus US-findings.

	β (S.E.M.)	Wald	P-value	Exp(β) (95%-CI for Exp(β))
FN				
Intercept	-2.83 (0.17)	282.39	0.000	
Erosions at dorsal scanning	1.39 (0.51)	7.30	0.01	4.01 (1.46, 10.96)
Osteophytes	0.75 (0.28)	6.97	0.01	2.11 (1.21, 3.69)
Extensor tendinitis	1.29 (0.61)	4.41	0.04	3.63 (1.09, 12.06)
FP				
Intercept	-2.67 (0.16)	275.83	0.00	
Erosions at dorsal scanning	0.27 (0.78)	0.12	0.73	1.31 (0.27, 6.02)
Osteophytes	1.11 (0.26)	18.19	0.00	3.02 (1.82, 5.02)
Extensor tendinitis	0.92 (0.68)	1.86	0.17	2.51 (0.67, 9.41)

Multivariate nominal logistic regression; false classification (yes/no) as dependent variable and relevant ultrasonographic findings as independent variables. The reference category is true positive classification and true negative classification together. OST: optical spectral transmission, US: ultrasound, FN: false negative classification, FP: false positive classification. Exp(β): the odds ratios (ORs) for the predictors; the OR of having a false classification (false positive or false negative) with respect to true classifications (true positive and true negative) in the presence of a pathophysiological finding (erosions at dorsal scanning, osteophytes, or extensor tendinitis). The reference category is: True positive classification and true negative classification together.

Discussion

Our findings show that OST is a sensitive, and specific technique to assess inflammation not only in the hand but also wrist joints of RA patients. The new laser light source that was implemented indeed has improved diagnostic performance of OST in MCP, PIP and wrists, as shown by the ΔAUC-ROC, compared with previous research,[6] performed with similar methods. Still, diagnostic performance in the MCP and PIP joints was higher than that in the wrist. With US as a reference standard, at joint level, the optimal cut-off for OST yields a sensitivity higher than either SJC or TJC (60% vs 46% and 50% respectively), a specificity similar to SJC (89% vs 86% respectively), but higher than TJC (78%). This finding is supported by evaluating Cohen's Kappa, which in itself is a measure for accuracy, and is found to be higher when comparing US to OST than to SJC or TJC (47% vs 30% vs 24% respectively). Test-retest reliability of the duplicate OST measurement, reflected by the ICC and Pearson scores, at both patient and joint level (*supplementary material – Test-retest reliability of OST*) is especially high considering the possible variations between measurements caused by time in-between measurements, difference in hand position and joint manipulation necessary for US evaluation. A recent study on the intraobserver reliability of US evaluation of inflammation in hand and wrist joints of RA patients using the same semiquantitative scoring as our study, showed fair to excellent weighted Kappa coefficients. Although evaluated using different statistical analyses and in a slightly different set of joints, the reliability of assessing inflammation by OST seems at least as high as that of US (*supplementary material – Test-*

retest reliability of OST).[17]

At patient level, the inflammation determined by OST correlated moderately with the inflammation determined by US, whereas the correlation between DAS28 and US assessment was very weak. DAS28 components like VAS Pain are known to poorly correlate with inflammation as defined by GSUS or PDUS,[18] therefore correlations at joint level were evaluated for TJC and SJC separately. At joint level, inflammation determined by US strongly correlated with inflammation by OST, weakly with SJC, and very weakly with TJC, the latter could be expected in patients with low-to-moderate disease activity and being actively treated; inflamed joints are not necessarily painful. The prevalence of osteophytes, as scored by US dichotomically, was rather high. Osteophytes of all sizes were scored as present, even very small osteophytes (grade 1; scale 0-3), however, they seem a relevant source of both over- and underestimation of inflammation. Osteophytes decrease the amount of light passing through the joint, potentially leading to underestimation of inflammation (see supplementary figure 2). The false negative classification of inflammation associated with osteophytes cannot be explained yet. Erosions could cause an increase in the amount of light passing through the joint space and thus underestimation of inflammation. Extensor tendinitis may involve the whole length of the tendon or be localized; a tendon with inflammation within the reference area but no inflammation within the ROI of the joint might cause an underestimation of inflammation of the joint. The prevalence of pathological findings other than osteophytes however was low, as to be expected in an RA population without deformities of hands. Even if tendinitis/tenosynovitis would cause overestimation of joint inflammation, clinically the decision regarding RA treatment would be similar. The pathological findings that are clinically relevant when monitoring disease activity using OST seem specifically related to bone changes, especially osteophytes.
A relatively frequent (16%) physiological finding was presence of a vascular pattern adjacent to the extensor tendon, but it did not have a significant influence on misclassification of inflammation, probably because of usage of reference areas; a representative US example is provided in the supplementary figure 2.
Given these results, in our view OST is suitable as a disease monitoring tool in an RA population without clinically significant hand and wrist joint deformities.

Limitations of this study are the use of a single, though experienced, examiner for performing US (PvdM) and a single examiner (KS) for performing physical examination, not allowing testing of interobserver variability. A drawback of OST is that it assesses inflammation of hand and wrist joints only. However, in daily clinical practice, OST would probably be applied next to other assessments, e.g., the RA-patient's grading of disease activity and the ESR. Development and validation cohort were the same; while this is not optimal, several precautions have been taken to prevent overfitting of the OST model, among others: increasing the dataset (duplicate measurements), using leave-one-out cross validation,

and parameter reduction (see details in the *supplementary material – Development and validation of the OST model*).

Summarizing, OST is a sensitive, specific and objective technique to assess inflammation of joints of hands and wrist of RA patients, even though joint pathology of bone and tendons may increase the risk of misclassification. OST now should be further validated as a monitoring tool in early RA patients in a randomized-controlled-trial setting, comparing OST-guided and DAS-guided treat-to-target strategies.

Supplementary material

Development and validation of an OST model

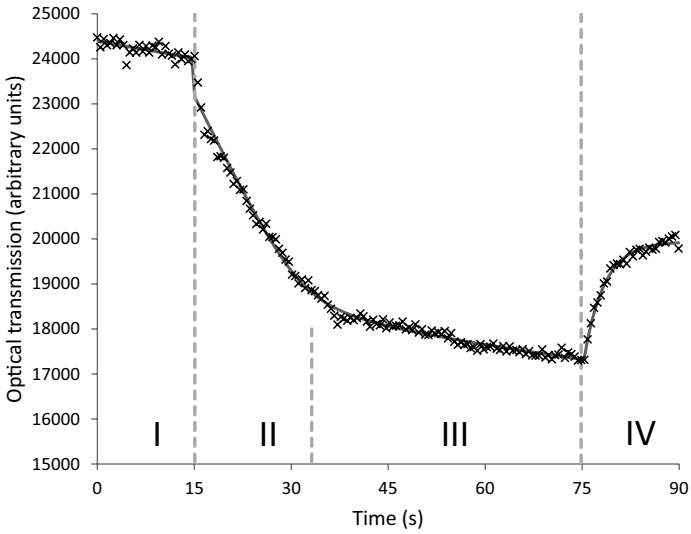
Image analysis was performed by PBLM using in-house developed software (*InFlame RA-160205, November 3, 2016*), blinded to the results of other study measurements. An OST measurement takes approximately 1.5 minute, and comprises four phases (see supplementary figure 1). In phase I, pressure cuffs are inflated to fit the arms of the patient, and then transmission of light is measurement in both the arterial and venous system. In the next phases, incremental increase in cuff pressure leads to venous occlusion and blood pooling (phase II and III). In phase IV cuff pressure is relieved and both arterial and venous flow is allowed again.[6] Light intensity (transmission) over time, as a response to obstructed blood flow, is fitted to a response curve, and was described by eight parameters. These parameters were acquired for the joint ROIs and reference ROIs. The parameters from the reference ROIs are then used to correct for systemic effects, yielding eight normalized parameters per wavelength; 16 normalized parameters in total. Multiple regression analysis was used to develop and validate an algorithm for detecting joint inflammation by OST with US as reference. Duplicate OST measurements were used as independent measurements, enlarging the dataset. The dependent variable was defined as the maximum of GSUS and PDUS scores for synovitis and tenosynovitis (US joint index), and the normalized joint parameters (derived from image analysis) as independent variables. A stepwise forward selection procedure with adjusted R2 testing was used to determine which variable to add. This was repeated until either R2 no longer increased (cut-off value of 0) or, to prevent overfitting of the model, a maximum of four parameters had been selected. This was done separately for each joint region sharing equal joint characteristics (such as thickness and orientation towards the light source), so separately for IP 1, PIP 2-5, MCP 1, MCP 2-5 and wrists. We tested whether individual observations exerting undue influence on the coefficients in the regression analysis (outliers) were present and if so they were removed from the development phase. Due to a limited amount of subjects, cross-validation was chosen as a model validation technique to assess how the results of the multiple regression would generalize to an independent dataset. The regression analysis with up to four parameters as independent variables per joint region was then performed using leave-one-out cross validation to detect and prevent overfitting. In this analysis, the model is repeatedly refit, leaving out at each fit both measurement of a single patient. The regression coefficients thus obtained are used to calculate OST values for the left-out observation.

Due to the implemented hardware changes, OST measurements from the previous study could not be used as a validation cohort for the current prediction model. Therefore, in this study, the development and validation cohort were the same. As shortly addressed in the section above, several precautions had been taken to prevent overfitting. Increasing the dataset reduced the risk of overfitting, using leave-one-out cross validation in a large

Supplementary table 1: Absolute and relative power Doppler (PDUS) grade distribution per joint type.

Joint types	0	1	2	3
Wrist (n=92)	60 (65%)	20 (22%)	12 (13%)	0 (0%)
MCP (n=460)	363 (79%)	23 (5%)	54 (12%)	18 (4%)
PIP (n=460)	413 (91%)	23 (5%)	16 (4%)	1 (0%)
Total (n=1012)	836 (83%)	66 (7%)	82 (8%)	19 (2%)

Power Doppler in these joints were scored according to the Outcome Measurements in Rheumatology Clinical Trials (OMERACT) criteria on a semiquantitive scale (0-3).



Supplementary figure 1: Four phases of an OST measurement. An OST measurement is performed within 100 seconds, and comprises four phases. In phase I, pressure cuffs are inflated to fit the arms of the patient, and then transmission of light is measurement in both the arterial and venous system. In the next phases, incremental increase in cuff pressure leads to venous occlusion and blood pooling (phase II and III). In phase IV cuff pressure is relieved and both arterial and venous flow is allowed again.

development sample has indicated to be valid in earlier work by correlating OST to MRI synovitis (MRI scores were not used in development of the algorithm).[6] Parameter reduction has been implemented to reduce the complexity of our model, increasing the generalizability to an independent dataset. Lastly, variation in the severity of inflammation helps to avoid overfitting. Ideally, there would be an equal distribution of the different grades of inflammation among the various joint types. This is not realistic for two reasons; patients included in this study were treated for RA with the objective to minimize disease activity, and inflammation in the hands and wrist joints of RA patients are known to occur primarily in the MCP and wrist joints, and to a lesser extent in the PIPs.[19] In our cohort, out of the 1003 joints assessed, when using PDUS to represent the severity of inflammation in this cohort (scale 0-3); 83% were grade 0, 7% grade 1, 8% grade 2, and 2% grade 3, confirming that there is no equal distribution of inflammation grades among all joints. In line with literature,

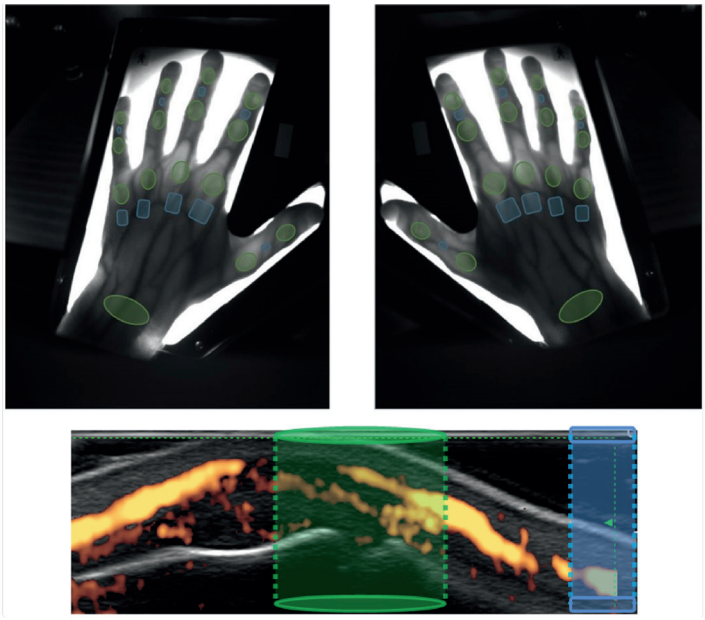
joint inflammation in our cohort was more frequently observed in the MCP and wrist than PIP joints, as can be seen in the supplementary table 1. Although explicit research on the distribution of different grades of inflammation per joint group in hand and wrist joints is limited, similar distributions are found, indicating that inflammatory distribution amongst joint in this study population is representative for the total early RA population, increasing the chance of successful applicability of our model in a validation cohort.[19,20]

Test-retest reliability of OST

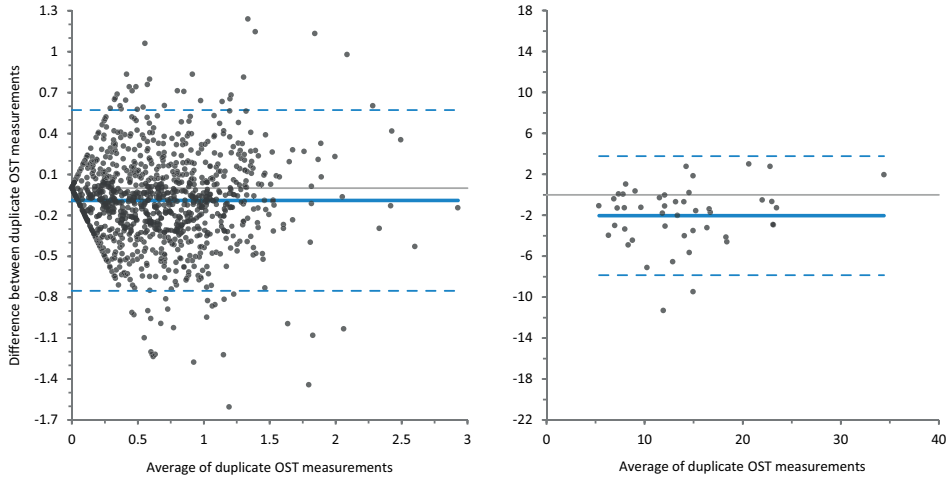
There was a good correlation between the first and second OST measurement at both patient (R = 0.76, 95%-CI: 0.73 to 0.76, p<0.001) and joint level (R = 0.89, 95%-CI: 0.81 to 0.94, p<0.001). As seen in supplementary figure 2, second OST measurements were on average slightly lower than the first measurements at both joint level (mean: -2.03, 95%-CI: -2.91 to -1.15) and patient level (mean: -0.09, 95%-CI: -0.11 to -0.07). The intra-class correlation coefficient (ICC) for two-way mixed single measurement (ICC 3,1) showed similar results, excellent correlation at both patient level (ICC = 0.86, 95%-CI: 0.76 to 0.92, p<0.001) and joint level (ICC = 0.76, 95%-CI: 0.73 to 0.79, p<0.001).

A direct comparison between of the reliability of OST and the reliability of ultrasonography cannot be easily made. Reliability of US assessment is most often expressed as (weighted) Kappa coefficients because of categorical scoring, whereas reliability of the continuous scoring of OST (0-3) is assessed using the intra-class correlation coefficient.

A recent study evaluated reliability of the semiquantitative OMERACT criteria in the US assessment of inflammation, separately for MCP and non-MCP joints (wrist, PIP, knee and MTP). MCP joints showed substantial intraobserver variability in the assessment of synovial hypertrophy (κ=0.44-0.94) and power Doppler (κ=0.29-1). Intraobserver reliability of scoring non-MCP joints was similar for synovial hypertrophy (κ=0.4-0.82), and slightly higher for power Doppler (κ=0.48-0.88). Although the intraobserver reliability was evaluated in a slightly different set of joints, it seems valid to conclude that the reliability of using the semiquantitative OMERACT criteria in the ultrasonograpic evaluation of inflammation in the wrist, (P)IP, and MCP joints in our study varies from fair to excellent and that this reliability is at least as high as that of US.



Supplementary figure 2: Automatic ROI and reference area placement in the image of the hands of a healthy volunteer. Automatic ROI (green circles, upper two panels) and reference area (blue squares, upper two panels) placement in the hands of a healthy volunteer. Extensor tendinitis of a PIP 5 (different volunteer, RA patient) is visualized using power Doppler (lower panel), including an abstract overlay of the areas where OST measurements are performed. In this example the ROI (green area) but also the adjacent reference area (blue area) would detect inflammation (a comparable OST score), and since there is no additional inflammation present in the PIP joint, a true negative classification would be given; no joint inflammation present.



Supplementary figure 3: Bland-Altman plots of duplicate OST measurements. Bland-Altman plots of duplicate OST measurements are shown at patient level (OST index range: 0-66) and at joint level (OST index range: 0-3).

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CHAPTER 3

Novel optical spectral transmission (OST) guided versus conventionally disease activity guided treatment

Study protocol of a randomised clinical trial on guidance of a treat-to-target strategy for early rheumatoid arthritis

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Abstract

Background

Assessment of disease activity is a critical component of tight-control, treat-to-target treatment strategies of rheumatoid arthritis (RA). Recently, the HandScan has been validated as a novel method for objectively assessing RA disease activity in only 1.5 minutes, using optical spectral transmission (OST) in hands and wrists. We describe the protocol of a randomized controlled clinical trial (RCT) to investigate whether HandScan guided treatment aimed at 'HandScan remission' (HandScan arm) is at least as effective as and more cost-effective than clinically guided treatment aimed at ACR/EULAR 2011 Boolean remission (DAS arm).

Methods

The study is a multi-center, double-blind, non-inferiority RCT of 18 months duration. Patients ≥18 years with newly diagnosed, disease modifying antirheumatic drug (DMARD)- naïve RA according to the ACR 2010 classification criteria, are randomised to the DAS arm or the HandScan arm. The efficacy of the arms will be compared by evaluating Health Assessment Questionnaire (HAQ) scores (primary outcome) after 18 months of DMARD-therapy, aimed at remission. The equivalence margin in HAQ scores between study arms is 0.2. Secondary outcomes are differences in cost-effectiveness and radiographic joint damage between treatment arms. The non-inferiority sample size calculation to obtain a power of 80% at a one-sided p-value of 0.05, with 10% dropouts, resulted in 61 patients per arm. In both arms, DMARD-strategy will be intensified monthly according to predefined steps until remission is achieved; in both arms DMARDs and treatment steps are identical. If sustained remission, defined as remission that persists consistently over 3 consecutive months, is achieved, DMARD-therapy will be tapered.

Discussion

The study protocol and the specifically designed decision-making software application, allow for implementation of this RCT. To test a novel method of assessing disease activity and comparing (cost-)effectiveness with the contemporary method in treat-to-target DMARD strategies in early RA patients.

Background

Rheumatoid arthritis (RA) is a chronic autoimmune disease with polyarthritis, frequently leading to joint damage and physical disability, especially if not treated adequately as soon as possible after diagnosis. Treatment in the first months is more effective than if the same treatment is applied later in the course of disease.[1] Early and intensive (tight-control) treatment of RA with disease modifying antirheumatic drugs (DMARDs) has significantly improved RA outcome.[2,3] The aim of tight-control treatment, i.e. tailoring treatment strategy to the disease activity of individual patients, is to achieve a predefined level of low disease activity, preferably remission (treat-to-target), within a reasonable period of time.

For tight-control and treat-to-target treatment strategies for the treatment of RA, typically frequent disease activity assessment is applied using the Disease Activity Score (DAS), a composite score of an acute phase reactant, such as C-reactive protein (CRP), patient global assessment (PGA), and swollen and tender joint counts (SJC and TJC, respectively) of 28 or 44 joints. Although commonly used in research and daily clinical practice, assessment of the DAS is rather time-consuming and subjective, and the DAS is only validated on the group level.[4] However, fast, objective, and easily implementable tools to assess arthritis and disease activity are lacking.[5] The HandScan (Hemics BV), using optical spectral transmission (OST), objectively measures the reduced transmission of light through joint tissues in presence of inflammation (e.g. synovitis, tenosynovitis).[6,7] HandScan results reflect disease activity, correlating moderately with DAS28 ($r=0.42$, $p=0.001$).[6] Moreover, HandScan correlates moderately with ultrasound (US) assessing synovial inflammation of hand and wrist joints (Spearman's correlation coefficient: $\rho=0.54$, 95%-CI: 0.28 to 0.73, $p<0.01$), while DAS28 did not correlate with these US results ($\rho=0.06$, 95%-CI: -0.26 to 0.36, $p= 0.71$).[7] Test-retest reliability of the HandScan was excellent at both patient level (ICC = 0.86, 95%-CI: 0.76 to 0.92, $p<0.001$) and joint level (ICC = 0.76, 95%-CI: 0.73 to 0.79, $p<0.001$).[7] In addition, HandScan has proven to be user-friendly, i.e. an assistant without medical background can operate the device, and fast: it provides the inflammation score within 1.5 minutes.

The aim of this study protocol was to determine the applicability of the HandScan in tight-control and treat to target treatment strategies of early RA patients. To facilitate this, specifically designed, decision-making software application was developed, allowing for double-blind comparison of HandScan guided treatment with the contemporary method of DAS-guided treatment.

Design

This is an investigator-initiated, multi-center, double-blind non-inferiority randomized controlled trial (RCT) of 18 months duration. Patients are being randomised (1:1) to a

HandScan guided treatment aimed at HandScan remission (HandScan arm), or a clinically DAS guided treatment, aimed at ACR/EULAR 2011 Boolean remission (DAS arm). The study is performed at five departments of rheumatology in the Netherlands, the University Medical Center Utrecht (UMCU), and four non-university hospitals: Meander Medical Center Amersfoort, Noord West Ziekenhuizen Alkmaar, Máxima Medisch Centrum Eindhoven, and Gelre Ziekenhuizen Apeldoorn.

The study has been approved by the Ethical Committee of the University Medical Center Utrecht, Utrecht, The Netherlands on April 6, 2017 (*NL50026.041.14*), and has been registered in the Dutch Trial Register (*NTR6388*). Privacy of patients will be protected according to the General Data Protection Regulation, using anonymized data.

Objectives

The overall aim of the study described in this protocol is to demonstrate clinical efficacy and cost-effectiveness of the HandScan arm, compared to the DAS arm, with identical treatment and treatment steps in both arms.

Table 1: Selection criteria

Inclusion criteria	
	Early (< 1 year) RA, fulfilling 2010 ACR/EULAR criteria.
	Age ≥ 18 years.
	Ability and willingness to give written informed consent.
	Ability to comply with the study protocol.
Exclusion criteria	
	Significant visual deformations of hands or fingers (impeding HandScan analysis).
Other (joint) disease	Concomitant or current inflammatory joint disease other than RA.
	Porphyria (HandScan risk analysis).
Drug-specific	Contraindication for methotrexate or prednisolone.
	Glucocorticoids used for RA < 6 weeks prior to baseline (NB: inhaled glucocorticoids are allowed).
	Previous treatment with any (biological) DMARD that is used in the treatment of RA.
	Treatment with any investigational agent within 4 weeks or period of 5 half-lives, whichever is longer, before screening.
	Patients using photodynamic therapy medication (HandScan risk analysis).
General medical	Pregnancy or breast-feeding.
	History of alcohol or substance abuse within the 6 months prior to screening. Alcohol abuse is defined as more than 3 units per day.
	Neuropathies or other painful conditions that might interfere with pain evaluation.

Figure 1: SPIRIT figure, trial visits and assessments

	Months																			
Assessments	-0*	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Patient screening	X																			
Patient informed consent	X																			
Patient randomization		X																		
HandScan (arm A)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Disease activity (DAS; arm B)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HAQ		X			X			X						X						X
SF36		X			X			X						X						X
EQ5D		X			X			X						X						X
Cost questionnaire		X			X			X						X						X
(S)AE		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X-rays (hands and feet)		X																		X

* The screening visit. Disease activity: blood sampling for C-reactive protein, swollen and tender joint count, and patient global assessment. HAQ: Health Assessment Questionnaire. SF36: the Short Form (36) Health Survey. EQ5D: standardized instrument for measuring generic health status. (S)AE: (serious) adverse event or effect.

The primary outcome is the Health Assessment Questionnaire (HAQ) score at 18 months, and the change in HAQ score over time. Secondary outcomes are cost-effectiveness, based on customized cost questionnaires (including direct and indirect costs) and radiographic damage of hand and wrist joints, assessed using a newly developed fully automated radiographic scoring system of hand joints,[8] as well as the conventional Sharp van der Heijde (SvdH) score.[9]

Patients

Patients ≥18 years with newly diagnosed, DMARD naïve RA according to the ACR/EULAR 2010 classification criteria,[10] are eligible for this study. Detailed selection criteria are shown in table 1.

All participating patients provide written informed consent, according to the ethical principles from the Declaration of Helsinki. At the screening visit, informed consent is signed by the patient and research/ rheumatology nurse or investigator, and selection criteria are checked.

Randomisation

Randomisation lists per centre will be prepared at UMC Utrecht, using *nQuery Advisor* in permuted blocks of 4; randomization lists are safeguarded at the UMC Utrecht. Randomisation data will be incorporated in the decision-making software application that is used in each of the institutes. This way, on-site research staff, patients, and sponsor trial personnel will remain blinded to treatment strategy. A patient will be allocated to the HandScan or DAS arm, as soon as baseline clinical data is entered into the software application. A patient specific study number and the year of birth will function as identifiers. Unblinding will not be necessary, as both arms receive identical medication in an open fashion.

Assessments

Patients will visit the outpatient clinics monthly (see figure 1). At each visit, disease activity will be measured, to ensure blinding both with HandScan and DAS44, consisting of serum CRP, a 44 joints assessment for swelling and tenderness, and a PGA (VAS 0-10 cm, 10=worst). At baseline, 3 months, 6, 12, and 18 months, patients will fill out the HAQ (1st outcome), the Short Form Health Survey (SF36), the EuroQol (EQ5D), and the questionnaire on direct and indirect costs (2nd outcome). Radiographs in the antero-posterior direction of hands, wrists and feet will be obtained at baseline and 18 months (2rd outcome). At each visit, patients' blood will be sampled (10 cc) for CRP evaluation and to monitor for adverse-effects of medication. Data from all centres, are collected digitally in one eCRF, through the online data-gathering tool *Research Online* (developed by *UMC Utrecht Julius Center*).

Definitions of remission

Aimed at predefined remission criteria, treatment is intensified in a similar fashion for both arms, according to a predefined schedule. In the DAS arm, remission is defined based on the ACR/EULAR 2011 Boolean remission criteria, all must be met:[11]

- TJC ≤1 of 44 joints: sternoclavicular, acromioclavicular, shoulder, elbow, wrist, metacarpophalangeal (MCP), proximal interphalangeal (PIP), metatarsophalangeal (MTP), knee and ankle joints
- SJC of 30 small joints (MCP, PIP and MTP joints) ≤1 AND SJC of 14 large and other joints (sternoclavicular, acromioclavicular, shoulder, elbow, wrist, knee and ankle joints)=0
- CRP ≤1 mg/dl
- PGA ≤1 (on VAS 0-10 cm, 10=worst)

In the HandScan arm, remission is defined based on the following HandScan criteria, all must be met:

- total optical joint score per patient ≤ 11 (based on ROC curves in a comparative study with DAS28 and ultrasonography)[6,7]
- ≤1 joint per patient with an optical score of > 1

The optical joint scores are shown by the HandScan shortly after assessment (see screen shot of the HandScan, figure 2).

Treatment strategy

After randomization, in both arms all patients will initiate a methotrexate (MTX)-based tight-control strategy, with 10 milligram per week (mg/wk) MTX orally as starting dose and with prednisolone (PRED) fixed dose of 10 mg/day orally.[12] Patients are evaluated monthly and at each visit a dosage decision is made based on efficacy and adverse events. In case remission is not achieved, MTX dose will be increased at each monthly visit until either remission or the maximum tolerable dose (MTD) is reached, see table 2. Escalation steps are 15, 20, 25, 30 mg MTX/wk orally; followed by 30 mg MTX/wk (or MTD) subcutaneously. At a dose of MTX of 25 mg/week (or MTD of MTX), hydroxychloroquine (HCQ) 400 mg/day for patients ≥60 kg (or 200 mg/day for patients ≤60 kg), will be added. After the final escalation step of MTX, in case of no remission, tumour necrosis factor inhibitor (anti-TNF), e.g. Adalimumab 40 mg s.c. every 2 weeks will be added to the MTX therapy, while HCQ will be stopped.

Figure 2: The HandScan user interface; total and individual optical joint scores. The total optical joint score of 9.43 (blue arrow) meets the HandScan remission criterion for total score. Individual optical joint criteria are shown below the picture. There are three joints (green arrows) that exceed the individual optical joint score criterion (>1). Regarding the HandScan remission criteria (total optical spectral transmission (OST) score ≤ 11 AND a maximum of one joint with OST>1), this (test) patient is not in remission.

If remission is achieved, treatment will be continued unchanged. If remission persists over 3 consecutive months (sustained remission, SR), treatment intensity will be de-escalated by one step; a following de-escalation steps will be taken every time remission persists for another 3 months (see table 3). De-escalation steps will vary with the dosages at remission, see figure 3 and table 3, however the first de-escalation step will always be tapering PRED to 7.5 mg/day. If disease activity flares (i.e. loss of remission according to the remission criteria), patients will return to the previous dosages at which they achieved remission, and will escalate medication, until remission is achieved again. If disease activity flares during medication-free remission, patients will return to MTX 10 mg/wk and PRED 10 mg/day (medication at start of the study) and will escalate the medication, until remission is achieved again. The treatment protocol is derived from the second Computer Assisted Management in Early Rheumatoid Arthritis trial (CAMERA-II).[12] Co-medications are calcium and vitamin D supplementation and a bisphosphonate during PRED treatment, and folic acid to prevent MTX toxicity, dosages of co-medication are according to guidelines. Each centre will provide their own preferred anti-TNF.

Adverse reactions to DMARDs

In case of adverse reactions to initial dose of MTX (10 mg/week), MTX will be substituted with Leflunomide (LEF) 20 mg/day. If LEF is well tolerated, and remission is not achieved, HCQ will be added; next escalation steps are as described above (table 2). Patients that will switch to LEF, and are consequently unable to achieve remission, will reach anti-TNF treatment faster than patients on MTX (with the exception of a MTD). In case of adverse

Table 2: Intensifying treatment strategy in case remission is not achieved

Week	MTX* mg/wk	PRED 10 mg/day	HCQ 400 mg/day	Anti-TNF
0= start of study	10	+	-	-
+4	15	+	-	-
+4	20	+	-	-
+4	25	+	+	-
+4	30	+	+	-
+4	same dose s.c.	+	+	-
+4	same dose s.c.	+	-	+
+4	same dose s.c.	+	-	+
+4	same dose s.c.	+	-	+

If remission is not achieved, after each period of four weeks, treatment is intensified stepwise. MTX: Methotrexate, PRED: Prednisolone, HCQ: Hydroxychloroquine, anti-TNF: anti-tumour necrosis factor, s.c.: subcutaneously. * Same dose s.c.: the dose at the previous step, given s.c. at a dose of 30 mg, or earlier. In case of dose-dependent adverse reactions to MTX (>10 mg/wk), previously tolerated dose will be administered s.c., and this will then be the maximum tolerable dose (MTD) for that patient. Further intensifying treatment would be adding or continuing HCQ. HCQ is given for three consecutive months in every scenario.

Table 3: Tapering and stopping treatment in case remission is maintained after 12 weeks of unchanged treatment*

	Weeks of remission	MTX* mg/wk	PRED mg/day	HCQ mg/day	Anti-TNF
MTX + PRED	**<12**	same dose	10	-	-
	12	same dose	7.5	-	-
	>12 , every 12 wks	decrease with 5 mg/week until 10 mg/wk	7.5	-	-
	+12	10	5	-	-
	+12	5	5	-	-
	+12	5	2.5	-	-
	+12	5	stop	-	-
	+12	2.5	-	-	-
	+12	stop	-	-	-
MTX + PRED + HCQ	**<12**	same dose	10	400	-
	12	same dose	7.5	400	-
	>12 , every 12 wks	decrease with 5 mg/week until 10 mg/wk	7.5	stop	-
	+12	10	5	-	-
	+12	5	5	-	-
	+12	5	2.5	-	-
	+12	5	stop	-	-
	+12	2.5	-	-	-
	+12	stop	-	-	-
MTX + PRED + aTNF	**<12**	same dose	10	-	same dose
	12	same dose	7.5	-	same dose
	>12 , every 12 wks	same dose	7.5	-	½ frequency#
	+12	one step back until 10 mg/wk	7.5	-	stop
	+12	10	5	-	-
	+12	5	5	-	-
	+12	5	2.5	-	-
	+12	5	stop	-	-
	+12	2.5	-	-	-
	+12	stop	-	-	-

* Tapering treatment depends on the combination of medication at the moment of sustained remission. # In this multi-centre study, centres prescribe their preferential anti-TNF, therefore, a more global approach in decreasing aTNF dose is applied: reduction of frequency of administration i.e. extension of dosing interval.MTX: Methotrexate, PRED: Prednisone, HCQ: Hydroxychloroquine, anti-TNF: anti-tumour necrosis factor.

reactions to MTX at dosages ≥10 mg/wk, the last well-tolerated dose will be administered subcutaneously, and considered to be the MTD. If remission is not achieved, treatment will be intensified according to table 2, e.g. at a MTD of MTX 15 mg/wk subcutaneously, HCQ will be added.

Prevention of under- and overtreatment in the HandScan arm

Since treatment guidance by the HandScan is a novel approach, measures are taken to prevent large deviation of the treatment strategy guided by the HandScan from treatment in the DAS arm, potentially leading to undesired under- or overtreatment. If treatment decisions dictated by HandScan are discordant with decisions that would have been dictated by DAS, at three consecutive monthly visits (see figure 3), patients will be switched to the clinical arm and medication decisions will be immediately guided by the clinical arm (without intervention of the researcher). Therefore, patients in the HandScan arm might only potentially be under- or overtreated (based on DAS guidance) for a maximum of two consecutive months.

Implementation of a software application for patient-tailored, tight-control treatment

Although treatment regimens and dosing steps are identical for all patients, actual medication will differ considerably between patients in this study, as the study protocol tailors treatment to every patient in a treat-to-target strategy, see figure 3. To account for these variations in treatments and to allow for double-blind treatment, a decision-making software application was developed. It uses a data trail (data log) of current and previous patient-specific input to check e.g. for consistent discordance between remission criteria, and prevent drug dosages higher than previous MTD, in case of a flare. At each visit, the patient identification number, year of birth, and parameters for both clinically and HandScan assessed disease activity will be entered into the application. Based on whether there is remission or not, according to the criteria of the randomized arm, the application will provide the next patient-specific medication step.

The application was developed and tested conform the regulations and guidelines for the development of a medical appliance class I and medical software class B (E*N 62304 Medical device software –Software life-cycle processes, EN 62366 Usability, ISO 14971 Medical devices – Application of risk management to medical devices*).

Statistical analyses

Sample size

Sample size was calculated for the primary outcome, HAQ score at 18 months, and the primary analysis of the non-inferiority design, using two-sample t tests and data from the CAMERA-II trial: mean (SD) HAQ score at 18 months 0.38(0.4).[12] An equivalence margin of 0.2 in HAQ scores between study arms was considered clinically acceptable. A sample size of 51 per group was calculated, to obtain a power of 80% at a one-sided p-value of 0.05. Taking

into account 10 percent drop-out, total sample size was set at 112 patients.

Primary outcome analyses

Primary outcome is HAQ score; primary analysis is change from baseline to 18 months, compared between the two arms according to intention to treat (ITT) using a one-sided, non-equivalence two-group t-test. The ITT population will include all randomized patients, as long as they have taken study medication at least once and at least one efficacy measurement was obtained. Secondary analyses are the same analysis in the per protocol population (i.e. all patients without major protocol violations or a switch between study arms) and a mixed model analysis of HAQ scores over time (baseline and 3, 6, 12, and 18 months) between study arms. For missing data, the last observation will be carried forward if missing data <10%; otherwise, missing HAQ data will be imputed using multiple impution. Imputations will be based on baseline characteristics and known predictors.

Secondary outcomes

Quality of life will be evaluated at baseline and after 3, 6, 12, and 18 months, using SF36 and EQ5D questionnaires with mixed model analyses between study arms.

Cost-effectiveness of the HandScan arm versus the DAS arm treatment will be calculated from actual data (i.e. a trial-based economic evaluation). Direct costs (health care) and indirect costs (loss of paid and household productivity) will be calculated from questionnaires, including the Health and Labour questionnaire.[13] In the HandScan arm, cost for a rheumatologist at the clinical visits will only be included if a rheumatologist would have actually been required, e.g. to change medication. To prevent overestimation of cost of the rheumatologist time, a visit rate of once per month during the first 6 months and once per 3 months thereafter will be assumed for cost calculation. Cost-effectiveness planes and acceptability curves will also be estimated, from the societal (base case), healthcare and hospital perspective, respectively. Differences in quality-adjusted life years (QALY's: life years multiplied by the utility value, as calculated using EQ5D) and costs (for drug cost, other direct costs and indirect costs) will be calculated using bootstrapping (5000 resamplings, with replacement). Costs and QALY's will be discounted by 4% and 1.5% according to the Dutch guidelines for pharmaco-economic evaluations. Sensitivity analyses will be performed for time spent by rheumatologists, number of visits, QALY's (according to either EQ5D or SF36), costing method (Human Capital Approach or Friction costs method), and discount rates. Missing data for costs and QALY calculation will be imputed using multiple imputation.

Radiographic joint space width and bone erosions of hands and feet will be measured by SvdH score,[14] total score and separately for joint space narrowing and erosion scores. Differences in change at 18 months from baseline between arms will be tested for statistically significant differences with Mann-Whitney-U tests. As sensitivity analysis, joint space narrowing scores by a novel,[8] fully automated assessment will be performed and analysed. Moreover,

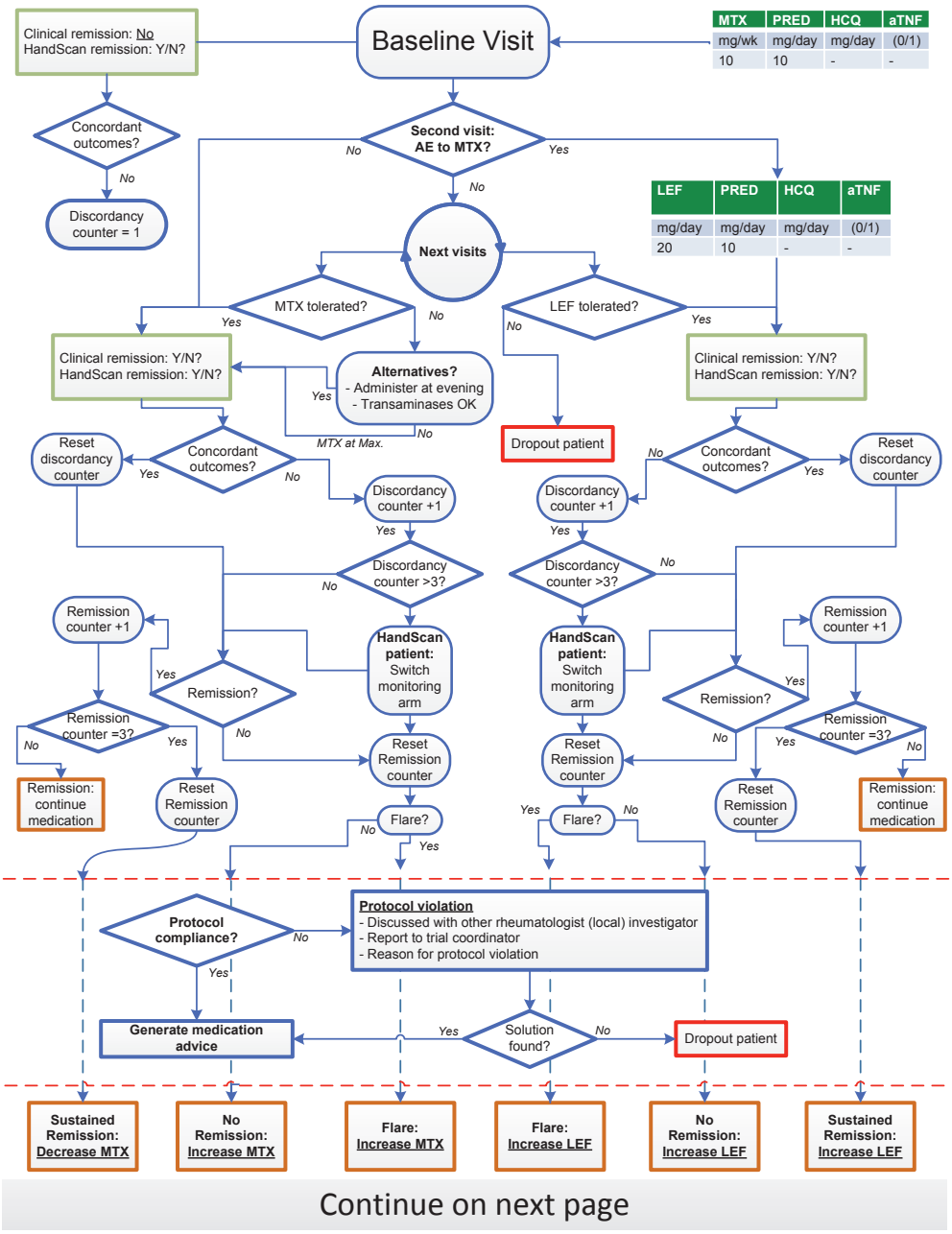

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Figure 3: Flowchart of the decision-making software application, generating medication advice.Green boxes indicate assessment of remission. Brown boxes indicate medication dosage related events (maintaining, intensifying, and tapering treatment). Red boxes indicate patient dropouts. Blue diamonds indicate decisions for patient-tailored treatment. Orange boxes refer to current medication dosages.

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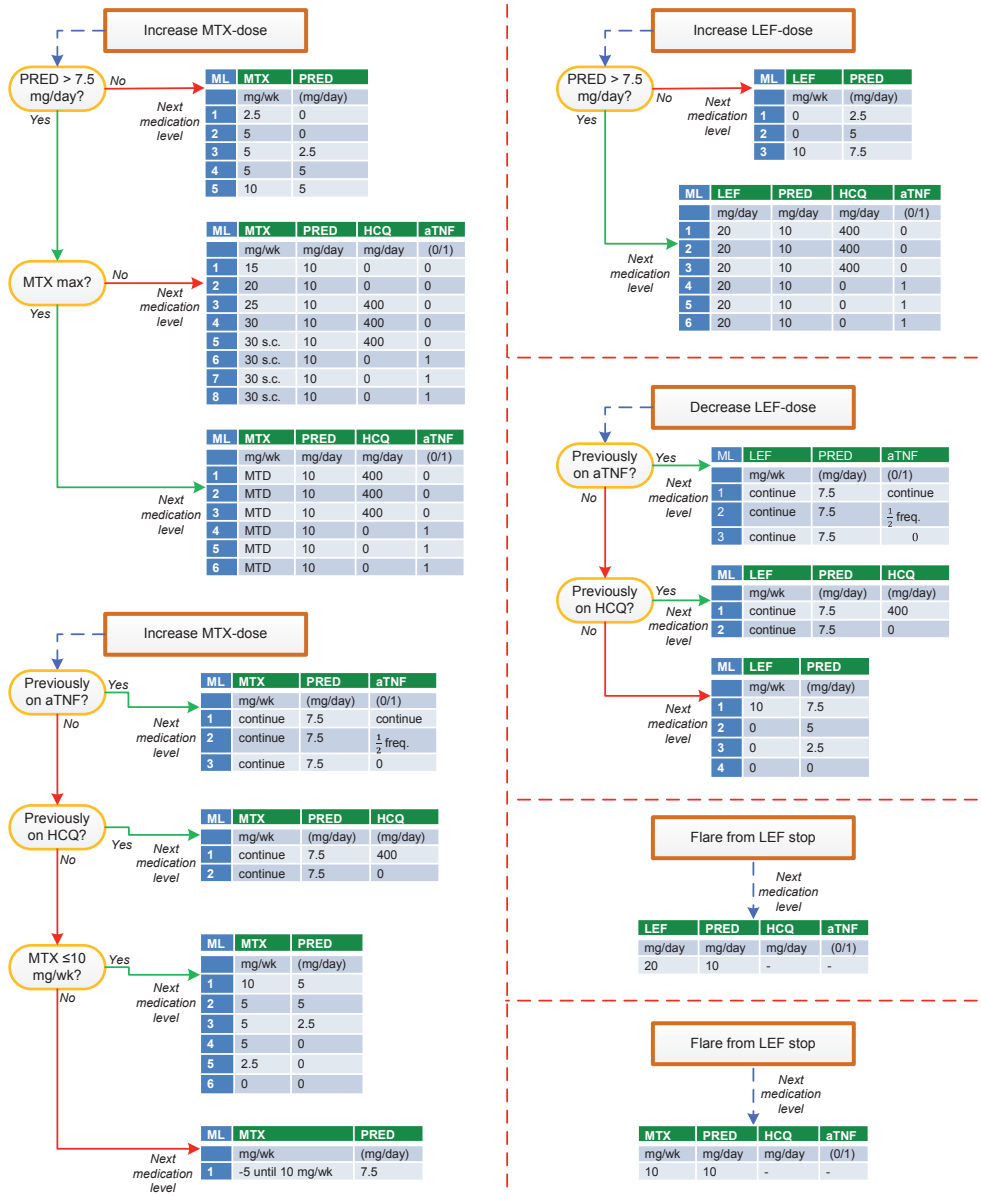

Figure 3 continued: Clinical remission: remission according to ACR/EULAR 2011 Boolean remission criteria (10). HandScan remission: remission is achieved if total optical spectral transmission (OST) score ≤ 11 AND a maximum of one joint with OST>1. MTX: methotrexate. LEF: leflunomide. PRED: prednisolone. HCQ: hydroxychloroquine. aTNF: tumour necrosis factor inhibitor. MTX at max: maximum (tolerable) dose; i.e. 30 mg or lower, or maximal tolerable dose. AE: adverse event or effect. ML: medication level.

cumulative disease activity according to area under the curve of HandScan data in each arm will be correlated to radiographic damage (total, narrowing and erosion scores) by SvdH and automated assessments by Spearman correlation analyses.

Patient safety
An independent researcher will analyse the number of study arm switches when the first 20 patients have 6 months of follow-up data. If more than 10 patients needed to switch, to the clinical arm due to discordant treatment decisions for 3 consecutive months, the study will be evaluated and if needed, stopped. If more than 20% of the first 20 patients have protocol violations within 6 months after inclusion (based on clinical judgement), the medical research ethics committee (MREC) will be informed, and protocol modifications will be communicated with the site investigators, treating rheumatologists, and if necessary, trial participants. All adverse events reported by trial participants or observed by investigator staff will be recorded. As the study does not involve experimental medication or treatment, no safety analysis will be performed and an independent data safety and monitoring board will not be installed.

Discussion

This study aims to evaluate clinical efficacy of a HandScan guided versus DAS guided tight-control and treat-to-target treatment strategy for early RA. The HandScan guidance has potential drawbacks, mainly because it relies solely on arthritic activity in hands and wrists. However, optical spectral transmission (OST) did correlate to DAS28 in a previous study,[6] as well as to US assessed synovial inflammation of hand and wrist joints, while DAS28 did not correlate with these US results. It is possible that, considering the strengths and weaknesses of both guidance methods, the optimal guidance for future treatment of early RA would be using both methods or a combination of parameters from both methods (e.g. HandScan with CRP measurement).

To enable a fair comparison between the randomised arms, we chose not to use DAS remission or the Boolean remission criteria with the components of DAS as primary outcome, because this would probably favour the DAS arm. Another rationale for this decision is that the Boolean remission criteria have a risk of false negative classification of remission, based on high scores on PGA and TJC in case of concomitant soft tissue rheumatism, fibromyalgia or other non-inflammatory chronic pain syndrome.[15] Therefore the HAQ score was chosen as primary outcome, which in early RA reflects both disease activity and physical disability. Secondary efficacy outcome is radiographic joint damage.

An economic evaluation of guidance of a tight-control strategy using the HandScan has been previously published.[16] Implementation of the HandScan as a monitoring tool was modelled

at comparable costs and comparable effects as using clinical assessments. To validate this result, cost-effectiveness in the current study will be calculated based on actual data (i.e. a trial based economic evaluation), this approach requires less assumptions, and therefore has a lower risk of bias.

This protocol describes a specifically designed software application to allow for double-blind, safe, and patient-tailored guidance of treatment. Implementation of the software application has the advantage that it allows for on-site patient randomization and double-blind (for the guidance method) treatment. Deviation from standard treatment schedules is allowed, for example by defining a MTD or giving the opportunity to switch from MTX to LEF in case of MTX intolerance. Three consecutive discrepancies in medication advice between the HandScan and DAS arm lead to switch of the respective patients in the HandScan arm to the DAS arm, as a built-in safety for potentially large differences between arms. Importantly, as neither the patient nor the physician is aware of a switch, the double-blind design will be maintained.

This specifically designed decision-making software application also allows for implementation of other RCTs testing future, novel methods of guidance of tight-control and treat-to-target treatment strategies in RA.

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CHAPTER 4

Synovium and capsule

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Oxford Textbook of Osteoarthritis and Crystal Arthropathy; 2016:55-72.

The synovial membrane

The most common and movable type of joint is the diarthrodial joint, consisting of (at least two) articulating bones, covered by articular cartilage, and a closed joint cavity, formed by the surrounding joint capsule (Figure 1). The combination of these components provides support and mobility. The low-friction cartilage surfaces allow for smooth flexible motion under high weight-bearing conditions. The joint capsule consists of an outer fibrous layer and an inner more cellular layer, the synovial membrane. The latter supports smooth motion by lubricating the cartilage joint surfaces.

The outer fibrous layer, the articular capsule, is made up of dense connective tissue, and attaches to the end of each bone. It is continuous with the periosteum, and thus surrounds the entire synovial joint. The dense fibrous collagen tissue of the capsule is firmly attached to the bone with so-called Sharpey's fibres. The capsule is densely innervated and is, together with tendons and muscles, responsible for joint stability and proprioception. These in turn are, in addition to smooth movement, responsible for optimal function of the synovial joint.

The synovial membrane consists of two distinct layers: the intimal lining and the supportive sublining layer. The intimal lining is in direct contact with the intra-articular cavity and is the source of lubricious synovial fluid. The two major lubricating components that are important in reducing friction are lubricin (also called proteoglycan 4; PRG4) and hyaluronic acid (HA).[1] These lubricants not only have a lubricating effect but are also reported to have joint protective effects by, for example, inhibiting inflammatory activities and adherence of cells and proteins to the articular surface.[2-7]

The articular cartilage, unlike the synovial membrane, is not vascularized or provided with lymphatic drainage and therefore depends on the synovium for providing all the essential nutrients. The semipermeable membrane does this by controlling molecular traffic in and out of the joint space. High-molecular-weight molecules like HA and PRG4 do not cross the membrane, whilst small molecules like cytokines and chemokines can. This leads to retention of lubrication molecules in the synovial fluid, and keeps other high-molecular-weight molecules, like plasma proteins, out.[2,8-10] In this way the synovial membrane is essential for nutrition and lubrication of cartilage (Figure 1).

The synovial surface is an integration of lining cells, vessels, and nerve endings.[11] The synovial lining lacks epithelial cells, tight junctions, and desmosomes and the synovial cells (synoviocytes) are not fixed on a basement membrane, but are loosely organized over three or four layers.[12] Synoviocytes are classically subdivided into two types, macrophage and fibroblast-like synoviocytes, also referred to as 'type A' and 'type B' synoviocytes, respectively.

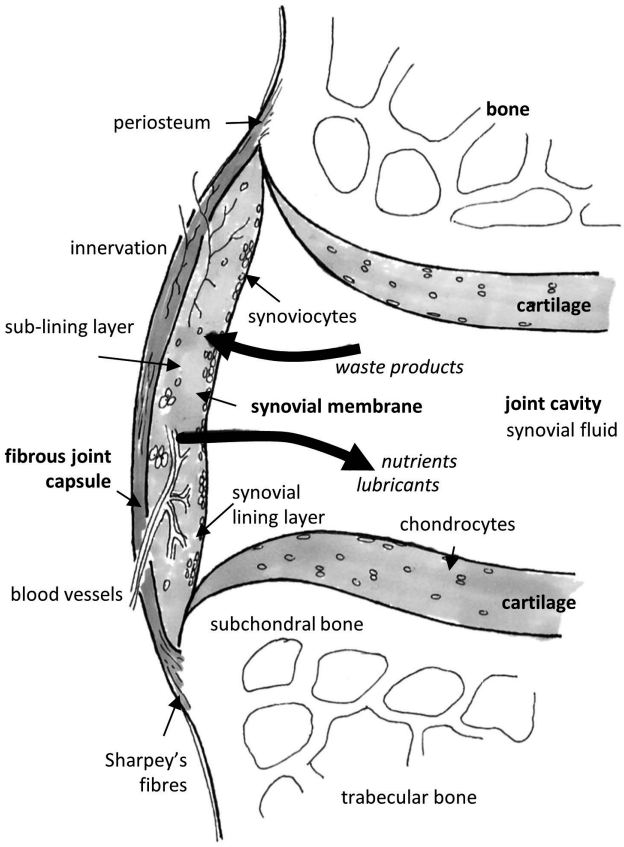

Figure 1: Synovial joint.

Type A synoviocytes express markers of haematopoietic origin, most similar to the monocyte/ macrophage lineage.[13] Being mainly phagocytic, these cells have lysosomes and a large Golgi complex. They are involved in removal of waste products from the synovial cavity as a result of tissue turnover. Type B synoviocytes are mesenchymal cells that display many characteristics of fibroblasts. They are involved in the production of molecules such as collagens, PGR4, and HA.[14] Note that production and intra-articular release of such molecules is not unique for the synovial membrane but that these molecules are also produced by the (superficial) cartilage chondrocytes.[3]

The synovial membrane is additionally a source of mesenchymal stem cells that are potentially able to differentiate into cartilage, bone, and adipose tissue. Therefore, the synovium is considered to contribute to the regeneration and repair of degenerated tissue in the joint although to what extent and how, still remains elusive.[15,16]

Synovial changes (synovitis) in osteoarthritis

Data on synovial changes in osteoarthritis have been obtained over the past decades from numerous ex vivo and in vitro human studies as well as in vivo animal disease models. It should be recognized that data from animal models in general and also data from synovial changes in animal models of osteoarthritis are not always translatable, or at least were not confirmed to be translatable, to human disease.[17-19]

Most of the literature on synovitis in human osteoarthritis originates from studies of the larger joints such as the knee. This is not only simply because this joint is more accessible for obtaining synovial tissue biopsies and synovial fluid, but of course also because of the high incidence of osteoarthritis in this joint. Additionally, the involvement of structures like cruciate ligaments, menisci, and (patellar) fat pads, of relevance to joint degeneration, can easily be studied in the knee joint.

However, synovial changes may differ between joint types, as there are clear differences between synovial joints. Specifically the large fat pad might be of relevance. This is an important source of inflammation,[20] and differs in the knee compared to, for example, the hip, with less surrounding adipose tissue.[21] Another example is the characteristic erosive hand osteoarthritis which is explicitly synovitis driven, not often as explicitly seen in other joints.[22-23]

Even within the same joint, synovial changes are not always equally distributed and can vary in location within the affected joint, being patchy in character and confined to areas near sites of cartilage damage.[24,25] Cartilage destructive properties and angiogenesis can be strikingly different in inflamed and non-inflamed areas of synovial tissue in individual patients with osteoarthritis.[26] The synovial tissue inflammatory cell infiltrate and synovial fluid proinflammatory cytokines can differ significantly between different forms of knee osteoarthritis.[27] Correlations have been reported between the region of inflammation and the severity of cartilage damage,[28] supporting this.

The location of inflammation can also determine the severity of symptoms; for example, in the knee, changes in the infrapatellar fat pad are most strongly related with changes in pain.[25] On the other hand, it has been reported that mononuclear cell infiltrates into the synovial tissue and the presence of lymphoid aggregates are not necessarily associated with clinical signs of inflammation like heat, pain, redness, and/ or effusion.[27,29]

These points should all be taken into account, as well as the variable character over the course of disease (early versus late, chronic versus acute, and flares), together with variable changes in synovial activity over time. As such, synovial inflammatory activity in osteoarthritis is not only variable between patients, but also between joints and within joints with different relations to tissue damage and clinical symptoms, all being variable over time. Therefore, it should be recognized that data from specific studies cannot simply be translated at all times to the role of synovitis in osteoarthritis in general.

Synovial reaction

Chronic synovitis is associated with synovial changes like hyperplasia as a result of proliferation and recruitment of synovial and inflammatory cells, angiogenesis, and nerve growth (Figure 2).[30-32] Acute synovitis may be apparent in osteoarthritis, but increasingly subclinical and low-grade clinical, more chronic inflammation is being recognized to be a driving force in the osteoarthritic process as well.[33] Acute synovitis, or flares, not only occur in non-inflamed joints but can also be superimposed upon chronic inflammatory activity.[34] The most common finding in synovitis is hyperplasia of the synovial lining with slender villous formation and limited layers of synovial membrane cells, which is already found early in the disease.[31] Actual inflammation with clear villi and thickening of the synovial membrane, in addition to hyperplasia, characterized by infiltration of inflammatory cells and hypervascularization, is seen in more advanced disease.[35]

Synovial cells

Synovitis consists of the activation and proliferation of the synovial lining cells and the infiltration of inflammatory cells into the sublining tissue, with both contributing

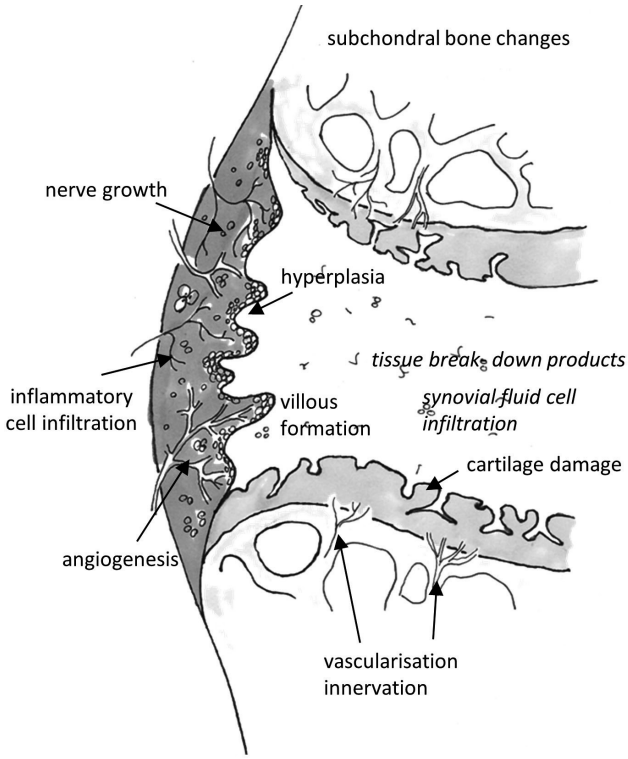

Figure 2: Osteoarthritic joint.

to thickening (hyperplasia) of the synovial membrane. Synovitis is characterized by an infiltration of mononuclear cells, predominantly macrophages and T cells but also infiltration of neutrophils, dendritic cells, NK cells, and even B cells have been reported.[32,36,37] These inflammatory cells have been shown to add to angiogenesis by the production of angiogenic factors, and as such might be involved in the (early) induction of angiogenesis.[29] In combination with the production of chemokines by many of these cells,[38] inflammation is enhanced in an autocrine manner (Figure 3).

Neutrophils have been found in acute synovitis in which removal of an irritant as a host response is suggested.[5,29] In case of acute inflammatory flares seen in osteoarthritis, these cells are suggested to be involved as well.[39] On the other hand, other researchers suggest that neutrophils are not found in the synovial joint.[40]

Macrophages are abundantly present in (chronically) inflamed osteoarthritis synovium and exhibit an activated phenotype, substantiated by the production of inflammatory mediators (e.g. tumour necrosis factor alpha (TNFA) and interleukin 1 beta (IL1B)), angiogenic factors

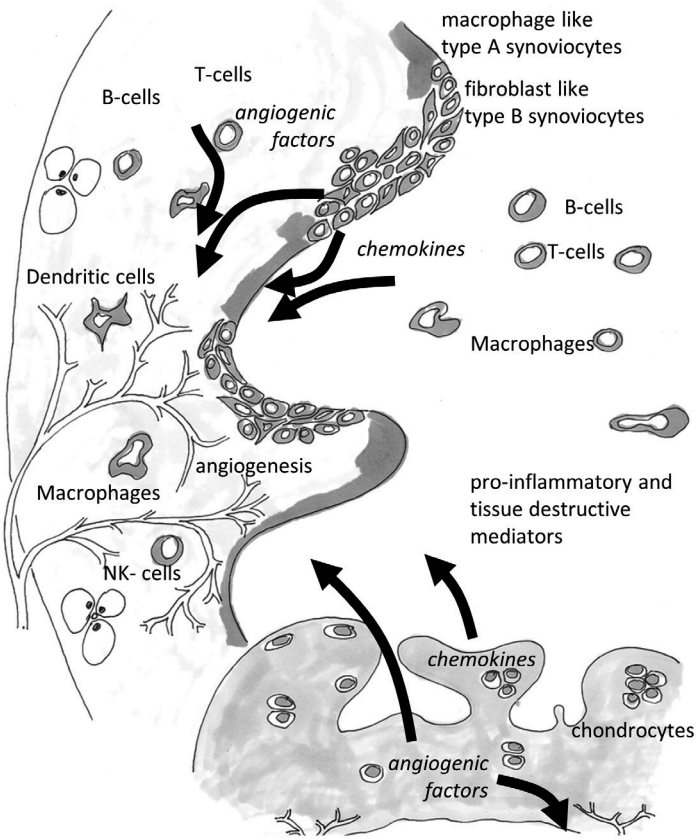

Figure 3: Angiogenesis and cell infiltration

(e.g. vascular endothelial growth factor (VEGF) and macrophage inflammatory protein 1 alpha (MIP1A)), as well as proteases.[30,41,42] Moreover, macrophages can stimulate other cells like endothelial cells and fibroblasts to produce proinflammatory mediators, angiogenic factors, and proteases, resulting in increased synovitis and tissue destruction.[43]

Dendritic cells have also been reported to be present in low numbers in osteoarthritic synovium of humans and rodents,[44,45] probably playing a role in T-cell antigen presentation in addition to macrophages providing this function. *T cells* are of CD4 as well as CD8 origin and express markers of immune activation such as major histocompatibility complex class II and have a Th1 polarized (proinflammatory) phenotype.[46] These T cells are also found in osteoarthritic synovial fluid, the majority of them being CD4+ T cells with a memory phenotype.[32,47] The T-cell response may potentially be directed against a common joint tissue-related antigen based on the T-cell receptor arrangement and oligoclonal expansion.[23,48] Also T cells add to neo-vascularization of the synovial tissue by producing pro-angiogenic factors.[29]

NK cells in limited[27] as well as high[49] numbers with elevated receptor expression for chemokines[50] have been reported in the osteoarthritic joint, although not many research groups have been studying these cells in this low-grade inflammatory disease. They have been reported to express a quiescent phenotype consistent with post-activation exhaustion,[38] possibly related to a role in the early phase under certain conditions in the disease.

The knowledge of the role of *B cells* in osteoarthritis is still limited. In general, these cells are rarely described,[23] although it has also been reported that they can be detected in half of the osteoarthritis patients tested.[51] Interestingly, if B cells are found in osteoarthritic joints, they are in an activated state.[23] Autoantibodies against breakdown products of collagen in osteoarthritis joints have been reported in the older literature, but such observations have not been reported recently.[52,53]

It is important to notice that not only mononuclear cells from the synovial tissue and in the synovial fluid are involved in the inflammatory activity. The cartilage chondrocytes are also able to produce most of the factors produced by these inflammatory cells including the inflammatory cytokines, chemokines, and angiogenic factors (Figure 3).[23]

Angiogenesis

The synovium is highly vascularized under healthy conditions to supply the cartilage with nutrients.[30] In osteoarthritis, angiogenesis and inflammation of the synovium are closely integrated processes and affect synovitis and related clinical symptoms.[30,39,54] Angiogenesis, neo-vascularization, the formation of new blood vessels, may be most important in potentiating inflammation rather than initiating it.[29]

Angiogenesis is a complex process, initiated via several pathways, in which in the end endothelial cells produce VEGF and angiopoietins, ensuring vessel stability.[29,55-57] Apart from being a potent stimulator of angiogenesis, VEGF also contributes to inflammation via plasma extravasation.[58,59] Also vascular regression is observed which does not lead to a decrease in vascular density, in fact, there is a redistribution of synovial vessel and a change towards a more immature phenotype.[60] A fully functional microvasculature is formed by the differentiation of the newly formed vessels into arterioles, capillaries, and venules. An inflammatory response is maintained by the supply of inflammatory cells through these new vessels. Angiogenesis can indirectly promote itself by increasing inflammatory cell infiltration, and increasing angiogenic factor release.[29,61] Also hypoxia, acting via hypoxia-inducible factor 1 alpha (HIF1A) can be involved in angiogenesis, HIF1A is to be co-localized with microvascularity in osteoarthritic synovium.

Synovial angiogenesis is found in all stages of osteoarthritis[30] but could contribute to the transition from acute to chronic synovitis by potentiating inflammatory pathways.[29,61] Synovial tissue from early osteoarthritis patients contains higher levels of angiogenic factors, suggesting a more active angiogenesis in early osteoarthritic synovium.[34] Importantly, angiogenic activity in osteoarthritis is also regulated through changes in the articular cartilage by promoting the expression and release of angiogenic factors from chondrocytes.[62] Release of these factors may also lead to ingrowth of blood vessels from the bone into cartilage at the bone– cartilage interface.[63] Angiogenesis in the synovium is associated with histological synovitis, but not clearly with cartilage changes, whereas vascular density at the osteochondral junction is more clearly associated with changes in the cartilage but not with histological synovitis.[64] In more severe osteoarthritis, vascular breaching of the tidemark to the cartilage is observed.[61] In this way, inflammation-related mediators from the bone (and vice versa) can also enter and influence cartilage damage (Figure 3).

Intracapsular fat pads

Fat pads are intracapsular, extrasynovial-located adipose tissues within joints, found most prominently in the knee but also in other joints. Adipose tissue in general secretes different adipokines, cytokines, and other inflammatory mediators contributing to inflammation.[65] In addition to adipocytes, the intra-articular fat pads in osteoarthritic joints contain a connective tissue matrix, nerve fibres, vascular cells, and immune cells (Figure 4).[66] The fat pads contain, in between the large adipocytes, macrophages, T cells, B cells, and mast cells. In the stromal vascular cell fraction of the osteoarthritic intra-articular adipose tissue, the T cells show a Th1 proinflammatory phenotype, whereas macrophages are of an M1 (proinflammatory) as well as M2 (anti-inflammatory) phenotype. Mast cells are more abundantly present in intra-articular adipose tissue than in subcutaneous fat, which might be related to angiogenesis, where vascularization of intra-articular adipose tissue is reported to be higher than that of subcutaneous tissue.[67] In addition to multiple adipokines produced

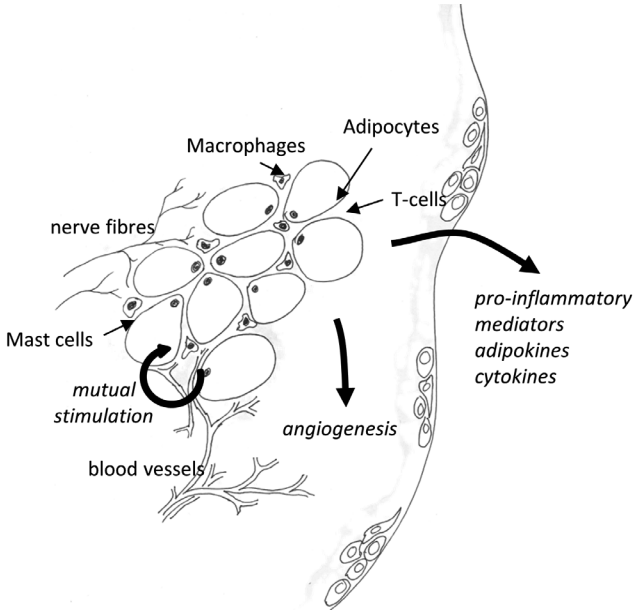

Figure 4: Intra-articular adipose tissue

by the adipocytes, the intra-articular adipocytes and the embedded inflammatory cells produce multiple proinflammatory cytokines including TNFA and IL6, the latter in higher levels than in subcutaneous fat.[65] As such, the intra-articular adipose tissue is considered to play an important role in joint inflammation as well.

The adipose tissue outside the joint is suggested to be systemically related to osteoarthritis. Specifically in cases of obesity, it produces and secretes large amounts of inflammatory mediators such as proinflammatory cytokines and adipokines, of which the production is changed in the presence of osteoarthritis.[20,68,69] As such the relation between obesity and osteoarthritis clearly goes beyond the influence of enhanced joint loading and the excessive fat is suggested to add to low-grade systemic inflammation adding to the osteoarthritic process.[70] Also there is an interplay and autocrine stimulation between adipokines, proinflammatory mediators, and angiogenic factors, the different cell types influencing each other,[20,71] contributing to low-grade inflammatory activity systemically.[72]

Moreover, obesity is associated with a disturbed lipid metabolism, leading to changes in the levels of high-density lipoproteins and levels of free fatty acids, triglycerides, and oxidized low-density lipoproteins,[73,74] suggested to play a role in osteoarthritis as well,[75,76] although so far this has been less extensively studied.

Clinical impact

Osteoarthritis is characterized by structural changes in bone and cartilage, muscle, and tendon weakness and/ or contracture, as well as (low-grade/ intermittent) synovial tissue inflammation with possible joint effusion. All influence each other at a mechanical and

biochemical level, and result in joint stiffness and most importantly pain. Pain is intermittent and typically intense during weight-bearing activities like walking and stair climbing.[77] The relationship between pain and the actual structural changes is not very clear yet. Subchondral bone changes seem to provide the best correlation, but also (changes in) synovitis and effusion as seen on magnetic resonance imaging (MRI) have been reported to correlate with knee pain[25,78,79] and functional outcomes.[80]

Synovitis relates to progression of disease

It is inevitable that synovitis in whatever way, low-grade chronic or intermittent flares, adds to the severity and progression of disease. Synovial inflammation (synovitis and effusion) is related to more cartilage pathology years later[24,81] and effusion assessed by ultrasound (US) evaluation predicts joint replacement.[82] More synovitis (higher synovial volume) is related to a higher Kellgren– Lawrence grade, representing more joint damage and also the proportion of patients with synovitis increases with the progress of tissue damage.[83] However, molecular cross-talk between the synovium, cartilage, and the other adjacent tissues can influence the final impact of synovitis on joint changes.[84,85]

Detecting synovitis

There are various ways to detect and characterize (score) synovitis: clinical evaluation, histochemistry of synovial tissue biopsies, arthroscopy, US, MRI, and even measurement of biochemical markers.[23,86]

Clinical evaluation

The cardinal signs of inflammation are redness, pain, heat, swelling, and restriction of motion.[87] The restriction of passive motion can be the first and only physical sign of osteoarthritis.[77] Palpable joint swelling due to thickening of the synovium or synovial fluid effusion is considered to indicate synovial inflammation.[88] Joint enlargement, resulting from joint effusion, bony swelling, or both, is present during osteoarthritic flares, but can also be present during chronic osteoarthritis.[77]

Histochemistry of biopsies

The synovial changes in osteoarthritis as described above, such as cell composition and vascularization, are predominantly based upon histological changes in synovial composition analysed from synovial tissue biopsies either taken by needle biopsies, during arthroscopic evaluation, at surgery (such as during meniscus treatment), or in the end at joint replacement surgery. Scoring histology in early osteoarthritis is difficult, since synovial tissue changes are often focal and can easily be missed by random biopsies.[89]

Arthroscopy

For orthopaedics, arthroscopy is the gold standard for imaging of cartilage damage and

synovial abnormalities. Arthroscopy has been used to show the association between knee effusion and synovitis.[23,24] Arthroscopic studies suggest synovial thickening to be associated with inflammatory changes in 50% of patients with osteoarthritis, and the presence of synovitis detected by arthroscopy is associated with more severe chondropathy.[23] Using a standardized macroscopic description of the synovial appearance, a distinction could be made between reactive and inflammatory synovium in which inflammatory synovium was suggested to have a direct effect on adjacent cartilage.[24] Arthroscopy is suggested to be used as a monitoring tool as well as a diagnostic or therapeutic procedure.[90] This is made possible by the development of arthroscopic scoring systems for determining the severity of synovial lesions (e.g. the Synovitis Score, a composite index incorporating intensity, extent, and location of synovial abnormalities).[90] However, arthroscopy is not comprehensive for assessment of overall synovitis and severity depends on the underlying cause.[90] Not all compartments of a joint can be fully visualized and not all joints are easily accessible.

Imaging modalities

Magnetic resonance imaging

MRI has good potential to objectively quantify the morphology and integrity of the synovium, however it has been predominantly used for evaluation of other joint tissues[91] and clearly more for the knee than for the smaller hand joints.[92] A comprehensive review has recently been provided by Guermazi et al. describing advances and limitations, acquisition sequences, and relations with severity and symptoms of disease for knee, hip, and hand joints.[92] Limitations are the acquisition time, complexity of the more advanced techniques, as well as the costs[77] and often but not necessarily the use of contrast agent.[93] Synovial scoring by MRI is mainly based on synovial thickening and joint effusion graded collectively ('effusion synovitis'); distinguishing between synovial fluid and synovial tissue needs the use of contrast. Different scoring methods have been described for knee, hands, and hips.[25,83,94–98]

Characteristics of the synovium on MRI correlate with histopathology of synovium biopsies, especially in early disease.[28,47,99–101] Severity of synovitis (enhanced synovial volume) correlates with increased severity of the disease on radiographs.[88,92] Synovitis can precede the development of radiographic knee osteoarthritis.[102] Longitudinally, synovial changes on MRI relate to cartilage loss over time.[25,103] Knee joint effusion synovitis and knee cartilage defects are correlated cross-sectionally and longitudinally.[104] However, others have found that synovitis does not relate to severity of cartilage damage.[28] It has been reported that 75% of patients with less than 4 years of osteoarthritis symptoms present with synovial thickening on MRI[47] and 37% in elderly persons without radiographic osteoarthritis.[105]

Ultrasonography

US has the ability to image synovium in several planes using grey scale, representing effusion

and synovial hypertrophy, and additionally power Doppler as a measure for vascularization of the tissue, representing more active inflammation.[106–109] It does not require a contrast agent and allows for real-time visualization. The penetration depth of the signal limits the tissue that can be assessed. As such, the technique is specifically suitable for the smaller joints affected by osteoarthritis, but is used for larger joints as well. A drawback is that US outcome is very dependent on the experience of the observer.[110] Several studies have been published over the years. Synovial involvement (synovitis and effusion) by US is found in 47% of patients with painful knee osteoarthritis.[111] The presence of knee effusion evaluated by US in addition to other parameters predicted the need for joint replacement surgery.[82] Inflammatory features evaluated by US, especially when persistently present, are associated with radiological progression of hand osteoarthritis.[112] Clearly synovial abnormalities on US are more common in osteoarthritic joints but the associations with severity and symptoms are not conclusive.[81,113,114]

Biochemical markers

Synovial tissue metabolism and inflammation can be assessed with biochemical markers. The more and the larger the joints involved, the higher the chance of detecting such markers in the peripheral compartment. A cluster of biochemical markers has been related to low-grade synovitis in osteoarthritis.[115] Serum cartilage oligomeric matrix protein (sCOMP) is present in synovial tissue, is produced by synoviocytes, and is associated with synovitis and/ or effusion. Serum hyaluronan and serum N-propeptides of collagen type III are two non-specific markers of synovial activity, segregated with sCOMP, all found to be associated with clinical synovitis.[115,116] General markers of inflammation are found to be raised in osteoarthritis including high-sensitivity C-reactive protein (related to IL6 and YKL40 levels)[117] and to be related to synovial cell infiltration[118] and to progression in early osteoarthritis.[119] However, correction of potential confounders such as body mass index decreases such relations. Recently it was suggested that evaluation of biochemical markers in the joint synovial fluid instead of the peripheral compartment is worthwhile to consider since better relationships with tissue changes are found.[120] Also systemic or local adipokines might be relevant markers of synovial (intra-articular adipose tissue) activity of knee and hand osteoarthritis.[121,122] Although promising, there are still some bridges to cross before such markers become of relevance to clinical practice.

Pathways that promote synovitis

There are numerous mediators involved in synovial activity, among them angiogenic factors, cytokines, chemokines, and proteases. Clearly, the number and diversity of these mediators in osteoarthritic joints, the complex roles and interactions of these molecules in inflammation, extracellular matrix (ECM) damage and repair, changes over time, and

the physiological roles of most of these mediators in normal ECM turnover and 'healthy' immunological defence, makes it virtually impossible to provide a comprehensive and still convenient overview of these molecules and their pathways.

Even in the absence of classical (overt) inflammation, healthy cartilage chondrocytes and (within normal physiology) 'surveilling' synovial cells, express mediators (stimulators as well as inhibitors) of inflammation including classical cytokines (such as IL1B, TNFA, and IL6), proteases (such as collagenases (including metalloproteinases; MMPs) and aggrecanases (such as a disintegrin and metalloproteinase with thrombospondin motifs; ADAMTS)) as well as other mediators (such as cyclooxygenase (COX), and nitric oxide (NO) as a result of mechanical or oxidative stress)[123] all being part of normal ECM turnover (Figure 5).

During the process of osteoarthritis, many of these mediators are upregulated. This upregulation may be restricted to the cartilage tissue, driven by biomechanical processes, and for a long period of time remain clinically unnoticed. Alternatively (or coinciding), synovial tissue inflammation can develop after (sub)acute, or chronic joint injury including mechanical derangement by, for example, meniscal extrusion or tears, joint overuse, hypermobility, mal-alignment, or ligament rupture.[124,125] Although an acute trauma may result in an acute inflammatory response, this inflammatory response is most often transient, demonstrated by a transient increase in inflammatory mediators in the synovial fluid.[126] However, even such acute responses may be critical in a degenerative process later on.[127] This initial response can also lead to a vicious cycle by which acute local tissue damage leads to acute synovial inflammation, which in turn leads to more chronic tissue damage and repair, resulting in a chronic inflammatory tissue destructive processes.[128] This may result in the intermittent or chronic low-grade inflammation in osteoarthritis.

Innate immune system

More recently, the networks of diverse innate inflammatory danger signals have gained attention in osteoarthritis research. Damage-associated molecular patterns (DAMPs), including alarmins (S100 proteins), high-mobility group box (HMGB) protein 1, ECM proteins (e.g. collagen, fibronectin, and proteoglycan), and free fatty acids and their receptors (pattern-recognition receptors; PRR), such as toll-like receptors (TLRs) and receptors of advanced glycation end-products (RAGEs), as well as elements of the complement system are elevated in the osteoarthritic joint and have become molecules of interest. Synoviocytes (specifically macrophages) as well as chondrocytes express a variety of TLRs and RAGEs, which are upregulated by tissue damage and inflammation in osteoarthritis.[129,130] Ligands for these receptors including over-expressed S100 proteins,[131] HMGB proteins, elevated levels of cartilage ECM components[130] such as tenascin C,[132] fibronectin isoforms,[133] small-molecular-weight species of hyaluronic acid[134] and biglycan,[135] but also certain plasma proteins[136] can activate the TLR cascade, stimulating the nuclear factor kappa B (NFKB) pathway and subsequent production of chemokines and cytokines. These in turn recruit and activate macrophages, and lymphocytes leading to downstream activation of inflammatory and

catabolic processes in the synovium as well as the cartilage, processes which in their turn perpetuate the upregulation of DAMPs.[137,138] The consequent induction and amplification of synovitis and chondrocyte-related inflammatory processes (including PPR activation[139]) amplify the catabolic processes of joint damage and thus osteoarthritis progression.[140] But clearly, the book of DAMPs and their PPRs is not closed because protective roles of TLRs have also been described; knockout of TLR2 in a murine model results in more severe disease, suggesting a protective as well as destructive role of TLR (Figure 5).[141]

Complement activation is also considered a factor in disease progression in osteoarthritis.[137] The complement cascade is a major player in the activation of the immune system. In osteoarthritic joints, complement can be activated by DAMPs (including cartilage matrix constituents), but also by cell debris, by crystals (e.g. hydroxyapatite and calcium pyrophosphate dehydrate), and by cartilage ECM components such as aggrecan and fibromodulin.[137,142] Increased expression and activity of manifold effector molecules of the complement pathways including formation of membrane attack complex (MAC) occurs in early human osteoarthritis, and synovial expression of multiple complement inhibitors are

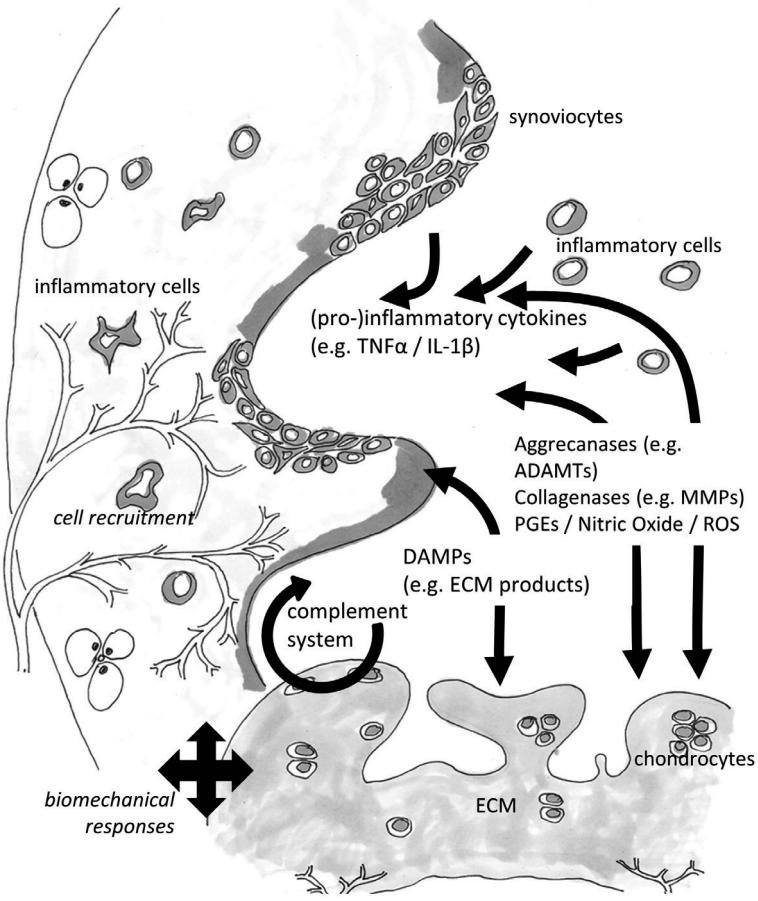

Figure 5: Synovitis perpetuation

decreased in human disease.[136,137,143,144] Activation of the cascade, resulting in MAC is essential for removal of many pathogens, however improper regulation can lead to tissue damage, as may be the case in osteoarthritis. Activated complement components accumulate in cartilage and change chondrocyte activity. MAC can directly lyse cells through formation of pores in the cellular membrane, and induce sub-lytic inflammatory signalling pathways that further promotes cartilage damage and results in an increase of ECM breakdown products, perpetuating the cycle of complement activation.[42]

Cytokine-related processes

Proinflammatory cytokines like IL1B, TNFA, IL6, IL8, IL17, their natural regulators such as the IL1Ra (receptor antagonist) and anti-inflammatory cytokines such as IL4, IL10, IL13 as well as proteases (collagenases and aggrecanases) and many other soluble components (NO, prostaglandins (PGEs), etc.) involved in inflammation are released during the inflammatory process by synovial (inflammatory) cells as well as chondrocytes and are found (in general elevated) in the osteoarthritic joint.[145– 147] These mediators play a pivotal role in initiating, perpetuating, and progression of osteoarthritis (Figure 5; Table 6.1).[148,149] For a more comprehensive overview of the additional inflammatory mediators see Table 6.1. During cartilage degradation, in both early and advanced osteoarthritis, synovial cells phagocytize ECM waste products released in the synovial cavity, and the synovial membrane releases proinflammatory mediators.[23] These in turn lead to increased cartilage breakdown and synovial inflammation, and an excess in the production of proteolytic enzymes.

L1B and TNFA

IL1B and TNFA are the two major cytokines (but clearly not the only ones) involved in the pathogenesis of osteoarthritis, mainly produced by activated synoviocytes, and mononuclear cells; but also chondrocytes themselves are capable of producing these cytokines that induce inflammation and cartilage degradation.[150] Patients with osteoarthritis have increased levels of IL1B and TNFA in the synovial membrane and synovial fluid, but also elevated levels in subchondral bone and cartilage. Both IL1B and TNFA have been found in higher concentrations in patients with early osteoarthritis than patients with advanced osteoarthritis.[34] These cytokines not only induce cartilage catabolism; osteoarthritic progression is also stimulated by suppression of anabolic processes by inhibition of cartilage ECM formation including production of aggrecan and collagen type II, the major ECM constituents of articular cartilage.[151,152]

In healthy individuals, IL1B is not produced as much as its natural antagonist, IL1RA; in osteoarthritis a disturbed balance is an additional enhancer to the catabolic effects of IL1B.[153] Activation of cells by IL1B is mediated through the receptor IL1r, extensively expressed on synovial cells and chondrocytes. IL1B can also bind to a second specific (decoy) receptor that is unable to transduce a signal. IL1RA is produced by several cells including synovial

Table 1: Important inflammatory mediators in osteoarthritis

Mediator	Description	References
IL1B	Proinflammatory cytokine elevated in synovial fluid/ membrane, subchondral bone, and cartilage. Supresses type II collagen and aggrecan expression. Stimulates the release of MMP1, MMP3, MMP13, NO, PGE2. Induces the production of IL6, IL8, MCP1, and CCL5 (RANTES)	136, 151, 152, 221–223
IL1RA	Anti- inflammatory cytokine that inhibits IL1B and PGE2 release	223, 224
IL4	Ambivalent cytokine, pro/ anti- inflammatory effects, found elevated in synovial fluid and in supranatural amounts in the synovium. Upregulates MMP1 and MMP13 in combination with IL1B and oncostatin. Activates B cells and T cells, and mediates recruitment of inflammatory cells to site of infiltration. IL6 also induces synovial fibroblasts to produce angiogenic factors and stimulates chondrocytes and synovial fibroblasts to release chemokines like IL8. Reduces expression of type II collagen. Induces the production of TIMP, but not MMPs, involved in feedback mechanism that limits proteolytic damage. Reduces proteoglycan loss in acute phase OA, but enhances osteophyte formation in the chronic phase of OA. Stimulates proliferation of chondrocytes	43, 223, 227
IL8	Proinflammatory chemokine synthesized by monocytes, macrophages, chondrocytes, and fibroblasts, and is found to be elevated in OA tissue. Induces iNOS, MMP1, IL1B, TNFA and IL6, and stimulates proteoglycan depletion	228
IL10	Anti- inflammatory cytokine elevated in OA tissue. Inhibits IL1B, TNFA, MMPs, and PGE2 release, and upregulates IL1RA and TIMP. Modulates TNFA by increasing release of TNFsr, downregulating receptor surface expression	223, 229
IL13	Anti- inflammatory cytokine elevated in OA tissue. Inhibits IL1B, TNFA, MMPs, and PGE2 release, and apoptosis of synoviocytes. Upregulates IL1RA and TIMP	223, 230
IL15	Proinflammatory cytokine elevated in synovial fluid in early OA (more than advanced OA). Closely associated with MMP1 and MMP3. IL15 receptor is present on the synovial lining layer and the endothelium. Involved in recruitment and survival of CD8+ T cells	128, 223, 231
IL17	Proinflammatory cytokine that induces IL1B, TNF, and IL6 production. IL17 also upregulates NO and MMPs and downregulates proteoglycan levels	223, 232
IL18	Proinflammatory cytokine elevated in human OA chondrocytes. Acts through IL1 dependent and IL1 independent pathways	223, 233
IL21	Proinflammatory cytokine, elevated the synovium during early OA	231
TNFA	Proinflammatory cytokine elevated in synovial fluid/ membrane, in subchondral bone and in cartilage. Supresses the synthesis of proteoglycan, link protein, and type II collagen in chondrocytes. Stimulates the release of MMP1, MMP3, MMP13, NO, and PGE2. Induces production of IL6, IL8, MCP1, and CCL5	136, 223, 234, 235
RANTES	Proinflammatory cytokine, also known as CCL5, elevated in OA tissue. Induces iNOS, MMP1, and IL6, and stimulates proteoglycan depletion	236
TGFB/ VEGF	Vascular growth factors, inducing angiogenesis	30, 237
Adiponectin	Protein hormone predominantly secreted by differentiated adipocytes, but also by synovial fibroblasts. Adiponectin receptors on synovial fibroblasts lead to an increased production of MMPs, cytokines, and PGE2	238, 239
Resistin	Proinflammatory adipokine that induces cartilage destruction and synovial inflammation. Upregulates IL6 and TNFA in macrophages and synovial fluid cells	242

(continued)

Table 1 continued

Mediator	Description	References
Leptin / visfatin	Proinflammatory adipokines	240, 241
PGE2	Prostaglandin that causes hyperalgesia, upregulated by IL1B and TNFA	243
NO/ NOS/ iNOS	Mainly produced by IL1B and TNFA. NO activates NFKB in peripheral blood mononuclear cells, an important transcription factor in iNOS gene expression in response to inflammation, and contributes to cartilage degradation	163
MCP1	Elevated in OA tissue. Induces iNOS, MMP1, and IL6. Stimulates proteoglycan depletion	244
ICAM1	Intercellular cell adhesion protein 1 is an adhesion molecule that mediates monocyte adhesion and regulates movement of mononuclear cells into inflammatory sites	245
VCAM1	Vascular cell adhesion protein 1 is an adhesion molecule that mediates monocyte adhesion and regulates movement of mononuclear cells into inflammatory sites	246, 247
MMP 1/ 3/ 9/ 13	Chemokine enzymes capable of degrading ECM. Involved in catabolic process and remodelling of ECM. MMP1 is produced by fibroblasts, chondrocytes and synovial fibroblasts at sites of synovial attachment to articular cartilage. MMP1 is capable of degrading type I/ II/ III collagens	248, 249
Substance- P	Neurotransmitter that mediates proinflammatory signals, vasodilatation and contributes to pain. Located in subintimal portion of synovial membrane and in areas with osteophytes and cartilage erosions. Induces PGE2 and collagenases by synoviocytes and induces proliferation of synoviocytes	5, 87
LIF	Leukaemia inhibitory factor (LIF) is a cytokine elevated in OA synovial membrane/ fluid. LIF is upregulated by IL1B and TNFA. LIF stimulates cartilage proteoglycan degradation/ resorption, MMP synthesis and NO production. LIF induces acute- phase protein synthesis, lipoprotein lipase activity and the expression of collagenase and stromelysin, but not TIMP Enhances IL1B and IL8 in chondrocytes and IL1B and TNFA in synovial fibroblasts. Regulates connective tissue such as cartilage and bone	23, 250
CXCL13	Attracts activated B cells in synovial membrane lymphoid aggregates	251
COX2	Upregulates PGE2, and is upregulated by TNFA, IL6, IL1B, and via TLR4 stimulation	173
Bradykinin	A neuropeptide generated in the synovium. Initiates and maintains inflammation and allows for excitation and sensitization of nerve fibres	23, 252
TIMP	Tissue inhibitor of metalloproteinases	234

fibroblasts and chondrocytes and is capable of binding to both receptors, as such providing anti-inflammatory properties.[153] TNFA has two receptors, TNFR55 and TNFR75, the latter only responding to membrane-bound TNF. Their expression is modulated by, among others, both IL1B and TNFA. IL1B and TNFA can independently initiate and propagate inflammation in osteoarthritis joints, however, it has been shown that a simultaneous injection leads to more cartilage destruction than either cytokine alone in animal models.[154,155] Both IL1B and TNFA can upregulate their own production, via the activation of NFKB.[156] Additionally, synovial fibroblasts are capable of upregulating IL1B and TNFA upon stimulation of their IL1B and TNFA receptors,[157] in turn activating synovial cell activation.[158] IL1B and TNFA can stimulate chondrocytes and synovial cells to produce many of the other inflammatory

mediators.[159]

Oxidative stress

IL1B and TNFA also induce the production of NO and reactive oxygen species (ROS) (mostly the superoxide anion), generating radicals capable of cartilage degradation.[160] Downregulation of antioxidant enzyme expression by IL1B and TNFA reduces the amount of ROS and NO that can be cleared, thus increasing the damaging potential of ROS and NO on cartilage.[161] NO has a major role in the modulation of chondrocyte function in osteoarthritis. NO is upregulated in the inflammatory osteoarthritic joint. In the affected joint, free radicals like NO and ROS are mediators of inflammation and tissue destruction contributing to disease progression.[160,162]

ROS are chemically reactive molecules originating from conversion of oxygen.[163] They are formed as a by-product of normal metabolism, and are increased during osteoarthritic conditions. ROS are involved in the regulation of biochemical factors that are involved in cartilage degradation and joint inflammation and influence certain intracellular signalling pathways.[162,163] ROS can directly cause damage to all matrix components, either by a direct attack, or indirectly by reducing matrix components synthesis by apoptosis of the cells or by latent activation of MMPs. ROS can regulate the activity of transcription factors through oxidative modifications of, for example, cysteines. ROS involvement in inflammation, fibrosis control, and nociception has been reported as well.[163] However, whilst the effect of ROS on synovial inflammation is clearly proinflammatory, ROS have also been shown to downregulate the expression of proinflammatory genes in chondrocytes.[164]

Epigenetic changes

At all different levels within the osteoarthritic joint and clearly also in the synovial membrane, epigenetic changes such as post-translational methylations, as well as the role of microRNAs are becoming a new area of research. Such changes, like in many diseases, are sure to be found to be of influence in the disease process, causative, and/ or epiphenomenal.[165,166]

Pain related to synovitis

Pain is the defining reason why most people seek care. Current pain management of osteoarthritis falls short of patients' needs in terms of providing adequate and sustained pain relief. Osteoarthritis-associated joint pain has a strong mechanical component, triggered by specific activities and relieved by rest.[167] The joint is a heavily innervated organ, and its sensory innervation is organized predominantly towards proprioception and nociception. Nociceptive fibres are located in the joint capsule, synovium, meniscus, bone marrow, periarticular ligaments, periosteum, subchondral bone, and the marrow cavity of osteophytes.[68,168] Consequently pain can originate from many articular tissues. Intra-articular local anaesthetics abolish knee osteoarthritis pain[169] suggesting that these

structures are in contact with the intra-articular environment as well. As cartilage is an aneural and avascular tissue, it is often considered as non-participatory in joint pain. However, osteoarthritic cartilage is potentially a large source of mediators that can act as nociceptive mediators, including cytokines, other mediators, and possibly extracellular fragments. Moreover, it has been demonstrated that nociceptors express TLRs, which are PRRs that recognize a large array of DAMPs released during tissue injury and contribute to pain generation.[170–172] In osteoarthritis, no information on the role of TLRs on nociceptors and pain generation currently exists, but TLRs play a clear role in synovitis in osteoarthritis as described above.[128,173] This indicates that TLRs contribute to pain in osteoarthritis indirectly by activating synovial fibroblasts and macrophages,[128] and potentially directly by sensitizing nociceptors.

Inflammation plays a critical role in the initial increase and processing of nociceptive input.[174] Many of the substances involved with inflammation are also neuroactive. These substances stimulate chemosensitive nociceptors and can be categorized into three groups (Table 2).[175] However, it is unclear to what extent the low and often varying level of inflammation in osteoarthritis can actually produce this enhanced nociceptive receptor sensitivity. Mediators related to tissue destruction might be involved, as well as sensitization by a vicious cycle of mediators. That explains why patients with osteoarthritis can react in a more exaggerated way to innocuous stimuli.

Table 2: Stimulation of chemosensitive nociceptors, subdivided by the release origin

Origin	Mediators	Description	References
Damaged cells	*Hydrogen ions (H+)* *Adenosine triphosphate (ATP)*	These factors are released by damaged tissue and activate the nociceptors, previously excited directly by the causal stimulus itself	253-254
Inflammatory cells	*Bradykinin* *Prostaglandins* *Leukotrienes* *Proinflammatory cytokines (TNFA, IL1B, IL6, IL17, High- mobility group protein B1 (HMGB1))*	Bradykinin increases capillary permeability and is among the most potent pain- producing substances identified to date. The factors in this group exert specific effects and sensitize the receptors to other factors. They cause primary hyperalgesia, a nociceptive stimulus that produces pain that is disproportionately severe compared to the intensity of the stimulus	215, 253-256
	Anti- inflammatory cytokines (IL4, IL10) *Nerve growth factor (NGF)*	Anti- inflammatory cytokines suppress pro- inflammatory cytokine expression, and macrophage/ microglial activation. These cytokines provide strong pain inhibition, but even more so with the combined IL4– 10 synerkine	
Nociceptors	*Substance- P*	Released by the nociceptor itself and can activate these receptors either directly or indirectly. These mechanisms result in a vicious circle of pain stimulation	182, 183, 253

Synovitis induces the release of prostaglandins, neuropeptides, and cytokines. These mediators are capable of causing hyperalgesia by activating threshold receptors or by sensitizing fine unmyelinated sensory nerves in the osteoarthritic joint. For example, TNFA and IL6 can cause prolonged mechanical hypersensitivity, whereas IL17 sensitizes joint nociceptors to mechanical stimuli.[176] The proinflammatory cytokines TNFA and IL1B can affect sensory neurons directly, but also indirectly by the downstream activation of other cytokines, chemokines, prostanoids, neurotrophins, NO, lipids, and via the complement and NFKB pathways.[176,177]

Persisting stimulation of nociceptors by these substances will lead to heightened neuronal activity; second-order neurons in the spinal cord increase their firing rate, enhancing the pain transmission and intensifying the pain sensation.[87] Joint damage can lead to joint inflammation and subsequent pain. However, reverse causality holds true as well, sensitization and heightened nociception can lead to an increase in inflammation, and thus an increase in joint damage. This happens at the same time as turnover within the synovial tissue and neurological restructuring of the joint. This means that cell activation, proliferation, and infiltration due to synovitis is accompanied by growth as well as retraction of nerve endings, associated with enhanced pain sensation.[178–18] Peripheral nerves do not proliferate, they grow by neurite extension or arborization, which is the terminal branching of nerve fibres in a treelike pattern.[181,182] This change in innervation pattern of the synovial layer is also demonstrated in osteoarthritis. Synovial material obtained at knee joint replacement with and without inflammation exhibited a similar vascular and neuronal network. However, the inflamed synovium had a decreased number of nerve fibres reaching the synovial lining layer depending on the degree of inflammation. The deeper areas (e.g. the capsule) were less affected.[183]

Moreover, the joint is innervated by postganglionic sympathetic efferents, but also by sensory fibres, that are distinguished based on their anatomic features,[184,185] and stimulation of these fibres is associated with a specific kind of pain. Different types of fibres can be identified in the joint:

- Aβ fibres (group II): thick myelinated nerves, innervating the capsule, fat pad, ligaments, menisci, and periosteum. These nerves may mediate sudden pain on movement or pressure.
- Aδ fibres (group III): thin nerves with myelin sheath that disappear in the terminal region, innervating capsule, ligaments, menisci, periosteum, and mineralized bone. These nerves may mediate sudden pain on movement or pressure.
- C fibres (group IV): thin unmyelinated nerves, innervating capsule, ligaments, menisci, periosteum and mineralized bone. These nerves may mediate slow, burning pain as described by many patients with osteoarthritis.

Inflammatory mediators can also stimulate nerves in the absence of mechanical stimulation. Over a period of hours or days, upregulation of genes within the synovium and recruitment of inflammatory cells enhance peripheral sensitization, that is, reduction of the pain threshold, whilst neuronal plasticity contributes to central sensitization, increasing the excitability of neurons within the central nervous system, resulting in multiple amplification of the pain sensation.[29] This is supported by the indications that hip osteoarthritis patients have a lower pain threshold compared to healthy controls.[186] In addition, there is also a tendency to note sensitivity to innocuous warmth and cold in patients with hip osteoarthritic pain.[187] The pain becomes more constant over time, from shorter periods of aching and throbbing to continuous periods of intense pain.[176]

Angiogenesis has proven to be a major contributor to inflammation and in a wide variety of tissues unmyelinated (C fibres) nerve growth follows angiogenesis.[181,182] However, the exact role of angiogenesis in pain is less established. In addition to the growth of these fibres and presence of classic nociceptors, there are also fibres present that only become active after damage or inflammation, called silent nociceptors. They can have a substantial contribution to pain sensation in osteoarthritis.

Before joint pain is reported, radiographic evidence of joint damage can be observed. Moreover, the extent of joint damage has little relation to the amount of pain experienced, especially in knee osteoarthritis.[188,189] However, more recent studies indicate stronger associations between structural changes and pain severity. For instance, radiographic osteoarthritis and individual radiographic characteristics were demonstrated to be strongly associated with knee pain.[190] Remarkably, especially joint space narrowing was more strongly associated with pain than were osteophytes.[191] Using imaging modalities like MRI, it has been shown that bone marrow lesions,[192] subarticular bone attribution,[78] effusion,[25] and synovitis are associated with knee pain.[192] In OA patients, more severe disease leads to worse structural outcome, which makes it difficult to determine the contribution of the individual tissue changes, but research has shown that changes in subchondral bone and synovial changes seem to predominate.[193]

Therapeutic approach to treat synovitis

Treatment of synovial inflammation in osteoarthritis is hampered by the fact that inflammation is, in general, low grade and variable over time. Treatment needs to be provided over a very long time period because of the chronic character of the disease with often several comorbidities such as obesity and diabetes. When multiple joints are involved, there is a need for systemic treatment. This all makes anti-inflammatory treatment for osteoarthritis a real challenge. Irrespectively, targeting synovitis may hold promise specifically for those patients in whom synovitis dominates the disease.

General anti-inflammatory treatment

General systemic and local anti-inflammatory treatment limiting synovial tissue activity in osteoarthritis such as non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX2 inhibitors as well as effects of corticoid-steroid injections and other supposedly inflammation controlling intra-articular injections will be discussed elsewhere, as will be the potential disease-modifying OA drugs. More recently, approaches with the anti-rheumatic drug methotrexate (MTX) have been dared.[194] However, because MTX is cytotoxic and has potential serious life-threatening side effects its use cannot be justified simply for treatment of the, in general, low-grade synovial inflammation in osteoarthritis.

Anti-angiogenic treatment

Targeting angiogenesis, and with that synovitis, could prevent disease progression and alleviate symptoms. Anti-angiogenic strategies have been implemented especially in the oncological field. Although the mechanism/initiation of angiogenesis differs between osteoarthritis and oncology, this does not mean that therapies efficient in one cannot be efficient in another. Broad inhibition of angiogenesis and inflammation with the use of drugs may however not easily be applicable due to potential toxicity of these drugs modifying biologically important physiological processes.[29]

Targeted anti-inflammatory (antibody) treatment

As has become clear from the above-discussed complexity of mediators involved, there are limitations in the ability to control inflammatory mediators in osteoarthritis collectively. This resulted in great interest in identifying and targeting the specific inflammatory mediators and pathways that contribute to the disease and through that developing an anti-cytokine therapy for osteoarthritis.[147] Strategies aimed at preventing excessive proinflammatory cytokine production, signalling, and downstream NFKB activation, by the use of highly specific drugs, small interfering RNAs (siRNAs), or other biological inhibitors,[195] are the focus of current osteoarthritis research. Animal models show hopeful results, however, as all these mediators are mutually interacting in human disease, clinical treatment is a challenge. Clearly these biological therapies will not be suitable for all types of osteoarthritis,[196] and biological therapies targeting single cytokines that are increased in osteoarthritis joint tissues (e.g. IL1B and TNFA) have not yet resulted in either effective or pragmatic treatment in human osteoarthritis.[153]

Anti-TNF to control synovitis

Thus far, despite compelling evidence suggesting the role of TNFA in the degenerative nature of osteoarthritis, no agents targeting the TNF family have been approved for osteoarthritis treatment. Small studies, in the case of clear inflammatory hand osteoarthritis injections of anti-TNFs, did not appear to be exclusively successful. Treatment with adalimumab (subcutaneously applied) did not significantly improve the signs and symptoms of erosive

hand osteoarthritis.[197] Adalimumab had no clear effect in erosive hand osteoarthritis but only appeared to slow down progression of joint damage in the most progressive subpopulation.[198] Adalimumab was not superior to placebo in patients with hand osteoarthritis not responding to analgesics and NSAIDs.[199] With infliximab, symptomatic effects were obtained in erosive hand osteoarthritis but disease-modifying action was not significant.[200] In a single case of knee osteoarthritis, in addition to treatment with a COX2 inhibitor, adalimumab seemed to alleviate symptoms of pain.[201] In an open uncontrolled study, patients with knee effusions treated with adalimumab showed promising short-term clinical benefit.[202]
Preclinical studies also suggest that monoclonal antibodies and single-chain antibodies against TNFA can potently inhibit inflammation and prevent cartilage damage.[203] In contrast to full antibodies, these smaller antibodies also penetrate into cartilage and might reverse the TNFA-induced catabolic state of articular cartilage in addition to targeting synovial TNFA-driven inflammation.

Anti-IL1 to control synovitis

In mouse models, the effects of ILRA have demonstrated promising results.[149,204,205] Also with use of gene therapy, where the IL1RA gene has been successfully transferred into synovial cells, the consequent increase in IL1RA in the joint protects the joint from IL1B-induced joint damage. This was proven to be protective in rabbit, dog, and horse models of osteoarthritis.[206] Overexpression of the decoy IL1 receptor prevents production of multiple proinflammatory tissue destructive signals.[207] However, an important issue for human gene approaches is safety, especially when applied in a non-fatal disease like osteoarthritis.
Also for anti-IL treatment, clinical studies were not conclusively positive. Treatment of knee osteoarthritis with the IL1RA anakinra was not associated with improvements in osteoarthritic symptoms compared with placebo.[208] A case series of three patients with erosive hand osteoarthritis treated subcutaneously with anakinra showed relief of pain.[209] Systemic administration of AMG108, a monoclonal antibody against IL1R in patients with knee osteoarthritis, showed minimal, if any, clinical benefit.[210] Treatment very early in the disease, where temporary high levels of IL1 are found, may be slightly more promising, at least in the short term, as two small studies reported.[211,212]

Pro-anabolic treatment

Instead of reduction of proinflammatory mediators, it is also an option to stimulate the production and activity of anti-inflammatory mediators. Along this line of thought, recombinant human IL4 (rhIL4) has been created and tested on osteoarthritic synovial tissue, showing evident IL1B or TNFA reduction.[213] IL13 has been shown experimentally to be useful by testing on human synovial membranes from osteoarthritis patients. A combination of IL4 and IL10 has been proven to be chondroprotective in mouse models.[214] A combined molecule (IL4– 10 synerkine) has also proven to protect cartilage from blood-induced damage.[215] This synerkine has been developed to overcome the low bioavailability of the separate cytokines,

and shows improved inhibitory activities (as compared to the combination of IL4 and IL10 monotherapy).[215] However of all these anti-inflammatory cytokine approaches, IL10 is the only one currently in clinical trials for treatment of rheumatoid arthritis.[153]

Summary

Synovium is an integrated tissue of the diarthrodial joints which interacts with all the other joint tissues and specifically is important in nourishment and lubrication of the articular cartilage, removal of waste products, and immunological surveillance. Much knowledge about the synovium and its numerous mediators in the healthy condition and during the degenerative process of osteoarthritis has been gained over the past decades. Chronic as well as recurrent low-grade synovial inflammation definitely contributes to progression and symptoms of certain patients with osteoarthritis. Low-grade inflammation may even be causative in the disease. The challenge is that osteoarthritis is a heterogeneous disorder with inflammation not only of the synovial tissue but with its mediators also present in cartilage and bone. Therefore, despite the presence of inflammatory mediators, in some cases synovitis may be seen as a bystander and not as a driving force in pathogenesis.[216] Further studies are needed to obtain a comprehensive understanding of its role in such a way that it is of use for routine diagnosis, prognosis, and specifically treatment of the disease.[40,217]

Anti-inflammatory therapy may have benefits for some phenotypes of the disease. The presence of 'systemic inflammation' in osteoarthritis of some patients may even provide a rationale for more aggressive anti-inflammatory drugs including biological therapy. Future research must be directed towards defining the risk-to-benefit ratio for biological therapy, especially if the purpose of the therapy is to target mediators of low-grade inflammation. This will be extremely challenging, because mediators of low-grade inflammation are likely to have important physiological effects on other organ systems. The representation of several subtypes with potentially certain specific set of cytokines, could allow for personalized anti-inflammatory medicine, thus increasing therapeutic efficiency.[218] Better stratification might also become possible using imaging modalities like MRI and US.[93,219,220] To develop highly efficient therapies we will probably need innovations in delivery systems, locally or such as nanotechnology, to selectively and safely target joints in a durable manner.

The absence of a clear effect of most anti-inflammatory therapies may be caused by treatment of a general osteoarthritis population, not taking into account subtypes of the disease with, for example, specific TNFA or IL1B involvement or involvement of certain inflammatory cell subsets, if existing. It might, on the other hand, suggest that in addition to the inflammatory component perpetuating the disease, a degenerative biomechanically driven component is able to drive the disease independently of inflammation (at least in certain phenotypes).

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CHAPTER 5

Can optical spectral transmission assess ultrasound-detected synovitis in hand osteoarthritis?

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Conditionally accepted for publication in PlosOne

Abstract

Objective

To determine whether optical spectral transmission (OST) can be used to assess synovitis in hand and wrist joints of patients with hand OA.

Design

Hand and wrist joints of 47 primary hand OA patients with at least one clinically inflamed hand or wrist joint were assessed for synovitis by OST and ultrasound (US). Associations between OST and US synovitis were studied in linear mixed effects models, across all joint types together and individually for wrist, PIP, and DIP joints, and adjusted for OA features that were associated with US synovitis. Diagnostic performance was determined using receiver operator characteristic (ROC) curves analysis, with US as reference standard.

Results

As a whole, 6.7% of joints showed US synovitis. Statistically significant associations between OST scores and US synovitis were found for all joints combined (Δ0.37SD, $p<0.001$) and PIP joints (Δ0.81SD,$p<0.001$), but not for DIP (Δ0.14SD, $p=0.484$) or wrist joints (Δ0.37SD, $p=0.178$). All associations were independent of OA features, i.e. osteophytes and dorsal vascularity. Diagnostic performance of OST, revealed an AUC-ROC of 0.74 for all joints together ($p<0.001$), 0.69 for PIP joints ($p<0.001$), 0.54 for DIP joints ($p=0.486$), and 0.61 for wrist joints ($p=0.234$).

Conclusions

There is a statistically significant association between OST scores and US synovitis, independent of osteophytes and dorsal vascularity. At this stage, OST performs fair in the assessment of synovitis in PIP joints of OA patients. These results seem to warrant future studies to determine the added value of OST in evaluating synovitis in case of hand OA clinical practice.

Introduction

Osteoarthritis (OA) is a highly prevalent, multifactorial joint disease that poses a huge and ever growing burden to affected individuals and society. It is increasingly recognised that OA may affect virtually all structures within and around joints. Synovitis can occur at any disease stage and is an acknowledged risk factor for OA progression,[1] probably through the release of pro-inflammatory mediators that affect joint tissues.[2] Also in patients with hand OA, synovitis in hand joints relates to joint swelling, and adds to pain, functional impairment, and progression of joint damage as compared to OA joints without synovitis.[3-5] The clinically recognizable subgroup of hand OA patients with so-called inflammatory or erosive hand OA is characterized by, among others, a rapid disease progression, more pain, functional impairment, inflammatory symptoms and signs, and more negative clinical, laboratory and sonographic outcomes as compared to other hand OA patients.[6] Early assessment and treatment of synovitis in hand and wrist joints might provide a potential opportunity to delay or even prevent joint deterioration.

Various imaging methods are used to assess signs of synovitis, e.g. magnetic resonance imaging (MRI) or ultrasound (US). While the sensitivity of MRI and US in detecting synovitis and tenosynovitis is higher than that of clinical examination, MRI and US are rather time-consuming, observer-dependent, and/or costly. Therefore, alternative methods for objective and fast assessment of synovitis are desired. For example, indocyanine green (ICG)-enhanced fluorescence optical imaging detected high signal intensities are suggestive of synovitis in wrists and PIP joints of primary hand OA patients.[7]

The novel optical spectral transmission (OST) technique might be an attractive alternative to US and MRI. OST objectively measures the reduced transmission of light through joint tissues in presence of inflammation (e.g. synovitis, tenosynovitis). A recent study showed that OST had a sensitivity of 60% and specificity of 89% in assessing US synovitis in hand and wrist joints of rheumatoid arthritis (RA) patients, even in the presence of bone pathology and periarticular tendon inflammation.[8] Also, OST correlated stronger with US signs of synovitis (ρ=0.64, 95%-CI: -0.43 to 0.78, $p<0.01$) than swollen joint count (SJC: ρ=0.30, 95%-CI: 0.11 to 0.46, $p<0.01$) did, with even no correlation between US and tender joint count (TJC: ρ=-0.02, 95%-CI: -0.21 to 0.17, p= 0.84).

The current study aims to assess performance of OST in assessing synovitis in hand and wrist joints of patients with hand OA and clinical signs of synovitis in at least one hand or wrist joint. Hitherto, we first, determine the test-retest reliability of OST measurements. Then, we compare OST levels between joints with and without US synovitis, adjusting for relevant other disease features. And thirdly, we determine diagnostic performance from receiver operator characteristic (ROC) curves, using US synovitis as a reference.

Methods

Subjects

Fifty consecutive outpatients with hand OA, according to the treating physician, were recruited at the outpatient clinic of the Department of Rheumatology & Clinical Immunology at the University Medical Center Utrecht (UMC Utrecht), between October 2016 and March 2017. Patients with primary hand OA could be included when they had at least one swollen finger or wrist joint (by clinical examination), were aged over 18 years, and were able to give informed consent. Exclusion criteria were obvious deformations of the hand, hand or wrist joint prostheses, concomitant diseases that could explain synovitis in hand joints (e.g. RA, psoriatic synovitis, gout), intra-articular injections with a glucocorticoid of hand or wrist joints within the past 3 months, trauma or surgery of the hand and wrist joints within the past 6 months, light hypersensitivity (e.g. due to erythropoietic protoporphyria), and photodynamic therapy in the past or near future. Of three patients, data could eventually not be used because of movement artefacts or incorrect positioning during scanning, leaving data of 47 patients for analysis.

Assessment procedure

Patients underwent OST and US of their hands, all systematically performed by separate experienced examiners, blinded for other study outcomes. The study complied with the Declaration of Helsinki. All patients gave written informed consent. The study protocol was approved by the ethics committee of the UMC Utrecht (*NL50848.041.15*).

Optical spectral transmission

OST measurements were performed with the HandScan (*Hemics BV., Eindhoven, The Netherlands*), operated by one rheumatology research nurse. In this procedure, the hands are inserted through cylindrical openings that contain pressure cuffs. Scanning laser light (wavelengths of 660 and 808 nm) then illuminates the CMC1, DIP, (P)IP, MCP, and wrist joints of both hands as well as reference areas, all from the palmar side. Regions of interest (ROI) are traced automatically for all joints regions (joint ROI), for regions proximal to each MCP, and for regions distal to all other joints (reference ROIs), based on pictures from the Complementary Metal Oxide Semiconductor (CMOS) camera. Reference ROIs allow for correction for systemic effects unrelated to inflammation, such as body temperature and use of vasoactive medication. Light transmitted through joints and reference areas is recorded continuously at the dorsal side by a CMOS camera at a rate of four frames per second, alternating between 660 nm and the 808 nm wavelengths. A complete measurement consists of three phases and takes less than 100 seconds: first, inflation of the cuff to 5 mm Hg (10 s); second, inflation of the cuff to 50 mm Hg (60 s) and finally deflation of the cuff to 5 mm Hg (30 s). For the development of an algorithm (see below) and to determine test-retest reliability, duplicate OST assessments were performed. OST assessment was

performed before and after US, with at least 20 minutes of rest after US examination (to enable normalization of blood flood).

Light transmission data were transformed into synovitis data through an algorithm. Previously created RA algorithms were not applied to the current OA cohort as these diseases differ in commonly affected joints, severity of synovitis, and presence of osteophytes.[9] Therefore, image analysis, algorithm development, and algorithm validation were specifically performed for the current OA cohort (for a more detailed description, see supplementary material – Development and validation of the OST algorithm). This algorithm provided an OST joint index for each joint (CMC1, DIP 2-5, (P)IP1–5, MCP1–5, and the wrists of both hands; range: 0-3), and a total OST index, being the average of all joints, scaled to a total OST index range of 0-66, to make comparison to total OST index for RA possible.[8]

Ultrasonography

US was performed by one experienced examiner (PvdM) using a MyLab 60 system (*Esaote, Genua, Italy*) with an 18-6 MHz linear array transducer, according to EULAR guidelines for patient and probe positioning.[10] Joint ROIs were the wrists, the carpometacarpal (CMC 1), metacarpophalangeal (MCP) 1-5, proximal interphalangeal (PIP) 2-5, interphalangeal 1 (IP1), and distal interphalangeal (DIP) 2-5 joints of the hands. Synovitis was assessed at both the dorsal and volar aspect of finger joints and at the dorsal aspect of radiocarpal (RC; radius-lunate) and intercarpal (IC; lunate-capitate) joints. Tendinitis and/or tenosynovitis of periarticular extensors and flexors were scored longitudinally. Dedicated scoring systems for US synovitis is hand OA were not available. The standardized scoring system in the Outcome Measurements in Rheumatology Clinical Trials (OMERACT) guidelines assess the same joints regions and components, and is therefore used to score greyscale US (GSUS) findings, combining joint effusion and synovial thickening,[11] and power Doppler (PDUS) findings on a semiquantitive scale (0-3).[12,13]

Also factors potentially leading to misclassification by OST were assessed: extensor tendinitis, flexor tenosynovitis, dorsal vascularity, osteophytes, and erosions. Dorsal vascularity at the MCP, PIP, and DIP joint regions was scored as present or absent. The hand's superficial venous system is mainly located at the dorsal side and follows a highly variable pattern. Dorsal vascularity was differentiated from tendinitis/tenosynovitis of extensors by the presence of interposed normal connective tissue.[14] Osteophytes, cortical protrusions, were scored dorsally at all joint regions, as present or absent. Erosions, discontinuities of the joint bone surface visible in two perpendicular planes, were scored at all dorsal and volar sides, as present or absent.[15]

GSUS grade 1 findings were found to be of limited clinical relevance, as they are also frequently found in healthy controls.[16] Therefore, US joint inflammation was defined as GSUS synovitis >1 or PDUS synovitis >0. Synovial inflammation (tenosynovitis) at the joints investigated was defined as GSUS/PDUS tenosynovitis score >0.

Statistical analysis

Associations between US and OST were assessed for all joints combined and for joint groups individually. Individual joint groups were only studied when US synovitis was present in >5% of joints, to increase the likelihood of sufficient relevance and statistical power. As explained before, associations between US and OST were adjusted for potential US confounders. Due to limited numbers of subjects and US findings, a preselection of potentially relevant confounders was performed. Potential confounders were selected when Chi-Square tests for the association between US synovitis and the potential confounder showed p-values <0.1.

The association between OST values and US synovitis in individual joints was studied using multilevel analysis (i.e. a linear mixed effects model) to account for the dependence of measurements within patients and side (left or right). For this analysis normalized OST values (raw OST values transformed into z-scores having a mean of zero and a standard deviation (SD) of one) were used as dependent (outcome) variable. The effects of US, joint type, side (left or right), and potential confounders for US were evaluated as fixed effects in the model. Random intercepts at the level of patient, joint type, side (left, right) were evaluated in all analyses and retained when they improved model fit (i.e. lower Bayesian information criterion).

Diagnostic performance of OST

Test-retest reliability of OST was evaluated by intra-class correlation coefficients (ICC), and Bland-Altman plots, at both joint (individual joint OST) and patient level (total OST).

Diagnostic performance of OST was determined using US as a reference (scoring synovitis as absent or present), by receiver operating characteristic (ROC) curve analyses with 95% confidence interval (95%-CI) estimation. This was also done for individual joint groups when more than 5% of the joints of that group showed US synovitis.

Multiple regression analyses to develop the OST algorithm were performed using Hemics in-house software (*InFlame RA-160205, November 3, 2016*). All other analyses were performed by using SPSS (*IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.*). All tests were two-sided; p-values < 0.05 were considered statistically significant.

Results

Patient demographics are reported in Table 1; 89% was female, the majority white Caucasian, and the average age was 64 years. No adverse events occurred.

US findings

As illustrated in table 2, 6.7% of all joint were inflamed according to US. Joint types of interest for evaluation at joint type level, exceeding the arbitrary cut-off of synovitis in >5%, were

Table 1: Patient demographics and clinical data.

	n=47
Age (years)	64.5 (9.9)
Female (N, %)	42 (89%)
US inflamed joints (0-32)	2 (IQR: 1 – 3, range 0 – 7)
Total OST score (0-66)	9.27 (0.83)

Numbers are presented as mean (SD) or median (IQR, range) unless mentioned otherwise. US arthritis was defined as GSUS synovitis >1 or PDUS synovitis >0. US, ultrasound; OST, optical spectral transmission. Total OST score: the average of all joints times 22, to maintain a similar total OST index as for the previous RA cohort.[8]

Table 2: Descriptives at joint level, for each separate joint type and all joints together.

		All	DIP 2-5	PIP 1-5	CMC 1	MCP 1-5	Wrist
N (US-synovitis)		100/1503 = 6.70%	23/375 = 6.10%	55/470 = 11.7%	2/94 = 2.10%	8/470 = 1.70%	12/94 = 12.80%
US GS	**0**	1403	352	415	92	462	82
	1	31	9	14	0	2	6
	2	64	14	38	2	6	4
	3	5	0	3	0	0	2
US PD	**0**	1435	362	431	92	465	85
	1	50	12	25	1	4	8
	2	16	1	13	1	1	0
	3	2	0	1	0	0	1
Erosions scanned dorsal		0/1314 = 0%	0/375 = 0%	0/469 = 0%	NA	0/470 = 0%	NA
Erosions scanned volar		4/1400 = 0%	2/370 = 0.50%	2/467 = 0.20%	0/93 = 0%	0/470 = 0%	NA
Flexor tendinitis		16/1307 = 1.10%	5/370 = 1.30%	6/467 = 1.30%	NA	5/470 = 1.10%	NA
Extensor tendinitis		3/1312 = 0.20%	0/375 = 0%	2/468 = 0.40%	NA	1/470 = 0.20%	NA
Vascular pattern		266/1313 = 17.70%	142/375 = 37.90%	121/468 = 25.70%	NA	3/470 = 0.60%	NA
Osteophytes		666/1314 = 44.30%	294/375 = 78.40%	317/469 = 67.40%	NA	55/470 = 11.70%	NA

An arbitrary cut-off for analysis of OST performance in individual joint types was set at 5%, to focus on the most relevant joint groups and maintain sufficient power, leaving DIP, PIP and wrist joints. GS: grayscale US scores (0-3), PD: power Doppler US scores (0-3).

the wrist (N=12, 12.8%), PIP (N=55, 11.7%), and DIP (N=23, 6.1%) joints. The prevalence of potential confounders varied, erosions were absent at the dorsal side and very rare at the volar side (4/1400). Tendinitis was also rare, with more flexor (1.1%) than extensor (0.2%) tendinitis. Dorsal vascularity was observed quite often (17.7%) and osteophytes were present in 44.3% of all joints. Only dorsal vascularity and osteophytes were related to presence of US synovitis (i.e. Chi-square test P < 0.1) and, therefore, included as potential confounders in further analyses, see table 3.

Table 3: Cross-tabulation of US arthritis and potentially confounding US variables.

		US arthritis	
		Absent	*Present*
Erosions scanned dorsally	*Absent*	1228	86
	Present	0	0
	Total	1228	86
Erosions scanned volarly	*Absent*	1309	87
	Present	3	1
	Total	1312	88
Flexor tendinitis	*Absent*	1206	85
	Present	15	1
	Total	1221	86
Extensor tendinitis	*Absent*	1223	86
	Present	3	0
	Total	1226	86
Dorsal vascular pattern	*Absent*	985	62
	Present	242	24
	Total	1227	86
Osteophytes	*Absent*	638	10
	Present	590	76
	Total	1228	86

Test-retest reliability of OST

As illustrated in figure 1, repeated OST measurements (with US measurement and 20 min of rest in between) were essentially similar to the initial measurements at both joint level (mean difference: 0.01, 95%-CI: -0.237 to 0.207, n.s.) and patient level (mean difference: -0.38, 95%-CI: -2.30 to 1.50). ICC for two-way mixed single measurement (ICC 3,1) showed excellent reliability at joint level (ICC = 0.82, 95%-CI: 0.80 to 0.83, p<0.001) and fair reliability at patient level (ICC = 0.54, 95%-CI: 0.29 to 0.72, p=0.001).

Associations between OST and US

In the final model (table 4), a statistically significant association of US synovitis with OST scores (an increase with presence of synovitis on US of 0.37 SD, p<0.001) was found, as well as for joint type (p<0.001) and side (right vs. left: Δ0.05 SD p=0.047). As joint type appeared to modify the association between US synovitis and OST values (p<0.001 for interaction), stratification of analyses for joint type (wrist, PIP, and DIP joints) was performed. Table 4 shows a strong association between US synovitis and OST in PIP joints (Δ0.81 SD, 95%-CI:

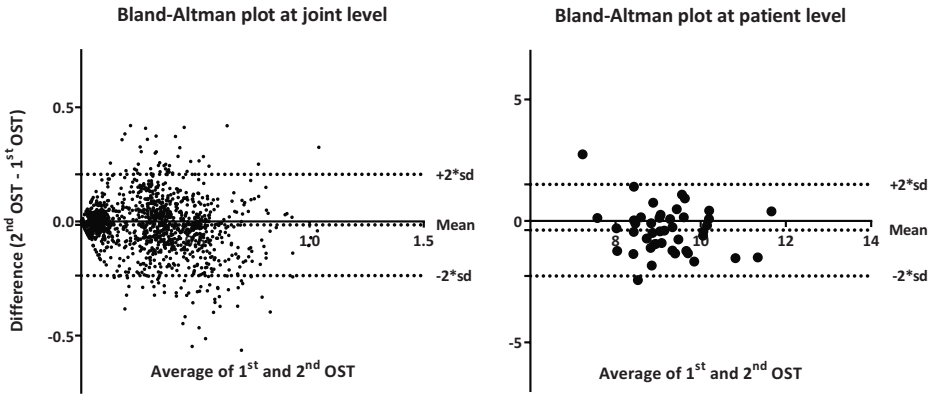


Figure 1: Bland Altman plots to show test-retest agreement of OST, at joint level and patient level.Bland-Altman plots of duplicate OST measurements at patient level (OST index range: 0-66) and at joint level (OST index range: 0-3). 1st OST: first OST measurement, 2nd OST: second OST measurement.

Table 4: Results of multilevel (mixed effects) linear regression model with OST values as outcome: association with US arthritis, for all joints combined and for individual joint types.

Parameter	Estimate[$] (95%-CI: lower, upper)	P-value
All joints combined		
Presence of US arthritis	0.37 (0.28, 0.46)	**P<0.001**
PIP vs. wrist	0.68 (0.55, 0.82)	**P<0.001**
DIP vs. wrist	-0.20 (-0.33, -0.06)	**P=0.005**
MCP vs. wrist	-1.34 (-1.48, -1.21)	**P<0.001**
CMC vs. wrist	-1.44 (1.59, -1.29)	**P<0.001**
Left vs. right side	-0.05 (-0.09, 0.00)	**P=0.047**
Separately for joint types		
Wrist[$]	0.37 (-0.17, 0.91)	P=0.178
PIP[$]	0.81 (0.56, 1.06)	**P<0.001**
DIP[$]	0.14 (-0.26, 0.55)	P=0.484

The association between OST values and US arthritis in individual joints was studied using multilevel analysis (i.e. a linear mixed effects model) to account for the dependence of measurements within patients and side (left or right). The effects of US, joint type, side (left or right), and potential confounders for US were evaluated as fixed effects in the model. Random intercepts at the level of patient and joint type were retained. Joint type appeared to modify the association between US arthritis and OST values (p<0.001 for interaction), therefore associations between OST and US arthritis are shown separately for each joint type as well. P-values <0.05 are written in bold.
[$] Estimates indicate the effect of one unit change in the parameter on SD units change in OST values.

0.56 to 1.06, p<0.001), but no statistically significant association in DIP (Δ0.14 SD, 95%-CI: -0.26 to 0.55, p=0.484) and wrist joints (Δ0.37 SD, 95%-CI: -0.17 to 0.91, p=0.178).
The potential US confounders osteophytes and dorsal vascularity were only available for PIP and DIP joints. In these two joint types, it appeared that osteophytes (p=0.603) and dorsal vascularity (p=0.813) had no statistically significant association with normalized OST scores or joint type (p=0.795). Accordingly, the regression coefficient for US synovitis, in DIP and PIP joints combined, remained similarly and statistically significantly (p<0.001) associated with normalized OST scores when these were removed from the model. No modification of the association between US synovitis and OST by osteophytes (p=0.267 for interaction term) and dorsal vascularity (p=0.409 for interaction term) could be established either.

Diagnostic performance of OST
For all OST assessments combined, data were available for 1503 joints (16 joints per hand in 47 patients, minus one joint that was excluded due to a ring that could not be removed). From these data an algorithm was developed to assess synovitis in this cohort. Details on development and validation of this algorithm are discussed in the *supplementary material – Development and validation of the OST algorithm.* The limited number of inflamed joints and the overall mild synovitis in this cohort made algorithm development considerably difficult, particularly for the DIP joints.
Accordingly, as shown in figure 2, OST performance of all joints together (AUC-ROC: 0.74, 95%-CI: 0.70 to 0.79, p<0.001) was higher than performance of the DIP (AUC-ROC: 0.54, 95%-CI: 0.41 to 0.68, p=0.486) but also of the wrist joints separately (AUC-ROC: 0.61, 95%-CI: 0.44 to 0.77, p=0.234), as a result of the higher performance of OST observed at the level of (P)IP 1-5 (AUC-ROC: 0.69, 95%-CI: 0.62 to 0.77, p<0.001).

Discussion

OST values are statistically significantly higher in the presence of US-defined synovitis, independent of osteophytes and increased vascularity. Associations for all wrist and hand joints combined, is dominated by the strong associations at the level of the PIP joints.

Diagnostic performance for all joints combined was fair. When looking at each of the joint types separately though, performance was only fair for PIP joints, poor for wrists, and less for DIP joints. US synovitis was relatively uncommon in our cohort, even though patients were required to have at least one clinically swollen hand or wrist joint at inclusion. There were even 11 patients without any synovitis in hand or wrist joints according to US (i.e. GS>1 | PD>0). The low number of inflamed joints and low intensity of synovitis, although maybe typical for an OA cohort, posed difficulties for setting up an effective diagnostic algorithm for other joint types than the PIP joints. However, considering early detection to enable early treatment emphasizes the relevance of specifically such a mildly inflamed hand OA cohort.

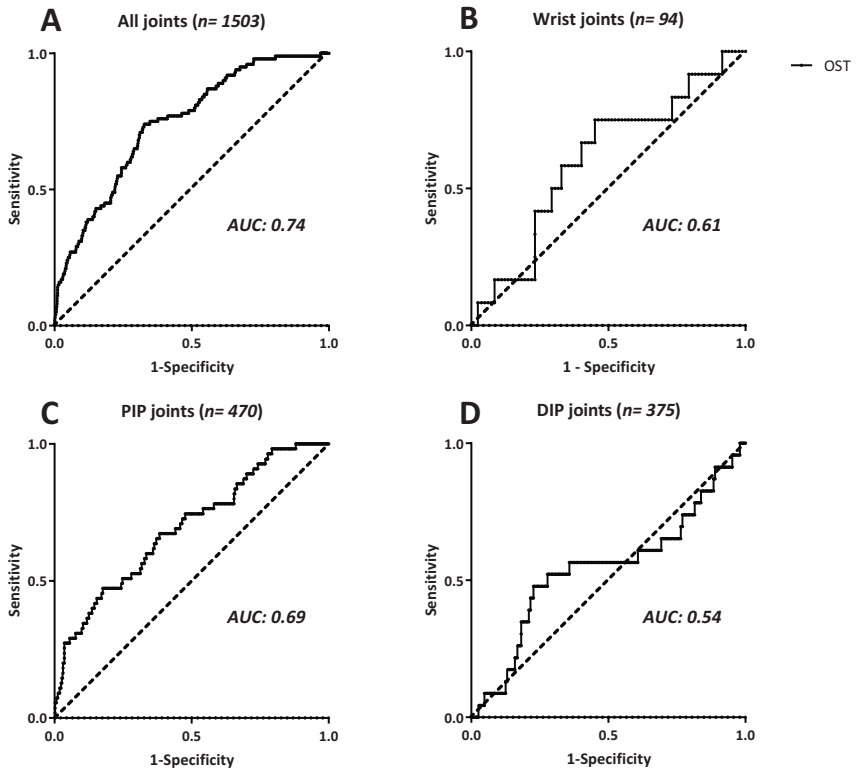


Figure 2: Receiver Operating Characteristic (ROC) curves. Areas under the curves (AUC) for optical spectral transmission (OST) versus ultrasonography (US) in A) all joints (*AUC-ROC: 0.74, 95%-CI: 0.70 to 0.79, p<0.001*), B) wrist joints (*AUC-ROC: 0.61, 95%-CI: 0.44 to 0.77, p=0.234*), C) PIP joints (*AUC-ROC: 0.69, 95%-CI: 0.62 to 0.77, p<0.001*), and D) DIP joints (*AUC-ROC: 0.54, 95%-CI: 0.41 to 0.68, p=0.486*), with US as reference, for all joints and per joint type.

The slightly worse performance of OST in the wrist is probably due to the more complex anatomy of the wrist as compared to the PIP and DIP joints, and is in line with our previous findings in RA patients.[8] While inflammation in DIP joints might be more prevalent in clinical practice, they are clinically less important, as they cause less limitations for the patient than an inflamed PIP joint.

In the current study, the diagnostic performance of OST was determined using GSUS and PDUS defined synovitis as the reference standard. In previous studies in hand OA patients, when defined according to both GSUS and PDUS, there appeared to be a dose-response association between US synovitis and 5-year radiographic OA progression. The odds for 5-year radiographic OA progression were 2,8 higher for joints with grade 1 versus 0 GSUS synovitis.[17] However, US also has its limitations. For example, GSUS≠0 can also be found in healthy individuals and especially GSUS grade=1 is found to relate less to synovitis.[16] Using the alternative definition for synovitis of GSUS grade ≥ 1, irrespective of PDUS grade, would have yielded 263 more arthritic joints in our study. When evaluating this alternative definition

in ROC curve analysis, diagnostic performance of OST appeared to be almost similar (ROC-AUC: 0.737 to 0.722, p=0.654). Moreover, in a study with both US and MRI assessment of synovitis in MCP and PIP joints of RA patients, US was demonstrated to have a sensitivity and specificity between 0.6 and 0.8 as compared to MRI. Importantly, US performed less in joints with subclinical synovitis, as might have been more prevalent in our current OA cohort.[18] OST performance in the current study might be underappreciated because of a higher sensitivity than the US reference standard. The current reference standard could in turn have an underappreciated sensitivity because of the implemented scanning and scoring guidelines limiting assessment of synovitis to predefined areas and planes for time and standardization purposes.

This is the first study evaluating OST in the assessment of synovitis in hand OA, and in which reliability is evaluated, but with some limitations. First, US was performed by a single, though very experienced, examiner, and reliability assessment was therefore not possible. However, the reliability of ultrasonography on hands and wrists joints, assessed using the same semiquantitative scale, was found to be high.[11,19] Second, the number of arthritic joints and the severity of synovitis in this study were low. This caused suboptimal development of the OST algorithm. Future studies in hand OA patients with more and more heavily inflamed joints might be useful to increase the performance of OST measurements in OA, although validation in a cohort with minor inflammation, regarding clinical relevance, is needed subsequently. Third, the development and validation cohort for the algorithm were the same. Yet, several precautions have been taken to prevent overfitting of the OST algorithm, such as increasing the dataset (duplicate measurements), using leave-one-out cross validation, and parameter reduction (see details in the *supplementary material – Development and validation of the OST model*). Fourth, the performance of assessing synovitis by an (improved and validated) OST algorithm should be compared to physical examination, and alternative reference standards like MRI should be evaluated. Fifth, although fast and objective assessment of synovitis with OST is an obvious advantage, using alternative imaging techniques like MRI, have the added advantage of simultaneously being able to assess subchondral bone, cartilage, and fat tissue.

In summary, OST values are statistically significantly higher in the presence of US synovitis, where associations for all wrist and hand joints combined rely on strong associations at the level of the PIP joints. OST performs well in the assessment of synovitis in the PIP joints of OA patients. These results seem promising, and future studies, preferably in cohorts of hand OA patients also including more severe synovitis and also including MRI data on synovitis, are warranted to determine the added value of OST as compared to current clinical practice and trials would be of great interest.

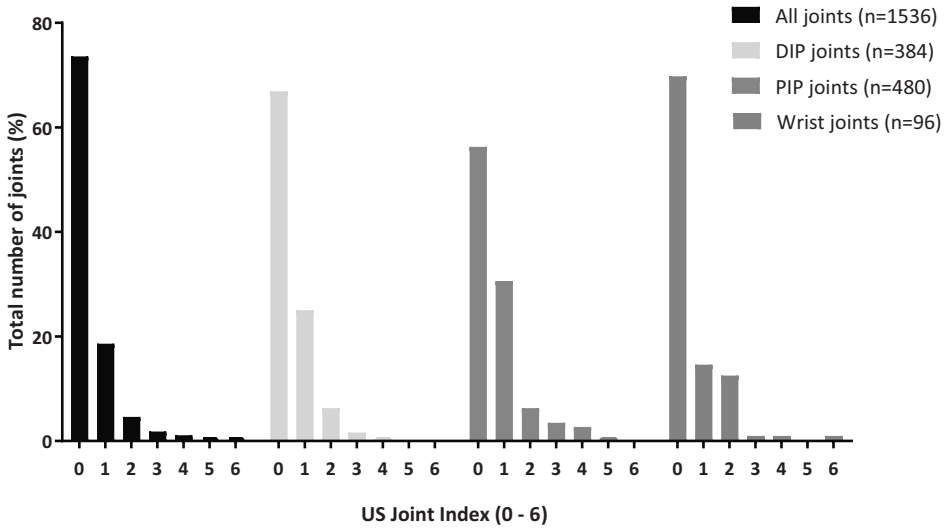
Supplementary material

Development and validation of an OST algorithm

Image analysis was performed by P.B.L Meijer, Hemics programmer, using in-house developed software (*InFlame RA-160205, November 3, 2016*), blinded to other study results. Multiple regression analysis was used to develop and validate an algorithm for detecting arthritis by OST with US as reference. Duplicate OST measurements were used as independent measurements, enlarging the dataset. Dependent variables were the sum of the maximum of GSUS and PDUS grades for synovitis and tenosynovitis (US joint index), and independent variables were normalized joint parameters derived from image analysis (confidential). A stepwise forward selection procedure with adjusted R^2 testing was used to select contributory independent variables. This was repeated until either R^2 no longer increased (cut-off value of 0) or, to prevent overfitting of the model, a maximum of four parameters had been selected. This was done separately for each joint region sharing equal joint characteristics (such as thickness and orientation towards the light source), so for DIP 2-5, IP 1, PIP 2-5, MCP 1, MCP 2-5, CMC1, and wrists separately. We tested whether individual observations exerting undue influence on the coefficients in the regression analysis (i.e. outliers) were present, and if so, these were removed from the development phase. Due to a limited sample size, cross-validation was chosen as a model validation technique to assess how results of the multiple regression would generalize to an independent dataset. The regression analysis with up to four parameters as independent variables per joint region was then performed using leave-one-out cross validation to detect and prevent overfitting. In this method, the model is repeatedly refit, each time omitting both measurements of a different, single patient. The regression coefficients thus obtained are used to calculate OST values for the left-out observation.

As the last OST study was performed in RA patients and the current study intended to develop an OST algorithm for OA patients, the previously developed algorithm could not be validated in our cohort. In the current study, as stated above, the development and validation cohort were the same and several precautions were taken to prevent overfitting. Firstly, by performing duplicate measurements the dataset was increased in size and the risk of overfitting reduced. The validity of the then used leave-one-out cross validation has been shown before.[20] Secondly, the maximum of four independent variables was implemented to reduce the complexity of our model, increasing generalizability to an independent dataset. Lastly, variability of the severity of inflammation would help avoid overfitting. Ideally, there would be an equal distribution of inflammation severity among the various joint types. In our hand OA cohort, out of the 1536 joints successfully assessed by US, there was no equal distribution of inflammation grades among all joints, as can be observed from supplementary figure 1. Moreover, apart from the higher number of PIP joints that were assessed than DIP or wrist joints, PIP joints showed a higher percentage of the more severe US joint indices

(US joint index >2) in our cohort. Although explicit research on the distribution of different grades of inflammation per joint group in hand and wrist joints is limited, similar distributions are found, indicating that inflammatory distribution amongst joint in this study population is representative for other OA populations without severe deformations, increasing the chance of successful applicability of our model in a validation cohort.



Supplementary figure S1: Percentage of joints per US Joint Index per joint type. The sum of the maximum of GSUS and PDUS grades for synovitis. Distribution of joints per US joint index, for all joints pooled together, and separately for the DIP, PIP, and wrist joints. US joint index range: 0 – 6.

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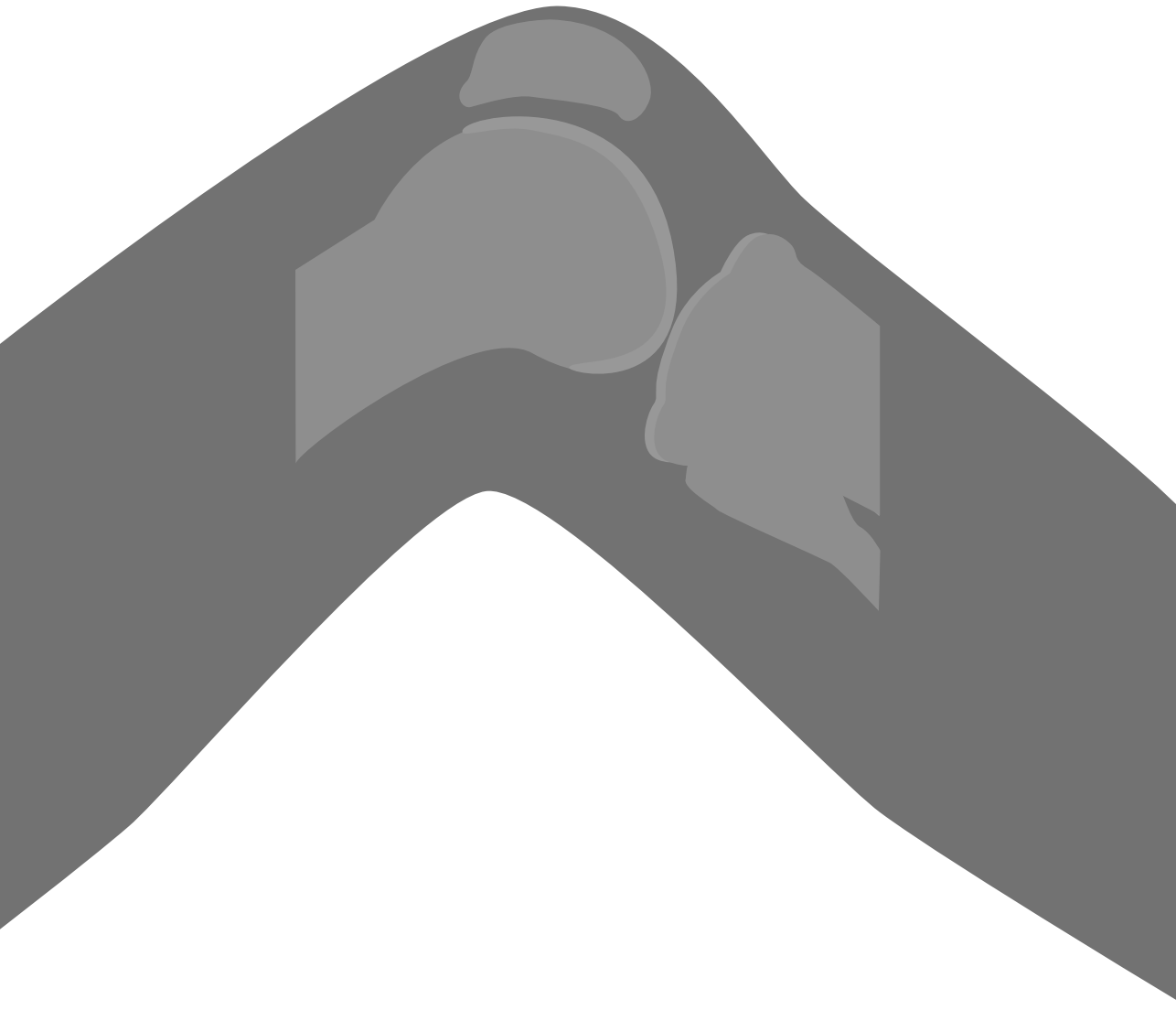
PART II

Monitoring tissue damage and repair in knee osteoarthritis



CHAPTER 6

Knee joint distraction compared with high tibial osteotomy and total knee arthroplasty

Two-year clinical, structural, and biomarker outcomes

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Submitted

Abstract

Background

Knee joint distraction (KJD) is joint-preserving surgery, that, like high tibial osteotomy (HTO), postpones total knee arthroplasty (TKA) in younger knee osteoarthritis (OA) patients, thereby decreasing the chance for revision surgery later in life. The present study evaluates the two-year clinical and structural follow-up for KJD vs. TKA and KJD vs. HTO.

Methods

Knee OA patients indicated for TKA were randomized to KJD (n=20; KJD_{TKA}) or TKA (n=40). Medial compartmental knee OA patients considered for HTO were randomized to KJD (n=23; KJD_{HTO}) or HTO (n=46). Patient reported outcome measures were assessed over two years of follow-up. The radiographic minimum and mean joint space width were measured yearly. In the KJD groups, serum-PIIANP and urinary-CTXII levels, were measured as markers for collagen type-II synthesis and breakdown. Normalized Z-indexes were used to express net collagen type-II synthesis.

Results

Significantly improved clinical (for all groups) and structural (for KJD_{TKA}, KJD_{HTO}, and HTO) outcomes sustained for at least two years post-treatment ($p<0.05$). At 2 years, outcomes of the KJD_{HTO} and HTO groups were similar, while the TKA group showed slightly better clinical results than KJDTKA. The net collagen type-II synthesis decreased initially (3 months) and subsequently increased over time (2 years: both $p<0.05$).

Conclusions

Sustained improvement of clinical benefit and cartilage thickness increase after KJD is demonstrated for patients with medial compartmental knee OA indicated for HTO and patients with severe knee OA indicated for TKA. The cartilage repair observed on radiographs is supported by net collagen type-II synthesis. For the HTO-indicated population, results of KJD and HTO patients were comparable. For the TKA-indicated patients, TKA appeared to result in a slightly better clinical outcome, but at the expense of the native knee joint. Level of evidence: Randomized controlled trial, level I.

Introduction

In patients with severe knee osteoarthritis (OA), total knee arthroplasty (TKA) is generally performed effectively to reduce pain and function impairment. However, younger patients have a higher risk of failure and future revision surgery later in life.[1] With up to 40% of TKAs performed under the age of 65, joint-preserving surgery is of major importance to postpone a first prosthesis, decreasing the risk for revision surgery.[1,2]

High tibial osteotomy (HTO) is a well-established surgical treatment for patients with medial uni-compartmental OA in varus malalignment and shows good long-term survival with significant improvement of patient-reported outcome measures.[3-7] Also, cartilage tissue repair activity has been reported following HTO.[8-11]

Knee joint distraction (KJD) is a more recently introduced joint-preserving surgery used for bi-compartmental tibiofemoral knee osteoarthritis or unilateral OA with limited malalignment. Long-term significant clinical benefit as well as cartilage tissue repair has been reported in an open prospective long-term follow-up study.[12-14]

In two independent randomized controlled trials (RCTs), KJD has been compared with TKA and KJD has been compared with HTO.[15] At one-year follow-up KJD was non-inferior to both other treatments with respect to patient reported outcome measures.[16,17] Cartilage repair activity appeared more pronounced in case of KJD as compared to HTO and was present in case of KJD when compared to TKA, being obviously absent in case of TKA.[16,17] The present study presents the two-year follow-up results of these two independent trials at the level of patient reported outcomes, radiographic (joint space width), and systemic biochemical (collagen type-II) marker changes.

Methods

Patients

Knee OA patients were included in a randomized controlled trial comparing TKA with KJD. Patients considered for TKA were randomized (2:1) to either TKA (n=40) or to KJD (n=20; KJD_{TKA}) treatment. The trial was granted ethical approval (No 10/359/E) and was registered in the Netherlands National Trial Register (NTR2809). In a separate RCT, patients with medial compartmental knee OA considered for HTO and less than 10° varus were randomized 2:1 to either HTO (n=46) or to KJD (n=23; KJD_{HTO}) treatment. The trial was granted ethical approval (No 11/072) and was registered in the Netherlands National Trial Register (NTR2900).
The similarities and differences in selection criteria of both trials are listed in table 1.
Both trials were performed in accordance with the ethical principles from the Declaration of Helsinki and all patients gave written informed consent.[15]

Table 1: In- and exclusion criteria of the two randomized controlled trials (KJD vs TKA and KJD vs HTO).

	Both KJD vs TKA and KJD vs HTO	KJD vs TKA only	KJD vs HTO only
Inclusion criteria	Age < 65 years	Patients considered for TKA according to regular clinical practice	Patients with medial or lateral tibio-femoral compartmental OA considered for HTO according to regular clinical practice
	Radiological joint damage: Kellgren & Lawrence score above 2		
	Intact knee ligaments		
	Normal range-of-motion (min. of 120° flexion)		
	Normal stability		
	Body Mass Index < 35.		
Exclusion criteria	Psychological inabilities or difficult to instruct	An infectious susceptible prosthesis (joint replacement) in situ	Mechanic axis-deviation (varus-valgus) of less than 10 degrees
	Not able to undergo MRI examination (standard protocol)		
	Inflammatory or rheumatoid arthritis present or in history		Contralateral knee OA that needs treatment
	Post-traumatic fibrosis due to fracture of the tibial plateau		
	Bone-to-bone contact in the joint (absence of any joint space on X-ray)		
	Surgical treatment of the involved knee < 6 months ago		
	Primary patello-femoral OA		

KJD = knee joint distraction, TKA = total knee arthroplasty, HTO = high tibial osteotomy.

Treatments

Both TKA and opening-wedge HTO were performed according to standard care with routine rehabilitation after surgery.[15] Distraction surgery was performed with a proof-of-concept device consisting of two dynamic monotubes (medial and lateral) bridging the knee joint. Each monotube was fixed to two bone-pins on each end (tibia and femur). The tubes were distracted by 2 mm during surgery and by 1 mm every day post-surgery, until a total distraction of 5 mm was reached, confirmed on radiographs. Afterwards patients were discharged, with heparin prescribed for nine weeks, and allowed full weight bearing of the distracted knee, supported by crutches if needed. Three to four weeks after surgery, radiographic evaluation of distraction and clinical evaluation of pin tracts was performed in the outpatient clinic. After six to seven weeks the frame and pins were surgically removed.

Patient reported outcome measures (PROMS)

Primary outcomes were the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC, version 3.1) and the validated Dutch Knee injury and Osteoarthritis Outcome Score (KOOS) to score clinical improvement (normalized to a 100-point scale; 100 being the best condition). As secondary measure, the Intermittent and Constant Osteoarthritis Pain

score (ICOAP) for the knee was assessed (0–100, 0 reflecting no pain). As tertiary measures, a visual analogue scale for pain (VAS pain; 0–100 mm, 0 reflecting no pain), the EuroQol (EQ)-5D-3L for quality of life (transformed to an EQ-5D index score; 0–1, 1 being the best), and the Short Form 36 (SF-36) for general health (transformed to the physical (PCS) and mental (MCS) component summary score; 0-100, 100 being the best) were assessed. All clinical outcome parameters were assessed at baseline (0), and after 3, 6, 12, 18, and 24 months except for the SF-36, which was not assessed at 3 months.

Radiographic evaluation

Standardized weight-bearing, semi-flexed posterior-anterior radiographs were obtained at baseline (0), 12, and 24 months post-treatment to assess structural outcome for the KJD_{TKA}, KJD_{HTO}, and HTO groups. An aluminum step wedge was used as a reference standard for linear measures and density. The images were evaluated using knee images digital analysis (KIDA) software[18] to analyze the minimum and mean joint space width (JSW) of the most affected compartment (MAC) of the knee. All image analysis was performed by a single, experienced observer, blinded to patient characteristics, and the intra-observer variation of this measurement method was shown to be good (ICC = 0.73-0.99).[18]

Systemic biochemical marker analyses

Serum and urine samples were collected from all but solely KJD patients at baseline (0), 3, 6, 12, 18, and 24 months and stored at −80°C. Cartilage collagen type-II synthesis and breakdown were determined by serum N-propeptide of type IIA procollagen (PIIANP; Linco, EZPIIANP-53K) and urinary C-telopeptide of type-II collagen (CTXII), Cartilaps; corrected for urine creatinine), respectively. Longitudinal samples of each patient were analyzed in a single plate to prevent influence of variability between kits.

Statistical analyses

Two-sided paired t-tests were used to evaluate changes between follow-up and baseline scores, for each group separately. Differences in changes between groups were evaluated using linear regression, corrected for baseline. For all graphs and changes over time, the mean and standard error of the mean (SEM) are given.

Biochemical marker measurements outside the 95% confidence interval (95%CI) of each group (KJD_{TKA} or KJD_{HTO}) were defined as outliers and removed. Outlier exclusion was validated by a sensitivity analysis. Since there were no differences in biochemical marker reponse between the two KJD groups anticipated, the groups were combined to increase statistical power. For both biomarkers, combined normalized Z-scores were calculated, and the net collagen type-II synthesis was expressed as a Z-index ($Z_{index} = Z_{PIIANP} – Z_{CTXII}$).
A p-value < 0.05 was considered statistically significant. SPSS v.22 software (IBM, Armonk, New York) was used to perform statistical analyses.

Results

Over the two years of follow-up, in the KJD_{TKA} group, one patient was lost to follow-up after undergoing TKA surgery because of unsatisfactory clinical benefit (after nine months). In the TKA group, four patients withdrew consent before surgery and two patients were lost to follow-up due to comorbidities discovered after treatment.

In the KJD_{HTO} group, one patient was excluded before surgery due to inoperability and two patients were lost to follow-up after undergoing a TKA and HTO because of unsatisfactory treatment benefit (both after twelve months). In the HTO group, one patient was excluded before treatment due to anxiety and four patients were lost to follow-up because of comorbidities interfering with follow-up but unrelated to the procedure. Of the remaining 114 patients (out of the original 129), the baseline characteristics are presented in table 2.

Table 2: Baseline characteristics of patients from the two RCTs.

	KJD vs TKA		**KJD vs HTO**	
	KJD_{TKA} (n = 19)	***TKA (n = 34)***	***KJD_{HTO} (n = 20)***	***HTO (n = 41)***
Male gender *(n,%)*	8 (42)	12 (35)	15 (75)	24 (58)
BMI *(kg/m2)*	27.1 (3.8)	28.4 (6.0)	27.4 (3.3)	27.1 (3.3)
Age *(years)*	55.7 (7.4)	55.4 (6.0)	51.2 (5.8)	49.3 (6.3)
Axis *(degrees)*	2.1 (7.0)	2.8 (6.2)	5.9 (2.7)	6.1 (2.2)
Kellgren-Lawrence grade	4 (1.0)	3 (0.0)	3 (1.8)	3 (1.0)
Grade 0*, n (%)*	0 (0)	0 (0)	0 (0)	1 (2)
Grade 1*, n (%)*	0 (0)	0 (0)	5 (25)	4 (10)
Grade 2*, n (%)*	1 (5)	7 (21)	4 (20)	11 (27)
Grade 3*, n (%)*	8 (42)	21 (62)	10 (50)	21 (51)
Grade 4*, n (%)*	10 (53)	6 (18)	1 (5)	4 (10)
Flexion *(degrees)*	121 (10.5)	123 (7.7)	130 (7.2)	132 (8.5)
Total WOMAC *(0-100)*	39.2 (15.6)	44.7 (20.6)	52.5 (20.5)	46.5 (19.6)
Total KOOS *(0-100)*	38.4 (9.2)	35.8 (11.6)	45.7 (14.4)	40.6 (12.8)
VAS pain *(100-0)*	63.8 (19.0)	71.9 (15.7)	52.3 (22.1)	64.7 (17.9)
EQ-5D *(0-1)*	0.66 (0.25)	0.61 (0.24)	0.70 (0.20)	0.72 (0.18)
ICOAP Combined *(100-0)*	57.7 (12.0)	64.9 (17.2)	54.2 (16.3)	58.5 (15.1)
SF-36 PCS *(0-100)*	33.6 (9.0)	31.3 (7.2)	37.7 (6.7)	35.8 (8.1)
SF-36 MCS *(0-100)*	54.5 (8.4)	54.0 (9.8)	55.0 (8.2)	55.1 (8.5)
Minimum JSW *(mm)*	0.65 (1.3)	-	0.49 (0.7)	0.54 (1.0)
Mean JSW *(mm)*	1.93 (2.0)	-	1.99 (1.5)	1.89 (1.2)

TKA = total knee arthroplasty, HTO = high tibial osteotomy, KJD_{TKA} = knee joint distraction patients from the clinical trial comparing KJD with TKA, and KJD_{HTO} = knee joint distraction patients from the clinical trial comparing KJD with HTO. Mean values and standard deviation are given for all continuous parameters. For the categorical Kellgren-Lawrence grade the median and interquartile range are given. Separate Kellgren-Lawrence grades and gender are given in numbers and percentages. Ranges from worst to best are indicated for the clinical parameters.

Patient reported outcome measures

As primary outcome, a clear improvement in total WOMAC score (figure 1) was still present two years after treatment for all four groups (KJD_{TKA} Δ38.9 (±4.7); TKA Δ42.1 (±3.7); KJD_{HTO} Δ26.8 (±4.7); HTO Δ34.4 (±3.1); all p<0.001). The total KOOS (figure 2) remained significantly improved for all four groups as well (KJD_{TKA} Δ28.7 (±3.9); TKA Δ43.3 (±2.2); KJD_{HTO} Δ21.6 (±3.4); HTO Δ30.0 (±2.5); all p<0.001). All three subscales of the WOMAC and five subscales of the KOOS as well as the VAS pain score, the EQ-5D, the SF-36 PCS, and the ICOAP showed similar positive trends, while only the SF-36 MCS showed almost no change compared to baseline (table 3; all secondairy and tertiairy outcomes).

Table 3: Two-year changes in clinical and structural parameters.

		KJD vs TKA			KJD vs HTO		
		KJD_{TKA} (n = 19)	***TKA (n = 34)***	***p-value***	***KJD_{HTO} (n = 20)***	***HTO (n = 41)***	***p-value***
WOMAC *(0-100)*	*Total*	38.9 (4.7)*	42.1 (3.7)*	0.066	26.8 (4.7)*	34.4 (3.1)*	0.413
	Stiffness	25.8 (5.5)*	32.7 (3.8)*	0.098	16.2 (5.3)*	24.5 (3.2)*	0.337
	Pain	28.4 (4.7)*	43.6 (3.2)*	**0.008**	23.6 (3.9)*	31.8 (3.2)*	0.408
	Function	26.3 (4.4)*	40.9 (2.6)*	**0.016**	21.5 (3.8)*	28.9 (2.9)*	0.318
KOOS *(0-100)*	*Total*	28.7 (3.9)*	43.3 (2.2)*	**0.002**	21.6 (3.4)*	30.0 (2.5)*	0.109
	Stiffness	28.3 (3.7)*	33.6 (3.0)*	0.212	16.7 (3.1)*	22.6 (2.4)*	0.276
	Pain	29.8 (4.5)*	47.9 (2.7)*	**0.001**	25.7 (3.9)*	32.5 (2.7)*	0.347
	Function	31.0 (3.8)*	42.5 (2.1)*	**0.034**	21.6 (3.8)*	28.9 (2.9)*	0.317
	Sport	28.3 (6.4)*	49.2 (4.0)*	**0.007**	25.7 (5.0)*	33.8 (4.2)*	0.314
	QOL	26.3 (5.9)*	44.5 (4.0)*	**0.015**	17.7 (3.6)*	32.2 (3.4)*	**0.013**
VAS *(100-0)*	*Pain*	-31.9 (7.8)*	-55.9 (4.1)*	**0.016**	-21.4 (5.5)*	-38.5 (3.8)*	0.120
EQ-5D *(0-1)*	*Index*	0.10 (0.06)	0.27 (0.05)*	**0.023**	0.16 (0.05)*	0.11 (0.04)*	0.564
ICOAP *(100-0)*	*Constant*	-28.0 (3.6)*	-39.2 (3.9)*	0.089	-19.8 (4.2)*	-22.9 (3.8)*	0.770
	Inter	-26.0 (3.6)*	-35.5 (3.3)*	0.284	-17.1 (4.5)*	-22.3 (3.2)*	0.669
	Comb	-26.9 (3.5)*	-37.2 (3.4)*	0.168	-18.3 (4.2)*	-22.6 (3.1)*	0.673
SF-36 *(0-100)*	*PCS*	5.3 (2.8)	17.9 (1.6)*	**<0.001**	6.5 (1.8)*	11.9 (1.5)*	0.051
	MCS	0.4 (3.0)	-0.6 (2.9)	0.728	1.0 (1.8)	-1.1 (1.7)	0.468
Flexion *(°)*	*Knee*	-	-	-	1.4 (1.7)	-2.0 (1.5)	0.254
JSW *(mm)*	*Min*	0.90 (0.32)*	-	-	0.93 (0.21)*	0.62 (0.15)*	0.233
	Mean	0.99 (0.31)*	-	-	0.83 (0.24)*	0.88 (0.15)*	0.884

Western Ontario and McMaster Universities Osteoarthritis index (WOMAC), knee injury and osteoarthritis outcome score (KOOS), visual analogue score (VAS), EuroQol (EQ)-5D, intermittent (inter), combined (comb) and constant osteoarthritis pain score (ICOAP), and Short Form (SF)-36 clinical scores and subscores (PCS: Physical Component Score and MCS: Mental Component Score), maximum knee flexion and mean and minimum joint space width, for each of the four patient groups (total knee arthroplasty (TKA), knee joint distraction (KJD) patients indicated for TKA (KJDTKA), high tibial osteotomy (HTO) and KJD patients indicated for HTO (KJDHTO). Mean and standard error of the mean are given and ranges from worst to best are indicated for the clinical parameters. Statistically significant change (p<0.05) compared to baseline is indicated with *. Changes between patient groups from each separate trial (KJD/TKA and KJD/HTO) are compared and corrected for baseline values using linear regression. Flexion parameters were not measured at two years in the KJDTKA and TKA groups.

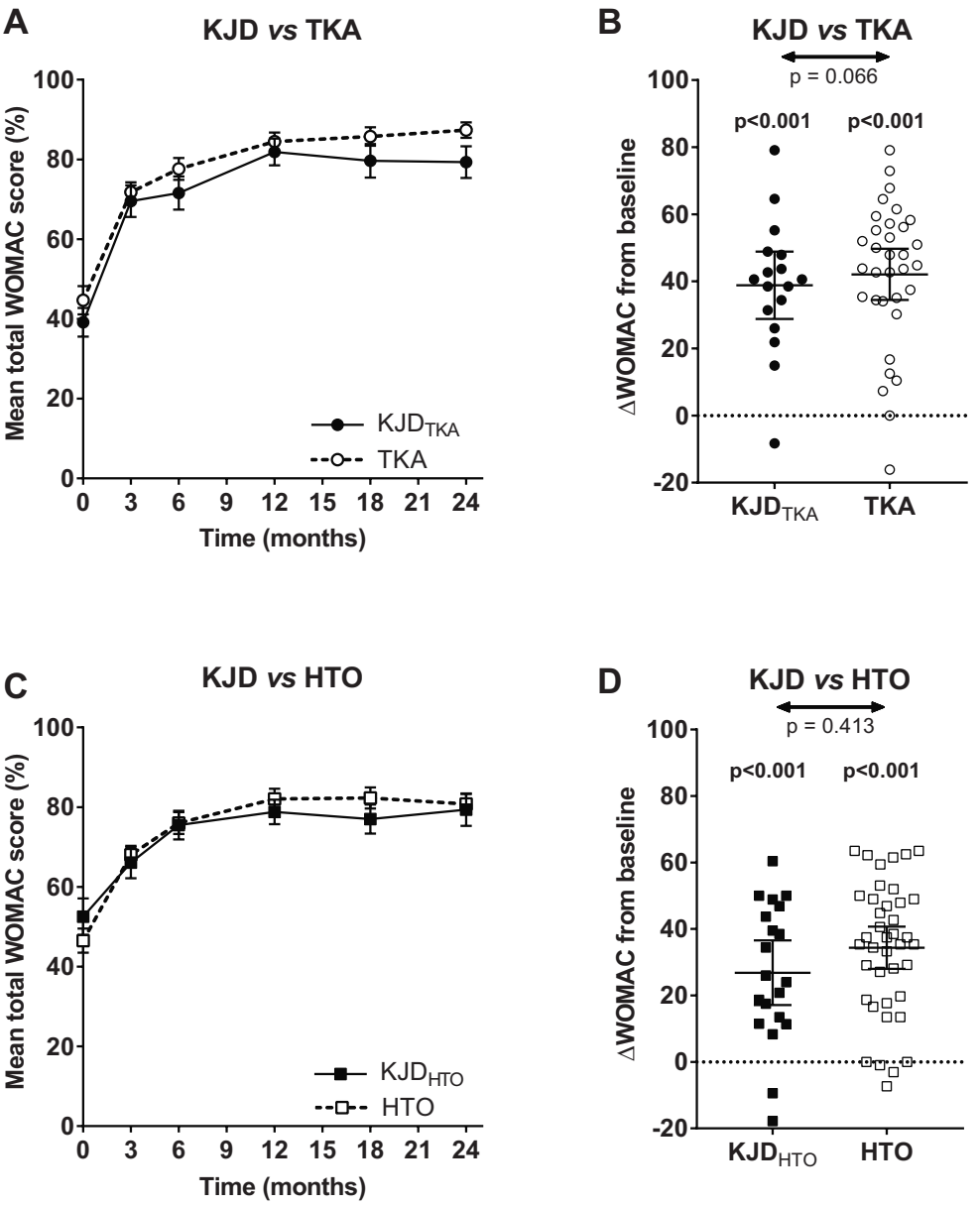

Figure 1: Total WOMAC.

(A) Total Western Ontario and McMaster Universities Osteoarthritis index (WOMAC) score over two years, for the TKA-indicated subgroups (KJDTKA and TKA), represented as mean ± standard error of the mean (SEM). (B) Two-year change in WOMAC score for each individual TKA-indicated patient (markers) and for the KJDTKA and TKA subgroups (average ± SEM, dashes). (C) Total WOMAC score over two years for the HTO-indicated subgroups (KJDHTO and HTO), represented as mean ± SEM. (D) Two-year change in WOMAC score for each individual HTO-indicated patient (markers) and for the KJDHTO and HTO subgroups (average ± SEM, dashes). The p-values above subgroups indicate significant two-year changes while the p-values between subgroups indicate the differences between each two groups.

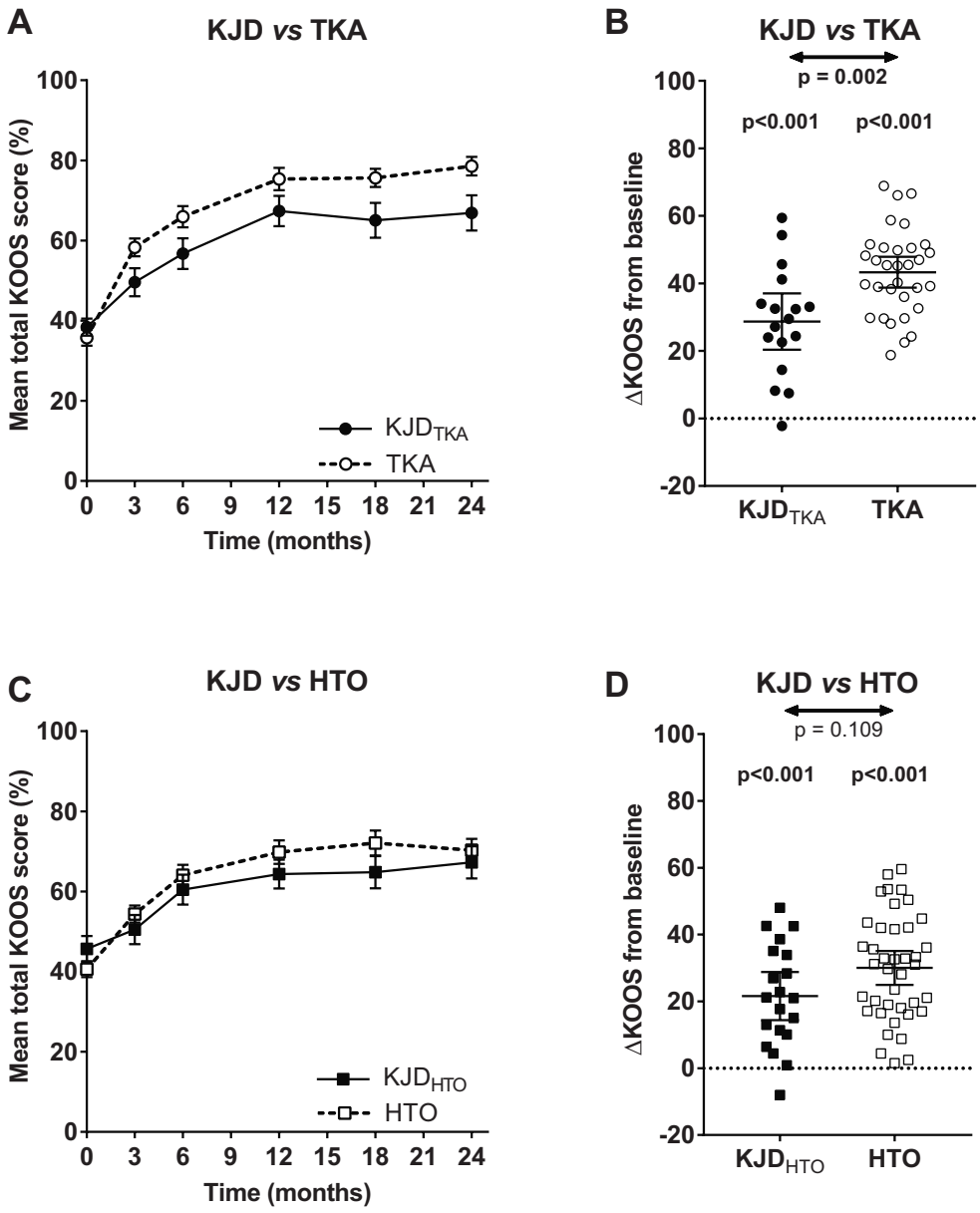

Figure 2: Total KOOS.

(A) Total knee injury and osteoarthritis outcome score (KOOS) score over two years, for the TKA-indicated subgroups (KJDTKA and TKA), represented as mean ± standard error of the mean (SEM). (B) Two-year change in KOOS score for each individual TKA-indicated patient (markers) and for the KJDTKA and TKA subgroups (average ± SEM, dashes). (C) Total KOOS score over two years for the HTO-indicated subgroups (KJDHTO and HTO), represented as mean ± SEM. (D) Two-year change in KOOS score for each individual HTO-indicated patient (markers) and for the KJDHTO and HTO subgroups (average ± SEM, dashes). The p-values above subgroups indicate significant two-year changes while the p-values between subgroups indicate the differences between each two groups.

KJD vs TKA

The TKA group showed statistically significantly greater improvements than the KJD_{TKA} group for most of the clinical parameters (table 3), including the total KOOS and most of its subscales (all p<0.035), the VAS pain (p=0.016), the EQ-5D (p=0.023), and the SF-36 PCS (p<0.001). There was no significant difference for the total WOMAC (p=0.066), WOMAC stiffness (p=0.098), KOOS stiffness (p=0.212), the ICOAP (p=0.089), and ICOAP subscales (both p>0.167).

KJD vs HTO

The HTO and KJD_{HTO} groups showed no statistically significant differences in change from baseline (table 3), except for the KOOS quality of life subscale, where HTO showed a greater improvement (p=0.013).

Radiographic evaluation

KJD vs TKA

In the KJD_{TKA} group, the minimum JSW increased significantly from 0.49 (±0.27) mm at baseline to 1.55 (±0.30) mm at two years (p=0.002) while the mean JSW of the MAC increased from 1.69 (±0.50) mm to 2.70 (±0.42) mm (p=0.009), as shown in figure 3. In the TKA group the JSW was not measured, since patients no longer had their native knee.

KJD vs HTO

In the KJD_{HTO} group the minimum JSW increased from 0.49 (±0.15) mm to 1.43 (±0.23) mm (p<0.001) and the mean JSW increased from 1.99 (±0.33) mm to 2.82 (±0.32) mm (p=0.002). In the HTO group, the minimum and mean JSW increased from 0.57 (±0.16) mm to 1.19 (±0.21) mm (p<0.001) and from 1.91 (±0.20) mm to 2.80 (±0.23) mm (p<0.001), respectively. For the two-year increase in both mean and minimum JSW, there was no statistically significant difference between the KJD_{HTO} and HTO groups (both p>0.232; table 3).

Biochemical marker analyses

In the KJD patients, normalized biochemical marker Z-scores showed a significant initial increase in collagen type-II degradation marker CTX-II, at 3 (p<0.001) and 12 (p=0.020) months, and a longer-term increase in collagen type-II synthesis marker PIIANP at 12 (p=0.008) and 24 (p<0.001) months. The Z-index, indicating normalized net collagen type-II synthesis, was statistically significantly decreased at 3 months (Δ-0.43 ±0.20; p=0.035) and statistically significantly increased at 24 months (Δ0.59 ±0.18; p=0.003) with respect to baseline, as shown in figure 4. In these analyses, 16 of 452 measurements were excluded as outliers (15 points above 95%CI, 1 point below 95%CI). The sensitivity analysis including these outliers resulted in a loss of statistical significance only at 3 months (p=0.231), the 24 months normalized increase of synthesis over breakdown remained statistically significant

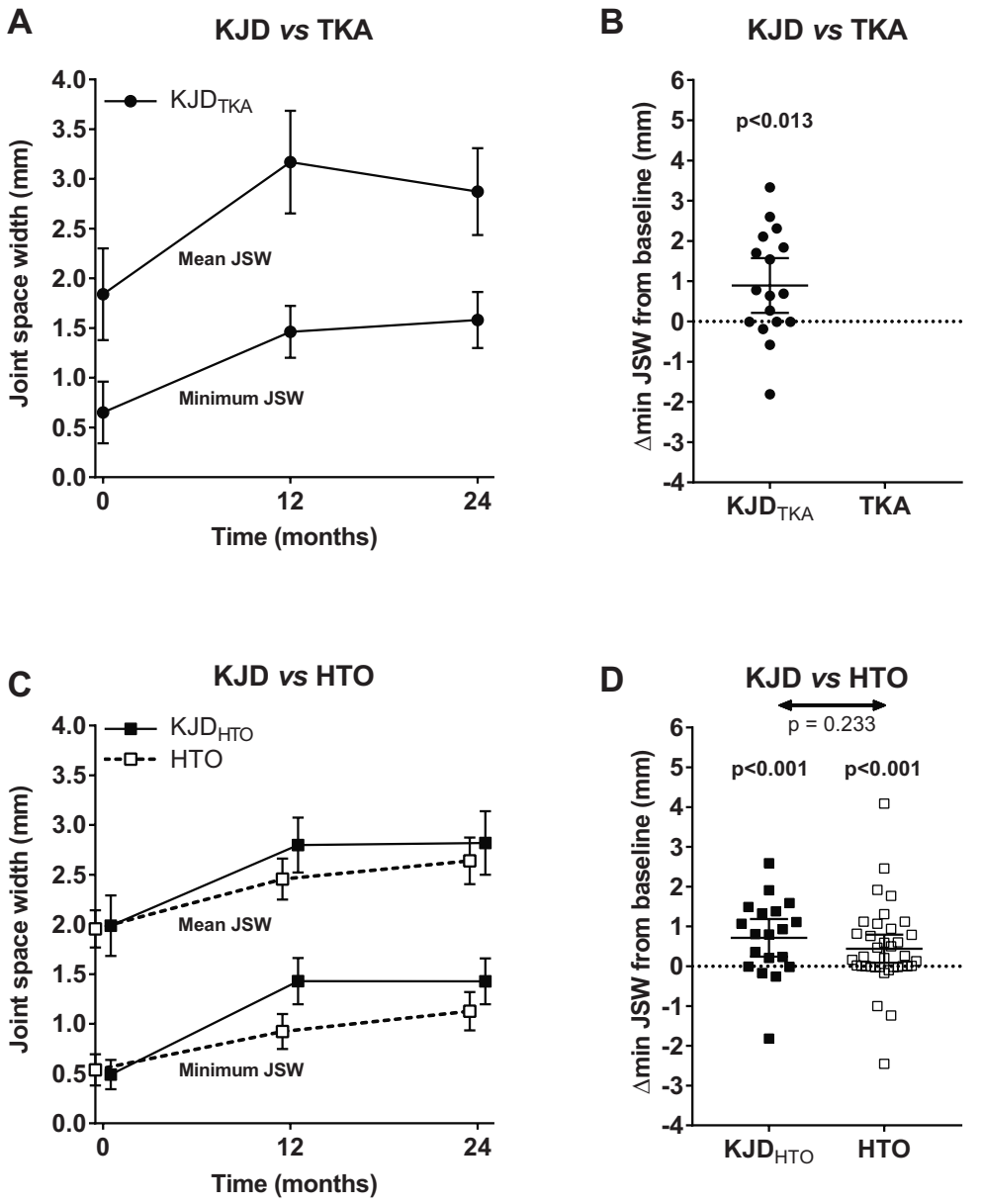

Figure 3: Joint space width.
(A) Mean and minimum joint space width (JSW) over two years, for the TKA-indicated subgroup that still has their native knee (KJDTKA), represented as mean ± standard error of the mean (SEM). (B) Two-year change in minimum JSW for each individual TKA-indicated patient (markers) and for the KJDTKA subgroup (average ± SEM, dashes). (C) Mean and minimum JSW over two years for the HTO-indicated subgroups (KJDHTO and HTO), represented as mean ± SEM. (D) Two-year change in minimum JSW for each individual HTO-indicated patient (markers) and for KJDHTO and HTO subgroups (average ± SEM, dashes).The p-values above subgroups indicate significant two-year changes while the p-values between subgroups indicate the differences between each two groups.

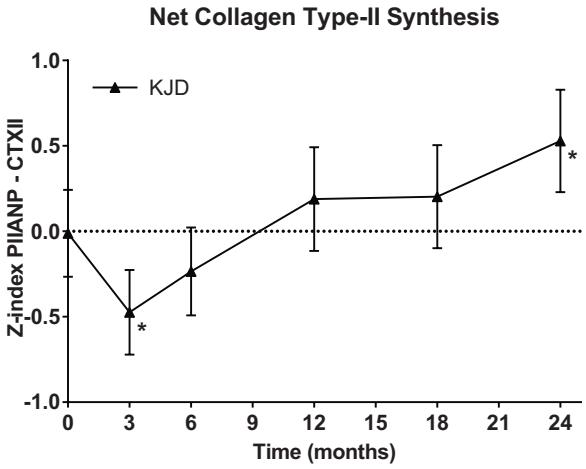

Figure 4: Collagen Type-II
Normalized biomarker Z-index over two years for all knee joint distraction patients combined, expressing net collagen type-II synthesis (Zindex = ZPIIANP – ZCTXII). Mean values ± standard error of the mean are given. Statistically significant changes compared to baseline are indicated with *.

(p=0.002). Performing the same analyses in the two KJD patient groups separately showed a similar pattern for both groups, although the differences from baseline were not statistically significant.

Discussion

Data from both independent randomized controlled trials demonstrated sustained patient-reported clinical benefit up to two years for all KJD, TKA, and HTO subgroups. For both JSW improvement and for clinical symptoms KJD was shown to be non-inferior to HTO. TKA showed better clinical efficacy than KJD for the primary and most additional outcome measures, but at the expense of the native knee joint.

While KJD could be considered an alternative to HTO, KJD is not meant to replace TKA, but to postpone a primary TKA and with that potentially prevent complex and costly revision surgery later in life. In patients where TKA has been performed after KJD, there were no complications, and similar beneficial outcomes were reported as TKA recipients that did not have prior KJD treatment.[19] A health technology assessment has demonstrated that a treatment strategy starting with KJD for severe conservative treatment resistant knee OA has a large potential for being a cost-effective intervention, especially for the relatively young patient.[20]

It should be noted that JSW measurements on radiographs depict the distance between bone ends, not actual cartilage thickness. Although in all cases weightbearing radiographs

were made, in case of HTO, opening of the joint space due to the correction[11,21] might have resulted in an overestimation of the observed JSW at the medial compartment not representing actual cartilage thickness.

Although not originally intended and powered to compare, looking at the two KJD groups KJD_{TKA} and KJD_{HTO} at two-year follow-up in figures 1 and 2, the KJD_{TKA} group seems to show a greater two-year improvement in total WOMAC and KOOS than the KJD_{HTO} group. This difference is statistically significant for WOMAC (p=0.028) but not for KOOS (p=0.070). The KJD_{TKA} patients were indicated for a TKA while the KJD_{HTO} patients were indicated for HTO in separate trials, and this difference in inclusion criteria led to a difference in baseline Kellgren-Lawrence grade that is less severe for the KJD_{HTO} group (p=0.002). However, on patient level, there is no significant influence of baseline Kellgren-Lawrence grade on the two-year improvement in primary outcomes total WOMAC or KOOS corrected for baseline, which was tested for all KJD patients together (both p>0.348). Apparently, patients with further progressed OA at baseline gain more clinical benefit after treatment with KJD on group level, but on patient level there might be multiple subtle differences that determine the level of clinical benefit after treatment.

Looking at the change in clinical outcome for all groups, almost all parameters are significantly increased (clinical, structural, and biochemical benefit) from baseline values. Data imputation of missing clinical data (including of those lost to follow-up) did not change significance of results or conclusions.

While these are data from the first two independent RCTs comparing two-year follow-up of KJD with TKA and with HTO, a prospective uncontrolled study has evaluated outcomes of 20 patients indicated for TKA that were treated with KJD.[12-14] The two-year clinical results were comparable with the two years follow-up data from this study and in particular with the KJD_{TKA} group, which is expected since the 20 patients in the uncontrolled study were indicated for a TKA as well. Given the similar pattern in the first two years of the prospective study, the continued clinical benefit that was found up to five years[14] and even nine years[22] after treatment should become evident in the follow-up of the current RCTs as well.

Despite the fact that TKA shows slightly better clinical benefit, twelve patients (age range 52-86 years) with varied clinical history attended a 'patient partners' meeting and were informed on the difference in clinical outcome between KJD and TKA. They were asked if, with KJD not giving as much pain reduction as TKA, they would still consider KJD over a tried and tested TKA procedure. Patients said that retaining their own knee was of utmost importance and they would choose KJD over TKA (*prof Pandit H, orthopedic surgeon, University of Leeds, personal communication March 2018*).

The clinical and structural benefit at two years corresponds with a significantly increased net collagen type-II synthesis, which suggests formation of (hyaline) cartilage. The increase in collagen type-II synthesis at two years is caused by significantly increased levels of PIIANP, while the synthesis decrease seen at three months is the result of a significant initial increase in CTXII. It is important to keep in mind that while CTXII is a cartilage breakdown marker, it is also a marker for (subchondral) bone turnover. Subchondral bone density decrease and bone normalization have been shown after distraction of the knee and the ankle, and the initial increase in CTXII could be a result of this bone remodeling process as well, alone or in combination with cartilage breakdown.[13,23] The repair of hyaline cartilage upon KJD is supported by canine *in vivo* studies demonstrating beneficial changes in proteoglycan and collagen turnover.[24] Moreover, beneficial changes regarding proteoglycan content in these canine studies is supported by recent dGEMRIC evaluation in clinical KJD studies.[25]

In conclusion, evidence up to two years suggests KJD can be considered a valid alternative to HTO in knee OA patients with (<10°) varus malalignment and a method to postpone primary total knee arthroplasty, potentially preventing revision surgery later in life.
While future follow-up of these patients will provide additional insight into long term follow-up, the results presented in this study indicate KJD is a clinically useful joint-preserving strategy for relatively young patients with knee OA.

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CHAPTER 7

The relevance of axial alignment for treatment efficacy of medial compartment knee osteoarthritis with knee joint distraction compared to high tibial osteotomy

a first exploration

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Submitted

Abstract

Objective

Knee joint distraction (KJD) and high tibial osteotomy (HTO) are treatments to (temporarily/ partially) unload the osteoarthritic (OA) joint in the pursuit of long term clinical benefit. In contrast to KJD, the efficacy of HTO relies on a permanent shift of the mechanical axis. However, previously reported radiographic outcomes of both HTO and KJD, indicate that KJD might also bring about a change in axial alignment. Therefore, in this exploratory study we investigate the potential relevance of axial alignment and a change in alignment for efficacy of KJD.

Design

28 KJD patients and 45 HTO patients, treated for medial tibiofemoral OA, were included. Radiographic and clinical changes were evaluated after one-year follow-up, and changes were compared between treatments. Furthermore, associations of preoperative axial alignment and change in axial alignment with changes in structural and clinical parameters were studied.

Results

Both KJD and HTO led to statistically significant clinical and structural benefit after one year. Axial alignment, defined by the femur-tibia-angle (FTA), increased statistically significantly after either treatment (*KJD:Δ0.90°, HTO:Δ0.73°*), with no statistically significant difference between KJD and HTO. Pain reduction was associated with the increase in FTA (*p=0.027*) and a higher preoperative FTA (*p=0.019)*, irrespective of treatment. Moreover, a higher preoperative FTA (more neutral alignment) was associated with a higher increase in structural improvement, for both KJD and HTO.

Conclusions

KJD, a joint-sparing treatment not necessarily focusing on changing axial alignment, causes a change in axial alignment that is associated with the reduction in pain from medial knee OA.

Introduction

Knee osteoarthritis (OA) poses a major and growing socio-economic burden. Along with current worldwide trends of increasing obesity rates, an ever growing and relatively younger population is at risk for developing knee OA.[1] The typical pain and functional limitations from OA presumably, at least in part, come from joint tissue damage. As such, joint-preserving treatments pursue long term clinical benefit through effectuating tissue repair.

Opening-wedge high tibial osteotomy (HTO) is a joint-preserving treatment that is primarily indicated for medial tibiofemoral (TF) knee OA. HTO probably obtains its efficacy from unloading the medial TF compartment through correcting the angular deformity towards the lateral TF compartment, i.e. shifting the hip-knee-ankle angle (HKA; mechanical axis) relatively from varus towards valgus. Many studies into HTO show high and prolonged joint survival rates[2] and even cartilage repair.[3,4] A systematic review showed that HTO delays total knee arthroplasty (TKA) for a median of seven years.[5] HTO has also been shown to improve success rates of cartilage restoration procedures like autologous chondrocyte implantation and microfracturing.[6,7]

Knee joint distraction (KJD) is an alternative joint-sparing treatment for either unilateral or bilateral TF knee OA. In KJD, an external fixation device distracts the TF joint for 6 weeks. Patients are mobile during the distraction period and springs in the device ascertain sufficient synovial fluid dynamics. KJD provides intra-articular conditions that allow joint tissue repair, as is evidenced by signs of cartilage regeneration on radiographs,[8-11] MRI,[8-12] and arthroscopy.[8,10,13-15] This structural benefit is accompanied by improvements in joint pain and function.[8-11] In a prospective, open, uncontrolled study, KJD proved to be successful in postponing TKA for at least five years in more than 75% of patients[16] and for at least 10 years in two-third of patients.[17]

KJD was compared to HTO in a recent randomized clinical trial (RCT) to report the efficacy of KJD compared to an established joint-sparing treatment.[18] At one-year follow-up, increases in radiographic medial TF JSW were observed upon both KJD (from 2.0±1.4mm to 2.8±1.4mm, p=0.004) and HTO (from 2.0±1.2mm to 2.4±1.3mm, p<0.001). The change in lateral JSW was not statistically significantly after both HTO and KJD (-0.2 ± 1.3 mm, n.s., for HTO and 0.2 ± 1.4 mm, n.s., for KJD).[19]

Theoretically, in contract to KJD, the efficacy of HTO would be expected to rely on successful shift of the mechanical axis. However, the increase in specifically the medial JSW on weight-bearing radiographs observed after both HTO and KJD, indicate that KJD might also bring about a change in axial alignment. Therefore, in this exploratory study we investigate the potential relevance of (changes in) axial alignment for the efficacy of KJD.

Methods

Patients

Data from the aforementioned KJD versus HTO trial were used (*NL35856.041.11*).[18] In this trial, patients with medial TF knee OA considered for HTO according to regular practice, were randomized to either KJD (N=22) or HTO (N=45). The relatively low number of KJD patients from this trial were supplemented with KJD patients from another KJD versus TKA trial (*NL34296.041.10*) as long as they would have complied to the inclusion criteria of the KJD versus HTO trial. These inclusion criteria were: Kellgren & Lawrence grade >2 radiographic knee OA, age younger than 65 years, intact knee ligaments and stability, relatively normal range-of-motion (≥120° knee flexion; flexion limitation ≤15°), and a BMI <35 kg/m^2. Exclusion criteria were any history of inflammatory arthritis, previous tibia plateau fracture of the index knee, complete joint space obliteration (i.e. bone-to-bone contact) on radiographs of the index knee, surgical treatment of the index knee over the last 6 months, predominating PF OA of the index knee, contralateral knee OA that required treatment, and joint prostheses in situ (due to a risk of hematogenous infection of prosthesis material). Of the 20 potentially eligible patients from this second trial, six patients complied to the inclusion criteria of the KJD versus HTO trial, and for the purpose of this study, had full-leg weight-bearing radiographs available. Eventually, data from 73 patients could be used for the current study, as they had whole-leg radiographs for preoperative axis alignment and weight-bearing semi-flexed knee radiographs available.

Ethical approval was received, and the study was performed in accordance with the ethical principles from the Declaration of Helsinki. All patients gave written informed consent (IC).

Treatment

KJD was performed by placing an external fixation device, distracting the TF joint space lateral and medial for 5 mm over six weeks. In HTO, the weight-bearing was shifted laterally until the postoperative mechanical axis ran laterally through the tibial plateau, at two-third of its entire width (measured from the medial side). HTO patients were instructed to limit weight-bearing activities over the first six weeks post-surgery. Both treatments were described in more detail before,[20,21] and in the supplementary information of **chapter 8**. Knee radiographs are shown in figure 1.

Function and pain

For the present study, data from shortly before treatment (baseline) and one year postoperatively were used. Clinical effectiveness was determined by the total WOMAC 3.0 index derived from the KOOS questionnaire (self-assessment reduced from five to three dimensions and using a five-point Likert scale, normalizing to a 100-point scale, where 100 is no pain). Pain was measured by a Visual Analogue Scale (VAS Pain), a continuous scale ranging from 0 (no pain) to 100 (worst imaginable pain).

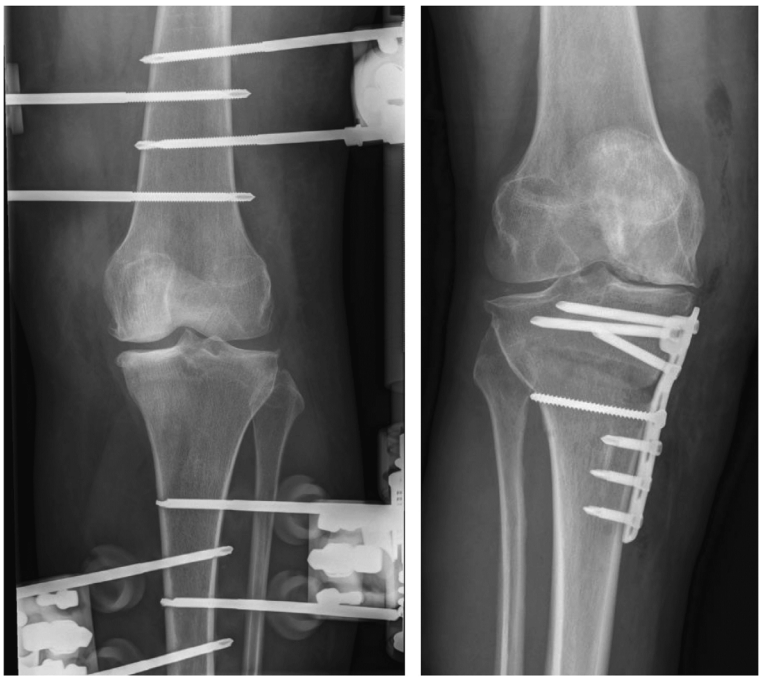

Figure 1: *Posteroanterior radiographs of knee joint distraction (left) and high tibial osteotomy (right).*

Weight-bearing knee radiographs and radiographic outcomes

The preoperative tibiofemoral axis (HKA) was measured on full-leg weight-bearing radiographs. For patients randomized to KJD, whole-leg radiographs were only obtained preoperatively. The HKA could therefore not be evaluated longitudinally in these patients. However, standardized weight-bearing, semi-flexed, posterior-anterior knee radiographs were obtained at inclusion and one year postoperatively in all patients. They provided the opportunity to use the FTA (anatomical axis) as an alternative to HKA (mechanical axis). A schematic overview of preoperative HKA and FTA measurements is given in figure 2. The FTA was assessed using Knee Images Digital Analysis (KIDA) software by a single experienced observer[22] and defined as the angle of the intersection between the bone and cartilage interfaces of tibia and femur (in degrees). A moderate correlation between HKA and FTA (Pearson correlation coefficient = -0.46, 95% CI: -0.67 to -0.25, $p<0.001$, N=73) was found in our cohort (see figure 2). Still, anatomical alignment was recently found to predict TF cartilage loss as well as mechanical alignment.[23] KIDA also provided four JSW measures: mean medial JSW, mean lateral JSW, and mean overall and minimal overall JSW of the total knee.

Statistical analysis

Changes in WOMAC, VAS Pain, and JSW are presented as means with standard deviation

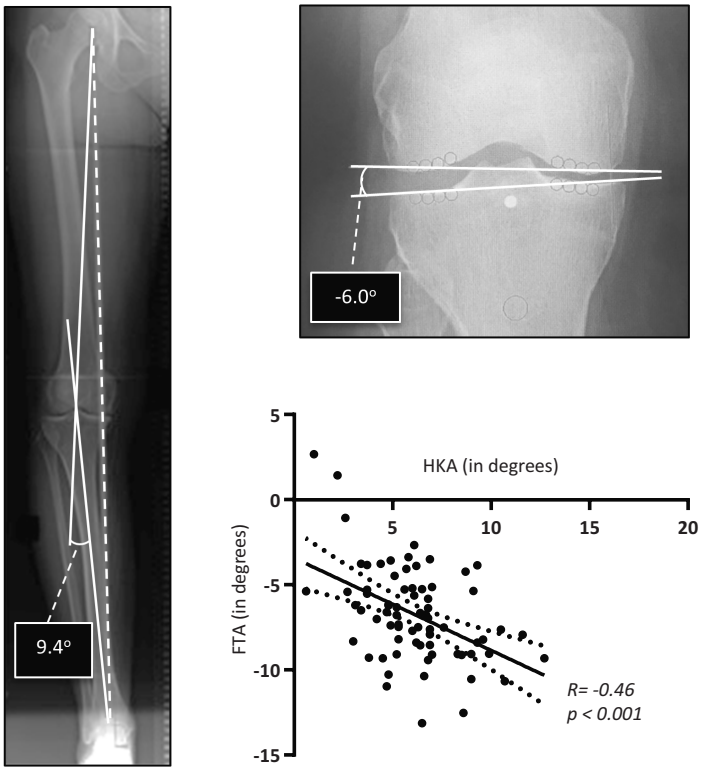

Figure 2: Schematic overview of a hip-knee-ankle angle (HKA) measurement on full-leg weight-bearing radiographs (left). A femur-tibia angle (FTA) measurement on weight-bearing, semi-flexed, posteroanterior knee radiographs (top right). Correlation between the preoperative FTA and preoperative HKA (bottom right).

(SD). One-year changes of WOMAC, VAS Pain, and JSW were evaluated by paired t-tests. Differences between KJD and HTO were evaluated by linear regression, with change in either WOMAC, VAS Pain or radiographic changes (JSW and FTA) as dependent variables, and baseline values, treatment (KJD/HTO), and age, as independent variables.
To evaluate the relevance of axial alignment in the successful treatment of knee OA, relations between change in FTA and change in WOMAC and VAS Pain were evaluated using linear regression, with change in either WOMAC or VAS Pain as dependent variables, and change in FTA, treatment (KJD/HTO), the interaction between the change in FTA and treatment, and baseline values as independent variables. In addition to the change in axial alignment, also the influence of the preoperative axial alignment (both FTA and HKA) in the successful treatment of knee OA by HTO and KJD was evaluated by association to clinical and structural changes over 12 months.

All tests were two-sided, and a probability of $p<0.05$ was considered statistically significant unless specified otherwise. Statistical analyses were performed using SPSS (*Version 21.0. Armonk, NY: IBM Corp*).

Results

Patients

Baseline characteristics including FTA were comparable between patients that underwent HTO or KJD, except for the range of motion, which was statistically significantly lower for KJD patients (132.2° vs. 127.6°, P=0.045, see table 1).

Axial alignment in HTO (n=45) versus KJD (n=28)

Axial alignment, as defined by FTA, increased statistically significantly one year after either treatment (*KJD: Δ0.90°, 95%-CI: 0.07° to 1.74°, p=0.04, HTO: Δ0.73°, 95%-CI: 0.23° to 1.23°, p=0.01*), see figure 3 lowest panel and table 2. There was no statistically significant difference in the change of FTA between KJD and HTO, see table 2.

Clinical and radiographic changes at one year after HTO (n=45) or KJD (n=28)

A statistically significant increase in WOMAC was observed after either treatment (*KJD: Δ23.1, 95%-CI: 16.8 to 29.4, HTO: Δ28.5, 95%-CI: 23.5 to 33.6, both p<0.001*). Similarly, a decrease in VAS pain was observed after either treatment (*KJD: Δ-22.3, 95%-CI: -32.7 to*

Table 1: Baseline patient characteristics

	High tibial osteotomy (n = 45)	***Knee joint distraction (n = 28)***	***P-value***
Male gender *(n,%)*	27 (60%)	19 (68%)	n.s.
Height *(m)*	1.77 ± 0.1	1.78 ± 0.1	n.s.
Weight *(kg)*	85.2 ± 14.0	86.2 ± 14.3	n.s.
BMI *(kg/m2)*	27.2 ± 3.3	27.1 ± 3.6	n.s.
Index knee *(left: n, %)*	20 (44)	12 (43)	n.s.
Age *(years)*	49.4 ± 6.3	51.9 ± 6.1	n.s.
Kellgren-Lawrence grade			
Grade 0, *n (%)*	1 (2.2)	0 (0)	n.s.
Grade 1, *n (%)*	5 (11.1)	6 (21.4)	
Grade 2, *n (%)*	12 (26.7)	5 (17.9)	
Grade 3, *n (%)*	23 (51.1)	12 (42.9)	
Grade 4, *n (%)*	4 (8.9)	5 (17.9)	
Total WOMAC *(0-100)*	52.3 [47.1 to 57.4]	53.0 [45.7 to 60.4]	n.s.
WOMAC *Function*	53.8 [48.5 to 58.9]	54.2 [46.6 to 61.8]	n.s.
WOMAC *Pain*	50.0 [43.8 to 56.2]	51.4 [43.8 to 59.1]	n.s.
WOMAC *Stiffness*	45.8 [39.6 to 52.1]	47.3 [39.6 to 55.0]	n.s.
VAS pain *(100-0)*	65.0 [58.8 to 71.1]	57.7 [48.8 to 66.6]	n.s.
Range of motion index knee *(°)*	132.2 [129.8 to 134.7]	127.6 [124.3 to 131.4]	0.045
Mean overall JSW *(mm)*	4.7 [4.4 to 4.9]	4.8 [4.4 to 5.2]	n.s.
Mean medial JSW *(mm)*	2.0 [1.6 to 2.3]	1.9 [1.3 to 2.5]	n.s.
Mean lateral JSW *(mm)*	7.5 [7.1 to 7.9]	7.7 [6.9 to 8.5]	n.s.
Minimum overall JSW *(mm)*	0.60 [0.29 to 0.90]	0.56 [0.20 to 0.91]	n.s.
Hip-knee-ankle angle *(HKA, °)*	6.2 [5.5 to 6.9]	6.0 [5.0 to 6.9]	n.s.
Femur-tibia angle *(FTA, °)*	-6.6 [-7.2 to -5.9]	-7.0 [-8.4 to -5.6]	n.s.

Table 2: Outcomes

		Knee joint distraction (KJD) n = 28		High tibial osteotomy (HTO) n = 45		KJD vs. HTO *	
		Mean (95%-CI)	***P-value***	***Mean (95%-CI)***	***P-value***	***Unst. B$ (95%-CI)***	***P-value***
Δ WOMAC *(0-100)*	*Total*	23.1 (16.8, 29.4)	**<0.001**	28.5 (23.5, 33.6)	**<0.001**	6.4 (-0.5, 13.3)	0.07
	Stiffness	24.2 (18.0, 30.4)	**<0.001**	28.4 (23.3, 33.6)	**<0.001**	5.2 (-1.8, 12.3)	0.14
	Pain	23.8 (15.5, 32.1)	**<0.001**	31.2 (25.5, 37.0)	**<0.001**	8.3 (0.6, 16.0)	**0.04**
	Function	12.1 (3.3, 20.8)	**<0.01**	23.1 (16.8, 29.4)	**<0.001**	11.7 (3.2, 20.2)	**0.01**
Δ VAS *(100-0)*	*Pain*	-22.3 (-32.7, -11.9)	**<0.001**	-37.9 (-45.6, -30.3)	**<0.001**	-13.9 (-24.9, -2.9)	**0.01**
Δ JSW *(mm)*	*Overall*	0.46 (0.12, 0.80)	**<0.01**	0.14 (-0.10, 0.38)	0.24	-0.39 (-0.78, 0.007)	0.05
	Medial	0.83 (0.35, 1.31)	**<0.01**	0.47 (0.25, 0.69)	**<0.001**	-0.38 (-0.83, 0.07)	0.09
	Lateral	0.06 (-0.48, 0.60)	0.82	-0.17 (-0.57, 0.23)	0.41	-0.32 (-0.99, 0.34)	0.33
	Minimal	0.82 (0.44, 1.19)	**<0.001**	0.35 (0.19, 0.51)	**<0.001**	-0.49 (-0.85, -0.13)	**0.01**
Δ Femur tibia angle *(FTA, °)*	*Mean*	0.90 (0.07, 1.74)	**0.04**	0.73 (0.23, 1.23)	**0.01**	0.008 (-0.89, 0.91)	0.99

* Differences between KJD and HTO were evaluated by linear regression, with WOMAC, VAS Pain or radiographic changes as dependent variables, treatment (KJD/HTO) as independent variables. $ Unstandardized coefficients of HTO vs KJD. All adjusted for age and baseline values. P-values <0.05 are in bold.

-11.9, HTO: Δ-37.9, 95%-CI: -45.6 to -30.3, both p<0.001). Both KJD and HTO led to statistically significant increases in mean medial JSW (*KJD: Δ0.83 mm, 95%-CI: 0.35 to 1.31, p<0.01, HTO: Δ0.47 mm, 95%-CI: 0.25 to 0.69, p<0.001*). Similar, an increased minimal overall JSW (*KJD: Δ0.82 mm, 95%-CI: 0.44 to 1.19, p<0.001, HTO: Δ0.35 mm, 95%-CI: 0.19 to 0.51, p<0.001*) was detected. While mean lateral JSW did not change statistically significantly after either treatment (*KJD: Δ+0.06 mm, 95%-CI: -0.48 to 0.60, HTO: Δ-0.17 mm, 95%-CI: -0.57 to 0.23, p=0.41*), only KJD led to a statistically significant increase in mean overall JSW (*KJD: Δ0.46 mm, 95%-CI: 0.12 to 0.80, p<0.01, HTO: Δ0.14 mm, 95%-CI: 0.10 to 0.38 , p=0.24*), see table 2 and figure 3.

While both treatments showed clear clinical and radiographic improvement, there were differences between treatments. HTO led to statistically significantly more clinical improvement, in WOMAC Pain (*P=0.04*), WOMAC Stiffness (*P=0.01*), and VAS Pain (*P=0.01*). In contrast, KJD led to more JSW increase than HTO, statistically significantly for minimal

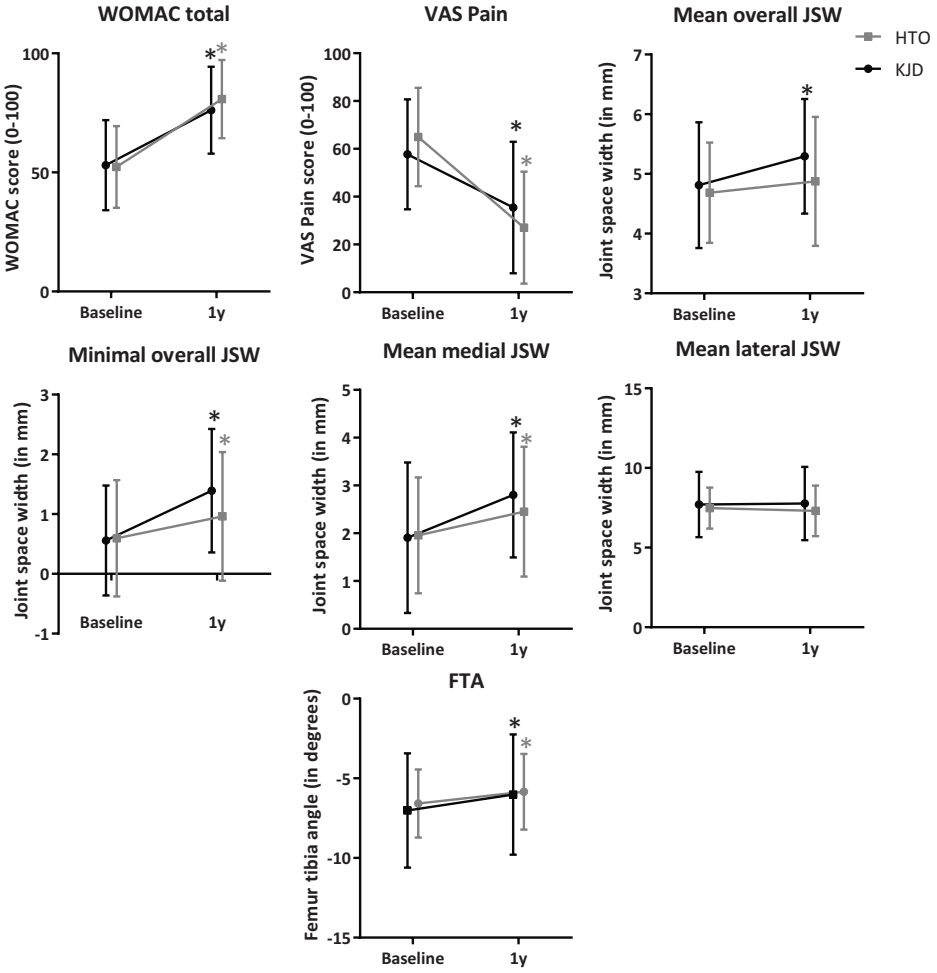

Figure 3: Changes in WOMAC, VAS Pain, and radiographic outcomes at one year after KJD and HTO. Changes are presented using means with standard deviation (SD). Changes over time were evaluated using paired t-tests. WOMAC: 100 is no pain, VAS Pain: 0 is no pain. JSW: Joint space width, FTA: femur-tibia angle. Asterisks indicate statistically significant changes from baseline.

JSW (P=0.01), and near-significantly for both mean medial (*P=0.09*) and mean overall JSW (*P=0.05*), see table 2.

Associations between changes in FTA and clinical improvement

Although changes in FTA and clinical improvement were observed after either treatment, no statistically significant associations between the change in FTA and change in total WOMAC (*mean: 1.1, 95%-CI: -0.7 to 2.9, p=0.232*), WOMAC function (*mean: 1.0, 95%-CI: -0.85 to 2.86, p=0.284*), WOMAC Stiffness (*mean: -0.4, 95%-CI: -2.7 to 1.9, p=0.727*), and VAS Pain (*mean: -0.6, 95%-CI: -3.5 to 2.4, p=0.707*) were found. There was, however, an association

Table 3: Effect of preoperative axial alignment, and change in axial alignment

		Baseline FTA (n=73)*		Baseline HKA (n=73)*		Change in FTA (n=73)#		Change in HKA (n=45) #$	
		Unst. B$ (95%-CI)	*P-value*	*Unst. B$ (95%-CI)*	*P-value*	*Unst. B$ (95%-CI)*	*P-value*	*Unst. B$ (95%-CI)*	*P-value*
Δ WOMAC *(0-100)*	*Total*	0.81 (-0.41, 2.03)	0.191	0.04 (-1.41, 1.50)	0.953	1.09 (-0.71, 2.88)	0.232	0.57 (-1.43, 2.57)	0.564
	Stiffness	0.90 (-0.35, 2.14)	0.155	0.14 (-1.35, 1.62)	0.853	1.00 (-0.85, 2.86)	0.284	0.64 (-1.44, 2.72)	0.534
	Pain	0.52 (-0.87, 1.90)	0.460	-0.11 (-1.76, 1.54)	0.893	2.18 (0.25, 4.10)	**0.027**	0.52 (-1.52, 2.57)	0.602
	Function	0.69 (-0.84, 2.21)	0.372	-0.09 (-1.89, 1.70)	0.919	-0.40 (-2.67, 1.87)	0.727	0.22 (-1.87, 2.32)	0.828
Δ VAS *(100-0)*	*Pain*	-2.31 (-4.23, -0.39)	**0.019**	0.35 (-2.01, 2.72)	0.767	-0.56 (-3.53, 2.41)	0.707	-1.84 (-4.38, 0.70)	0.148
Δ JSW *(mm)*	*Overall*	-0.01 (-0.08, 0.06)	0.853	0.01 (-0.07, 0.10)	0.728				
	Medial	0.11 (-0.01, 0.23)	0.066	-0.09 (-0.19, 0.01)	0.072				
	Lateral	-0.02 (-0.20, 0.16)	0.838	0.08 (-0.06, 0.22)	0.246				
	Minimal	0.09 (0.02, 0.17)	**0.019**	-0.03 (-0.11, 0.05)	0.450				

*Associations between baseline axial alignment (FTA or HKA) and change in structural and clinical parameters were evaluated using linear regression, with change in WOMAC, VAS Pain or joint space width (JSW) as dependent variables, and baseline axial alignment, and treatment (KJD/HTO) as independent variables. #Associations between the change in axial alignment and the change in clinical parameters was evaluated using linear regression, with change in WOMAC or VAS Pain as dependent variables, and change in FTA and treatment (KJD/HTO) as independent variables. $Only HTO patients had preoperative full-leg weight-bearing radiographs available, necessary to measure the hip-knee-ankle angle (HKA). All adjusted for baseline values. P-values <0.05 are in bold.

between an increase in FTA (more neutral axis) and improvement in WOMAC Pain (*mean: 2.2, 95%-CI: 0.3 to 4.1, p=0.027*), see table 3. Associations were not different for the two treatments (*interaction between change in FTA and treatment*; *P>0.05; n=73*).

Associations between changes in HKA and clinical improvement in HTO patients
Associations with change in HKA could only be evaluated for HTO patients, and no statistically significant association was found between a change in HKA and a change in total WOMAC (*mean: 0.6, 95%-CI: -1.4 to 2.6, p=0.564*), WOMAC Function (*mean: 0.6, 95%-CI: -1.4 to 2.7, p=0.534*), WOMAC Pain (*mean: 0.5, 95%-CI: -1.5 to 2.6, p=0.602*), WOMAC Stiffness (*mean: 0.2, 95%-CI: -1.9 to 2.3, p=0.828*), or VAS Pain (*mean: -1.8, 95%-CI: -4.4 to 0.7, p=0.148; n=45*), see table 3.

Associations between preoperative axial alignment and clinical and structural changes
The association between a higher preoperative FTA (more neutral axis) and clinical change over one year was statistically significant for a reduction in VAS Pain (*mean: -2.3, 95%-CI: -4.2 to -0.4, p=0.019*), independent of the type of operation. There were no statistically significant associations between preoperative HKA and clinical changes, see table 3.

Type of operation (KJD/HTO) appeared to modify the association between preoperative axial alignment and several JSW changes (*interaction terms: P<0.1*), therefore stratified analyses were performed. However, also after stratification, no statistically significant associations between preoperative axial alignment as defined by the HKA and changes in clinical or structural parameters were found for either KJD or HTO seperately. A higher preoperative FTA was associated with a higher medial JSW increase after KJD (*mean: 0.18, 95%-CI: 0.01 to 0.34, p=0.037*). Similarly, a higher preoperative FTA was associated with a higher minimal JSW increase after both KJD (*mean: 0.14, 95%-CI: 0.01 to 0.26, p=0.036*), and HTO (*mean: 0.10, 95%-CI: 0.00 to 0.19, p=0.043*), see table 3.

Discussion

In patients with medial compartment knee OA who were eligible for TKA otherwise, KJD and HTO both lead to a statistically significant clinical and structural benefit after one year. Both KJD and HTO appeared to improve axial alignment. An increase in FTA and a more neutral preoperative FTA were associated with more reduction in pain at one year after either treatment. A higher preoperative FTA was also associated with a higher increase in JSW after both treatments.

Clinical and structural improvement
As was also shown before, both the improvement in WOMAC and VAS Pain were higher after HTO.[18] On the other hand, structural improvement was more pronounced for KJD,

specifically the increase of minimal JSW. This suggests that the clinical benefit from HTO does not originate from the increase in joint space in the affected compartment per se, but that it may rather come from redistribution of the axial pressure away from the affected compartment. However, the change in FTA possibly reflects different processes between treatments. Although not fully evident in the current data, the redistribution of axial pressure, compresses the cartilage of the lateral compartment, while the medial compartment significantly increases, whereas after KJD, the 'normalization' of FTA seems to be caused especially by the increase in minimal and medial compartment JSW, even to a higher extent than after HTO.

Relation between (change in) axial alignment and clinical improvement
There was a statistically significant relation between change in FTA and improvement in WOMAC Pain and between higher preoperative FTA values (less deviation) and improvement in VAS Pain, for both treatments. Patient-reported pain outcomes are known to not be equally responsive,[24] possibly explaining the associations to different pain outcomes. Nonetheless, these findings suggest the relevance of both initial and change in axial alignment for pain reduction from KJD or HTO .

Relation between (a change in) axial alignment and radiographic improvement
Moreover, a higher preoperative FTA (less deviation) was related to a higher increase in medial and minimal JSW in the KJD group and a higher increase in medial JSW in the HTO group. The latter may indicate that less severely damaged cartilage has better regenerative capacity upon KJD and HTO than more severely damaged cartilage. In a previous longitudinal study in 829 patients with 1294 painful knees, outcomes were classified as either 'poor' (incidence, persistence or progression) or 'good' (no incidence, persistence, or progression) based on radiographic and clinical evaluations over five-year follow-up.[25] The 796 knees that had good outcomes had statistically higher preoperative FTA than the 189 knees that had poor outcomes (*FTA: -1.68°±1.70° for good outcomes, and -2.32°±2.03° for poor outcomes*). As such, initial FTA might be clinically relevant, as a lower initial FTA (more varus) is associated with a less increase in JSW and VAS Pain reduction over one year and is associated with more structural damage after five years.

Changes in axial alignment in the context of longitudinal radiographic assessment errors
There are several sources of error in the longitudinal assessment of radiographic parameters, that might also amplify one another; inconsistent patient positioning, intraobserver variation (using KIDA), and change in biomechanics. The semiflexed view according to Buckland-Wright has been shown to reposition the knee the best between radiographs.[26,27] Moreover, intra-observer variability of KIDA measurements on such radiographs was demonstrated to be low (ICC between 0.73 and 0.99 for the various radiographic outcomes of KIDA).[25] Yet, KJD and HTO both affect joint biomechanics and, with that, joint position. Previous research

into the influence of variation in clinically relevant knee positions on radiographic measures of axial alignment, also using the Buckland-Wright imaging protocol and KIDA,[28] revealed that only the extent of leg extension statistically significantly influenced FTA. However, the authors did not deem these effects clinically relevant, because changes were considerably smaller than the average change in FTA in patients with progressive radiographic knee OA.

Clinical relevance of the FTA

To put the order of magnitude of FTA changes from HTO and KJD in our cohort in perspective, they should be compared to other studies on changes in FTA over time. Ample literature is available on changes in the mechanical axis (HKA) after HTO, but not for changes in the anatomical axis (FTA) and for KJD these studies have not yet been performed. In the observational C*ohort Hip and Cohort Knee* (CHECK) study, 310 patients who had progressed from K&L grade < II to K&L grade ≥ II over a two-year follow-up, showed a mean FTA decrease of 0.77° degrees (SD 2.33). Therefore, we conclude that the joint-sparing treatments, KJD and HTO, not only affect FTA through delaying OA progression, but on average even reverse this process.

The FTA decrease observed in the current study are greater than the abovementioned measurement errors due to change in biomechanics over time (simulated by clinically relevant variations in knee position) and greater than the change in FTA seen in OA patients with radiographic progression. In context of these references, it seems likely that KJD actually causes a change in axial alignment, similar to HTO, one year after treatment.

Limitations of this study were the limited sample size in total and the relatively small number of KJD patients in particular. Changes in axial alignment are preferably evaluated by HKA, measured on full-leg weight-bearing radiographs, and considered the gold standard for measurement of the mechanical axis. However, full-leg weight-bearing radiographs were only available postoperatively for HTO patients, therefore, as a first exploration in comparing the longitudinal change in axial alignment between KJD and HTO treatment, the FTA was used as an alternative to HKA.

In summary, KJD and HTO both lead to a statistically significant clinical and structural benefit after one year in patients with medial tibiofemoral knee OA. Both KJD and HTO improve alignment. An increase in FTA and limited deviation in anatomical joint axis (higher preoperative FTA) were each associated with more reduction in pain over one year. Moreover, a higher preoperative FTA was associated with a higher increase in JSW after either treatment. This implies that KJD, a joint-sparing treatment not necessarily focused on changing axial alignment as HTO is, also causes a change in axial alignment in the successful treatment of medial compartment knee OA.

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CHAPTER 8

Cartilage quality (dGEMRIC index) following Knee Joint Distraction or High Tibial Osteotomy

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Cartilage. 2018; Epub ahead of print

Abstract

Objective
High tibial osteotomy (HTO) and knee joint distraction (KJD) are treatments to unload the osteoarthritic (OA) joint with proven success in postponing a total knee arthroplasty (TKA). While both treatments demonstrate joint repair, there is limited information about the quality of the regenerated tissue. Therefore, the change in quality of the repaired cartilaginous tissue after KJD and HTO was studied using delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC).

Design
40 patients (20 KJD and 20 HTO), treated for medial tibiofemoral OA, were included in this study. Radiographic outcomes, clinical characteristics, and cartilage quality were evaluated at baseline, and at one- and two-year follow-up.

Results
Two years after KJD-treatment, clear clinical improvement was observed. Moreover, a statistically significant increased medial (*Δ0.99mm*), minimal (*Δ1.04mm*), and mean (*Δ0.68mm*) radiographic joint space width (JSW) was demonstrated. Likewise, medial (*Δ1.03mm*), minimal (*Δ0.72mm*), and mean (*Δ0.46mm*) JSW were statistically significantly increased on radiographs after HTO. There was on average no statistically significant change in dGEMRIC indices over two years and no difference between treatments. Yet, there seemed to be a clinically relevant, positive relation between increase in cartilage quality and patients' experienced clinical benefit.

Conclusions
Treatment of knee OA by either HTO or KJD leads to clinical benefit, and an increase in cartilage thickness on weight bearing radiographs for over 2-years post treatment. This cartilaginous tissue was on average not different from baseline, as determined by dGEMRIC, whereas changes in quality at the individual level correlated with clinical benefit.

Introduction

Knee osteoarthritis (OA) is a major socio-economic burden.[1,2] End-stage knee OA is most often treated with a total knee arthroplasty (TKA).[3] When placed under the age of 65 the chance for revision surgery is significant.[4] Revision surgery is considerably more difficult, costly, and generally less effective, leading to increased complication and mortality rates.[4] In a population with increasing obesity, a relative younger population is increasingly at risk for development of OA. Moreover, life expectancy is increasing, increasing the risk for revision surgery later in life. Therefore, the need arises for joint preserving strategies.[5] Since structural tissue damage is a probable cause for pain and functional limitation, joint preserving treatment focusses on tissue repair, accompanied by clinical benefit.

High tibial osteotomy (HTO) is a well-known joint preserving procedure to treat unicompartmental knee OA by correcting a deviated mechanical leg-axis, with that unloading the damaged compartment.[6–8] Many studies show good clinical results, with high and prolonged survival rates,[6] and even structural cartilage repair.[9,10] A systematic review shows osteotomies can delay TKA with a median of seven years.[11]

Knee joint distraction (KJD) is a less known joint preserving treatment and indicated for both unilateral and generalized knee OA. KJD is performed by placing an external fixation device for 6 weeks, allowing for a renewal of the joint homeostasis, where anabolic activity takes over catabolic activity, providing a more healthy environment enabling tissue repair.[12,13] Studies have demonstrated a progressive decrease in pain, normalization of function, and a sustained increase in cartilage thickness as seen on weight-bearing radiographs.[14–17] Arthroscopy[14,15,18] and MRI[14,19] evaluation showed cartilage repair after KJD. As a surrogate marker for cartilage quality, biochemical markers for collagen type-II turnover demonstrated an increase of synthesis over release.[19] In a prospective open uncontrolled study, KJD proved to be successful in postponing TKA for at least five years in more than 75% of the treated patients.[20] Postponing a TKA over 10 years was reported to occur in two-third of patients treated with KJD based on data of small groups.[21]

HTO and KJD aim to permanently partially (HTO) or temporarily completely (KJD) alleviate the biomechanical load on the affected cartilage. Moreover, both treatments result in cartilaginous tissue repair and clinical benefit. Therefore, the effects of these treatments were directly compared in a randomized controlled trial (RCT). Recently, the one-year evaluation of this RCT was reported.[22] All patient-reported outcome measures were improved after one year ($p<0.02$) as well as an increased joint space width (JSW) of the medial compartment upon both KJD (0.8±1.0mm, $p=0.001$) and HTO (0.4±0.5mm, $p<0.001$). In the KJD group (in contrast to the HTO group), also the lateral compartment showed an increased JSW, resulting in a statistically significant increase in overall mean JSW ($p<0.02$).[22]

Following reports of structural repair, the next step is to assess cartilage quality, preferably using non-invasive techniques. Quantitative MRI analysis, in the form of delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC) relies on the relationship between the highly negatively charged glycosaminoglycans (GAG) and the negatively charged MRI contrast agent gadolinium, providing a measure of quality of the cartilaginous tissue, specifically with respect to GAG content.[23] In OA the highly negatively charged glycosaminoglycans (GAG) are lost and when intravenously injected, the MRI contrast agent gadolinium, reaches the patients' joints and penetrates the cartilage in an inverse proportional manner.[23] The qualitative state of the cartilage is thereby represented as dGEMRIC indices; low dGEMRIC indices represent low GAG content viz. degenerated cartilage, and high dGEMRIC indices higher GAG content, viz. more healthy cartilage.[23]

Although cartilaginous tissue repair is shown for both HTO and KJD, imaging data on cartilage quality are scarce. In case of HTO, there is only one case report series published and a few studies reporting on dGEMRIC changes; six[9,24], nine[25], twelve[9,24] and twenty-four[9] months post treatment in humans. Although positive results were obtained, none of these studies could confirm (statistically) significant cartilage quality changes upon treatment with HTO. For KJD such data are not present.

In the present study, the change in quality of the repaired cartilaginous tissue two years after KJD or after HTO treatment was investigated using dGEMRIC. In addition, it was evaluated whether these changes are related to radiographic changes and clinical outcome.

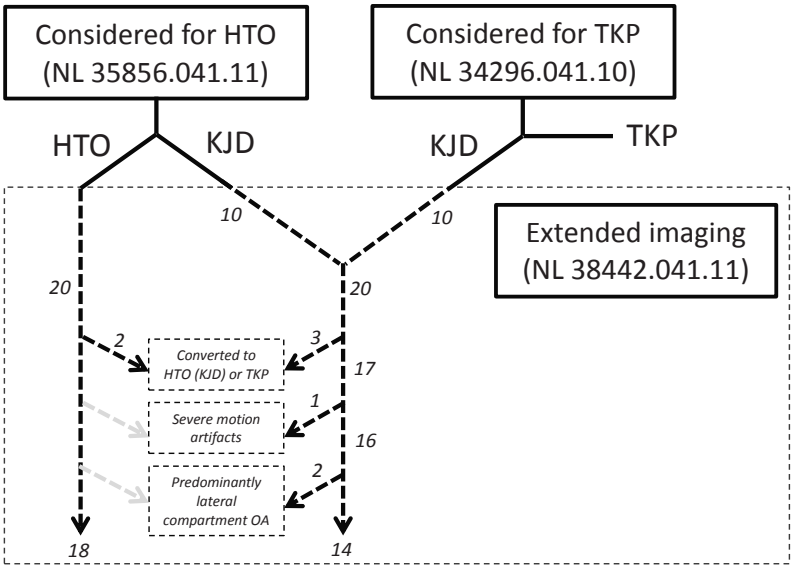


Figure 1: Inclusion flowchart. Patients considered for HTO or total knee arthroplasty (TKP), included in either of the randomized trials (*NL 35856.041.11* or *NL 34296.041.10*) were asked to participate in this extended imaging trial *(NL 38442.041.11*). Additional dGEMRIC imaging was performed at baseline and after 2 years for HTO patients, and at baseline, and at one and after two years for KJD patients.

Methods

Patients

For this explorative study patients were included originating from two independent RCTs (figure 1; *NL35856.041.11* and *NL34296.041.10*). Patients with medial compartmental knee OA considered for HTO according to regular practice[22], randomized to either KJD or HTO (1:2) were asked to participate in this extended imaging study. Due to the relative low number of KJD versus HTO patients, caused by the randomization ratio, KJD patients from an RCT comparing TKA with KJD[26] were additionally added to this study. These patients were, according to regular practice, considered for TKA surgery and randomized to either KJD or TKA (1:2).

For both studies, patients younger than 65 years, with varus deformity, Kellgren & Lawrence score >2, intact ligaments, normal range-of-motion (flexion >120°; flexion-limitation <15°), normal stability, and a BMI <35 were included. Exclusion criteria included any history of inflammatory- or rheumatoid arthritis, post-traumatic fibrosis due to fracture of the tibia plateau, full bone-to-bone contact (absence of any JSW on X-ray), surgical treatment of the involved knee <six months ago, and primary (isolated) patella-femoral OA. Patients with an infectious susceptible prosthesis in situ and/or contralateral knee OA that needed treatment were excluded as well.

After patients' written consent to participate in one of the two RCTs, they were additionally asked to participate in the current study extending the standard MRI measurements with additional imaging modalities, including dGEMRIC to measure proteoglycan content/ distribution. When comparing the demographics of the original KJD and HTO groups with those of this extended imaging study, only the proportion of males in the HTO group is statistically significantly higher, which was considered coincidental (table 1).

Ethical approval was received (NL38442.041.11), and the study was performed in accordance with the ethical principles from the Declaration of Helsinki. The first twenty patients whom gave written informed consent (IC) treated with HTO and the first twenty patients of both RCTs treated with KJD whom gave written IC were included.

Treatment

KJD was performed by placing an external fixation device, ensuring 5 mm distraction during a period of six weeks[26]. In HTO, the aim was to shift the weight-bearing line laterally, with the post-operative mechanical axis running laterally through the tibial plateau, at 62% of its entire width (measured from the medial side). HTO patients were hospitalized for three days, followed by six weeks of limited weight-bearing. At eighteen months, the plate was removed to allow MRI imaging at two years. Treatment radiographs are shown in figure 2. Both joint-preserving treatments have been described in more detail previously[27] and in the supplementary file.

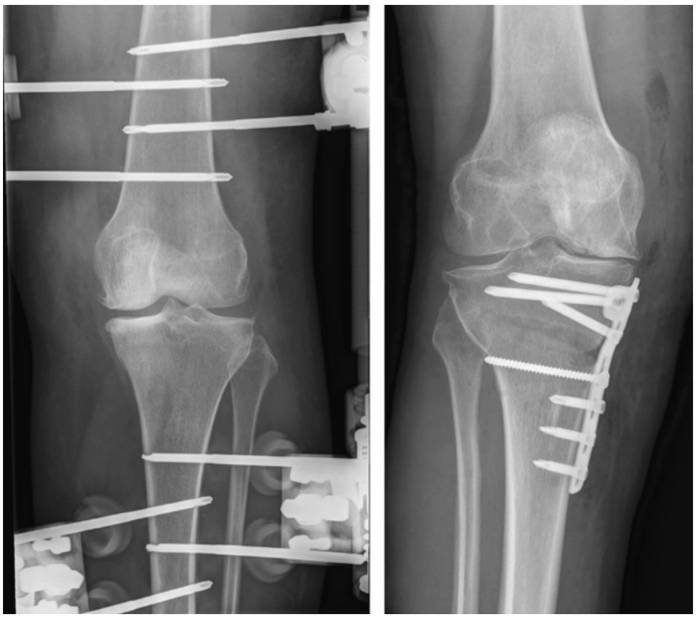


Figure 2: Posteroanterior radiographs of knee joint distraction (left) and high tibial osteotomy (right).

Study assessments

For the present study, evaluations were performed before treatment (baseline), at one-, and at two years after treatment. Patients undergoing HTO did not undergo dGEMRIC MRI at twelve months due to the metal-plate in situ.

Function and pain

Clinical effectiveness was determined by the WOMAC 3.0 index derived from the KOOS questionnaire (self-assessment reduced from five to three dimensions and using a five-point Likert scale, normalizing to a 100-point scale, where 100 is no pain). Pain was measured by a Visual Analogue Scale (VAS-Pain), a continuous scale ranging from 0 (no pain) to 100 (worst imaginable pain), upon which the patient indicated the amount of pain.

Weight-bearing radiographs and joint space width (JSW) measurements

Standardized semi-flexed weight-bearing radiographs were acquired at inclusion and two years after treatment to determine the Kellgren & Lawrence grade (K&L at baseline) according to a standardized protocol and to evaluate changes in JSW over time using Knee Images Digital Analysis (KIDA) software,[28] (single experienced observer) expressed in four JSW measures; mean medial, and mean lateral JSW, mean of the total joint (mean JSW), and minimal JSW of the total joint. The preoperative tibiofemoral axis was measured on full leg weight-bearing radiographs.

dGEMRIC acquisition

After scout images, dGEMRIC scans were performed on a clinical 3 tesla MRI scanner (Achieva 3T; Philips Medical Systems) using a 16-channel knee coil. The 3D imaging protocol consisted of a sagittal inversion recovery fast spoiled gradient-recalled echo (FSPGR) sequence with five settings for the inversion time (TI) (50; 150; 350; 650; 1650 ms), as previously described.[29] The repetition time (TR) was 10 ms. Other parameters were: flip angle=15°, echo time = 3 ms, field of view = 160×145.2×108 mm^3, in-plane voxel size = 0.625×0.625×3 mm^3 and matrix size = 260×234×36. Prior to scanning, patients received an intravenous injection of 0.2 mM/kg gadolinium-based contrast agent (Gd-DTPA; Magnevist by Bayer Schering Pharma). Subsequently, patients performed a standardized light exercise, by walking a pre-defined route for approximately 15 minutes, and rested until 90 minutes after contrast infusion before the MRI scan was made (dGEMRIC sequences including scout images took 20 minutes and 30 seconds).

dGEMRIC index estimation

Segmentation was performed on dGEMRIC images of every patient, acquired at baseline and follow up by two independent observers (NB, AC), blinded for time point and treatment. This segmentation provided a total of 12 Regions of Interest (ROIs), divided in anterior (a), central (c) and posterior (p) regions of the tibia (T) or femur (F) on the medial (M) or lateral (L) side of the knee (figure 3). ROIs were manually delineated on the sagittal images obtained in the dGEMRIC scan with inversion time of 1650 ms (TI=1650ms) according to the method described by Eckstein et al.[25,30] The central and both adjacent slices through both tibiofemoral joint compartments were manually selected. ROIs were delineated, using in-house developed software (*ImageXplorer, Image Sciences Institute*).

Phase–corrected real data reconstruction (allowing for noise-reduction), and image registration were performed on the 3D images with five different inversion time settings (TI=50;150;350;650;1650ms) before fitting. [31,32] Eventually, all sequences were rigidly transformed to TI=1650 ms using an intensity-based image registration, and alignment was visually inspected.

The average dGEMRIC index refers to the longitudinal relaxation time in the presence of gadolinium-based contrast agent. Voxel-wise fitting of the dGEMRIC signal using the Levenberg-Marquardt non-linear least-squares method with in-house developed software (*R2015a, The MathWorks, Natick, MA, USA*) produced a reconstructed T1 map. From this T1 map, the average dGEMRIC index was calculated for each compartment and ROI separately. The dGEMRIC index map was then superimposed onto the scan acquired for TI=1650ms, see figure 4. A color-scale was used, representing the condition of the cartilage, ranging from degenerated towards healthy (low GAG content results in a low dGEMRIC index, and *vice versa*).

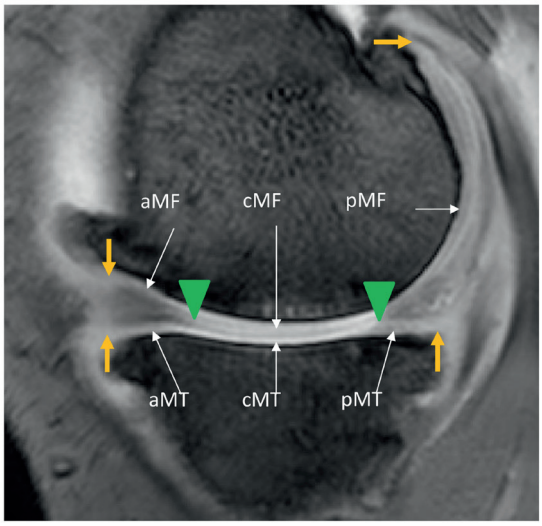


Figure 3: Delineating anterior (a), central (c) and posterior (p) regions-of-interest (ROIs) of the medial (M) and lateral (L) tibia (T) and femur (F). Regions are separated at the most anterior and posterior horn of the meniscus (green arrowheads), the anterior regions reach until the most anterior part of the tibia plateau (orange arrows). The posterior tibial region is bounded at the most posterior part of the tibia plateau, while the posterior femoral regions encompass all visible cartilage (orange arrows). Six regions are delineated per slice, for three consecutive slices in both the lateral and femoral compartment.

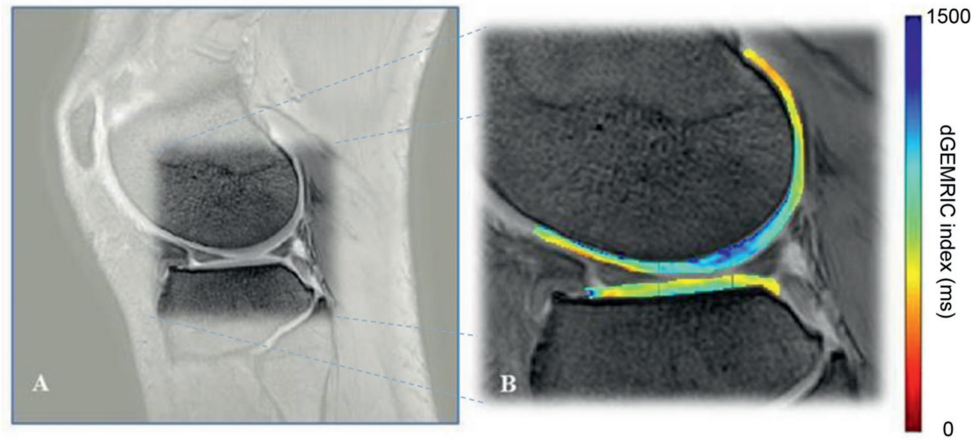


Figure 4A: Sagittal view of the lateral side of a tibiofemoral joint. 4B: Automated in-house developed algorithm used to reconstruct a quantitative T1 map. The dGEMRIC index map is then superimposed onto the scan acquired for TI=1650ms. A color-scale was used, representing the condition of the cartilage, ranging from degenerative (yellow) towards healthy (blue; low GAG content results in a low dGEMRIC index, and vice versa).

Statistical analysis

Changes in WOMAC, VAS Pain, radiographic JSW, and dGEMRIC signal (per side and region) were presented using mean with SD or median with interquartile range. WOMAC, VAS Pain, and JWS changes were evaluated (without correction for multiple testing) by paired-t-tests and differences in changes scores between KJD and HTO using independent-tests.

To account for clustering of dGEMRIC indices within the different regions analyzed, changes in dGEMRIC scores from baseline to follow-up, over all regions were analyzed using multilevel analysis (i.e. a linear mixed effects model) with a random intercept at region level. In this analysis the average change in dGEMRIC indices over time was estimated, as well as the effect of treatment and of side (medial or lateral) on this change. The association of change in dGEMRIC indices with change in WOMAC, change in JSW, and modification of these associations by side and by treatment was also evaluated with multilevel analysis and if relevant, based on size of regression coefficient of the interaction term and a $p<0.20$, subgroup analyses were performed.

All tests were two-sided, and a probability of $p<0.05$ was considered statistically significant unless specified otherwise. Statistical analyses were performed using SPSS (*Version 21.0. Armonk, NY: IBM Corp*).

Results

Patients

Three out of twenty KJD and two out of twenty HTO patients were lost-to-follow-up due to conversion to HTO (in case of KJD) or total knee arthroplasty (TKA; in case of HTO) within two years (figure 1). In addition, one KJD patient had severe motion artefacts in the dGEMRIC acquisition. As the HTO patients all have medial compartment OA, two KJD patients with predominantly lateral compartmental OA were excluded to allow for a proper comparison between groups. This resulted in a total of 14 KJD and 18 HTO patients analyzed, see figure 1. Baseline characteristics of these patients are given in table 1. There were no statistically significant differences in dGEMRIC indices at baseline between the KJD and the HTO patients.

Clinical and radiographic changes after HTO or KJD

One and two years after either treatment, a statistically significant increase in WOMAC and decrease in VAS-Pain compared to baseline was observed (figure 5). The one-year results of this sub-cohort are fully in line with the previously published one year results of the entire cohorts from both original RCTs.[22,33]

One year after KJD a statistically significant increase in medial, minimal, and mean JSW was found, this increase was still significant after two years. A statistically significant increase in medial and minimal JSW was found after one year in the HTO group, which also sustained

Table 1: Baseline characteristics.

	Extended imaging cohort			Total KJD cohort		Total HTO cohort	
	KJD (n = 14)	***HTO (n = 18)***	***p#***	***KJD (n=42)***	***p$***	***HTO (n=45)***	***p$***
Age *(years)*	54.14 (49.85-58.43)	48.94 (45.91-51.98)	**0.044**	53.14 (50.98-55.31)	0.662	49.58 (47.67-51.49)	0.715
Male gender *(n,%)*	9 (64%)	13 (72%)	0.644	25 (60%)	0.215	27 (60%)	**0.027**
BMI *(kg/m2)*	26.60 (24.46-28.74)	26.94 (25.52-28.36)	0.780	27.46 (26.34-28.59)	0.455	27.16 (6.18-28.15)	0.789
Index knee left side *(n, %)*	6 (43%)	9 (50%)	0.699	16 (50%)	0.350	20 (44%)	0.787
Kellgren-Lawrence grade	Median 3	Median 2.5		Median 3		Median 3	
Grade 0, *n (%)*	0 (0%)	0 (0%)		0 (0%)		1 (2%)	
Grade 1, *n (%)*	1 (7 %)	2 (11 %)	**0.039**	6 (14%)	0.486	5 (11%)	0.699
Grade 2, *n (%)*	1 (7 %)	6 (39 %)		5 (12%)		12 (27%)	
Grade 3, *n (%)*	8 (57 %)	8 (44 %)		19 (45%)		23 (51%)	
Grade 4, *n (%)*	4 (29 %)	1 (6 %)		12 (29%)		4 (9%)	
Tibiofemoral axis *(°)*	6.91 (4.50-9.33)	6.68 (5.33-8.03)	0.848	4.86 (3.26-6.45)	0.132	6.21 (5.53-6.89)	0.610
VAS Pain *(100-0)*	58.50 (45.40-71.60)	64.11 (55.79-72.43)	0.580	60.64 (53.78-67.51)	0.761	64.98 (59.47-70.49)	0.858
WOMAC *(0-100)*	49.19 (40.24-58.14)	49.42 (41.75-57.10)	0.966	51.78 (46.69-56.87)	0.599	52.28 (47.13-57.44)	0.525
Minimal overall JSW *(mm)*	0.23 (-0.16-0.62)	0.65 (0.08-1.22)	0.231	0.51 (0.22-0.80)	0.103	0.60 (0.29-0.90)	0.661
Mean overall JSW *(mm)*	4.80 (4.31-5.30)	4.73 (4.25-5.21)	0.943	4.70 (4.36-5.04)	0.929	4.69 (4.42-4.95)	0.756
Mean medial JSW *(mm)*	1.51 (0.53-2.49)	1.90 (1.27-2.54)	0.164	2.00 (1.32-2.69)	0.457	1.96 (1.58-2.33)	0.878
Mean lateral JSW *(mm)*	8.61 (7.91-9.31)	7.57 (6.82-8.31)	**0.044**	7.40 (6.72-8.09)	0.051	7.42 (7.00-7.83)	0.610

Data are presented as mean with 95% confidance intervals unless stated otherwise. # HTO and KJD patients' characteristics within the extended imaging cohort are compared. $ Demographics of the KJD and HTO patients from the extended imaging cohort are compared to their respective total cohorts. For difference in K&L grade between groups, Chi-square tests for trend are used. * $P < 0.05$ is statistically significant (outcomes in bold are statistically significant). As expected considering the original inclusion,[18] age, and baseline K&L score were statistically significant higher in the KJD group than in the HTO group. Also lateral JSW was higher for the KJD group, which was considered a coincidence. No other statistical differences were observed.

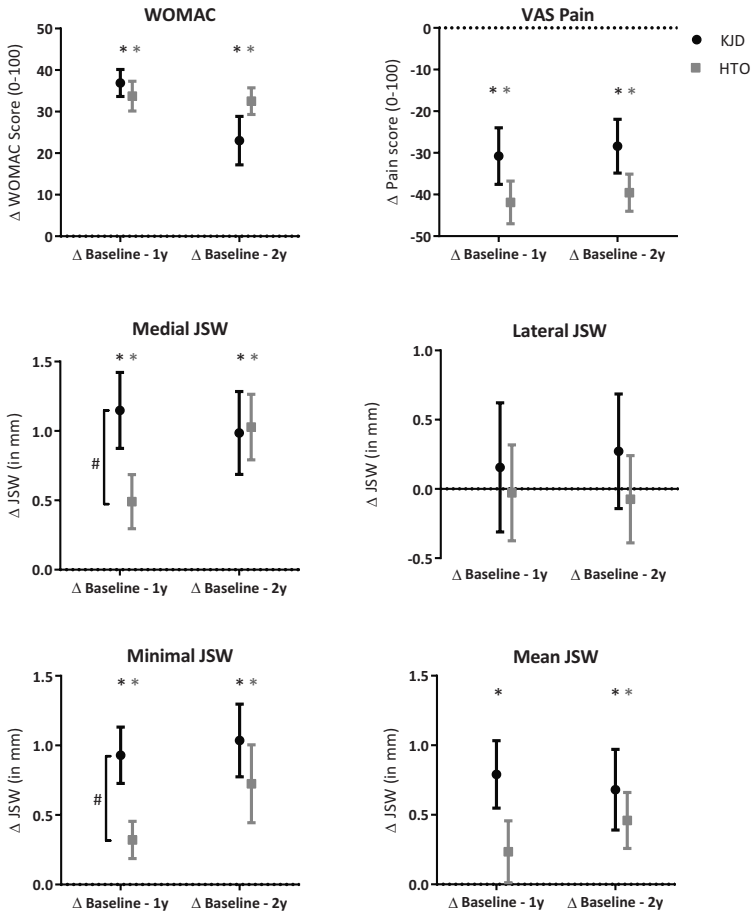


Figure 5: Change in WOMAC, VAS Pain, and medial / lateral / minimal / mean JSW, one year and two years after KJD or HTO. Visualized as mean change (± standard error of the mean) over 12 and 24 months, corrected for baseline. * Statistically significant ($p<0.05$) difference over time within treatment. # Statistically significant ($p<0.05$) difference in changes over time between treatments.

at two years. After two years, a statistically significant increase in mean JSW after HTO was observed, which was not present at one-year follow-up yet. JSW findings were substantiated by volumetric cartilage assessments of the delineated cartilage, ruling out biasing of JSW changes by an altered mechanical axis (data not shown).

Radiographic parameters did not change significantly between year 1 and 2 (figure 5 and supplementary table 1).

There was no statistically significant difference between both treatments with respect to the change in WOMAC, VAS-pain, and JSW parameters after two years. However, at one year after treatment these parameters were statistically significant different for medial JSW change (KJD:Δ1.28mm, HTO:Δ0.52mm, $p=0.049$), and minimal JSW change (KJD:Δ0.95mm,

Table 2.1: Average dGEMRIC indices (in ms) for the twelve ROIs, the medial and lateral compartments at baseline and after follow-up.

		Baseline	*1 year*	*2 year*	*Baseline–1y*	*Baseline-2y*	*1y–2y*
KJD	*aMF*	640,6 [561,5 to 719,6]	664,3 [595,4 to 733,3]	676,0 [603,3 to 748,7]	21,2 [-58,7 to 101,2]	29,9 [-47,3 to 107,2]	-11,9 [-98,3 to 74,4]
	aMT	586,6 [499 to 674,3]	649,7 [563,8 to 735,7]	606,6 [518,7 to 694,4]	63,1 [-45,3 to 171,5]	19,9 [-93,1 to 132,9]	-43,1 [-174,8 to 88,6]
	cMF	641,2 [571,9 to 710,5]	618,1 [546,6 to 689,5]	651,2 [581,1 to 721,4]	-15,6 [-106 to 74,8]	10,0 [-56,5 to 76,5]	18,0 [-86,7 to 122,7]
	cMT	565,4 [480,6 to 650,1]	602,2 [489,9 to 714,6]	611,6 [527,1 to 696,1]	-31,5 [-126,1 to 63,2]	41,3 [-43,8 to 126,4]	9,4 [-114,4 to 133,2]
	pMF	686,4 [635,3 to 737,4]	690,3 [638,3 to 742,2]	656,0 [603,5 to 708,5]	3,9 [-54,5 to 62,3]	-30,4 [-70,6 to 9,9]	-34,3 [-102,3 to 33,7]
	pMT	636,8 [548,0 to 725,5]	683,1 [605,2 to 760,9]	661,7 [568,4 to 755,0]	46,3 [-12,7 to 105,2]	24,9 [-56,8 to 106,7]	-21,4 [-110,6 to 67,9]
	Mean Medial	640,7 [594,3 to 687,2]	653,1 [602,9 to 703,3]	642,7 [597 to 688,5]	12,4 [-47,6 to 72,3] (1,9%)	2,0 [-47,0 to 51,0] (0,3%)	-10,4 [-83,9 to 63,2] (-1,6%)
	aLF	743,6 [663,6 to 823,5]	734,8 [664,5 to 805,2]	743,3 [665,8 to 820,8]	9,9 [-78,4 to 98,3]	-0,3 [-59,1 to 58,5]	-10,4 [-110,2 to 89,4]
	aLT	699,5 [621,0 to 778,0]	724,9 [631,4 to 818,3]	731,9 [622,0 to 841,7]	25,4 [-44,3 to 95]	32,4 [-26,3 to 91,0]	7,0 [-58,1 to 72,1]
	cLF	854,7 [725,9 to 983,5]	818,5 [719,1 to 918,0]	826,2 [693,7 to 958,7]	-12,9 [-81,6 to 55,8]	-36,5 [-113,6 to 40,5]	-26,7 [-56,9 to 3,6]
	cLT	754,4 [660,7 to 848,0]	752,4 [641,6 to 863,1]	733,2 [628,2 to 838,2]	-2,0 [-84,9 to 80,9]	-21,1 [-117,9 to 75,6]	-19,1 [-86,7 to 48,4]
	pLF	789,0 [721,4 to 856,6]	785,8 [715,4 to 856,1]	780,2 [712,1 to 848,3]	-3,2 [-71,1 to 64,7]	-12,2 [-78,1 to 53,6]	3,3 [-56,7 to 63,3]
	pLT	678,0 [610,5 to 745,5]	661,7 [587,7 to 735,7]	633,3 [567,5 to 699,0]	-16,3 [-67,7 to 35,1]	-44,7 [-118,4 to 29,0]	-28,4 [-81 to 24,1]
	Mean Lateral	763,4 [691,5 to 835,3]	754,4 [691,7 to 817,1]	754,4 [685,6 to 823,2]	-9,0 [-62 to 44] (-1,2%)	-9,0 [-61 to 43] (-1,2%)	0,0 [-40,7 to 40,7] (0,0%)

Table 2.2 continued.

		Baseline	*1 year*	*2 year*	*Baseline–1y*	*Baseline-2y*	*1y–2y*
HTO	*aMF*	640,6 [561,5 to 719,6]		676,0 [603,3 to 748,7]		29,9 [-47,3 to 107,2]	
	aMT	586,6 [499 to 674,3]		606,6 [518,7 to 694,4]		19,9 [-93,1 to 132,9]	
	cMF	641,2 [571,9 to 710,5]		651,2 [581,1 to 721,4]		10,0 [-56,5 to 76,5]	
	cMT	565,4 [480,6 to 650,1]		611,6 [527,1 to 696,1]		41,3 [-43,8 to 126,4]	
	pMF	686,4 [635,3 to 737,4]		656,0 [603,5 to 708,5]		-30,4 [-70,6 to 9,9]	
	pMT	636,8 [548,0 to 725,5]		661,7 [568,4 to 755,0]		24,9 [-56,8 to 106,7]	
	Mean Medial	640,7 [594,3 to 687,2]		642,7 [597 to 688,5]		2,0 [-47,0 to 51,0] (0,3%)	
	aLF	743,6 [663,6 to 823,5]		743,3 [665,8 to 820,8]		-0,3 [-59,1 to 58,5]	
	aLT	699,5 [621,0 to 778,0]		731,9 [622,0 to 841,7]		32,4 [-26,3 to 91,0]	
	cLF	854,7 [725,9 to 983,5]		826,2 [693,7 to 958,7]		-36,5 [-113,6 to 40,5]	
	cLT	754,4 [660,7 to 848,0]		733,2 [628,2 to 838,2]		-21,1 [-117,9 to 75,6]	
	pLF	789,0 [721,4 to 856,6]		780,2 [712,1 to 848,3]		-12,2 [-78,1 to 53,6]	
	pLT	678,0 [610,5 to 745,5]		633,3 [567,5 to 699,0]		-44,7 [-118,4 to 29,0]	
	Mean Lateral	763,4 [691,5 to 835,3]		754,4 [685,6 to 823,2]		-9,0 [-61 to 43] (-1,2%)	

The 12 ROIs are the anterior (a), central (c), and posterior (p) regions of the Lateral (L) or Medial (M) compartment of the Femur (F) and Tibia (T). Delta scores might deviate from the difference between time points due to missing dGEMRIC indices for specific ROIs at specific time points. Missing indices can e.g. be caused by cartilage being reduced to a volume so small, it is insufficient for dGEMRIC analysis.

HTO:Δ0.32mm, p=0.011); all in favour of KJD.

dGEMRIC evaluation

Inter-observer reproducibility of the segmentation process was evaluated by comparing the average dGEMRIC values of the ROIs (supplementary figure 1). The Inter-observer reproducibility was high (ICC=0.96), therefore dGEMRIC indices of both observers were averaged for all further analyses. Average absolute (and relative) changes in dGEMRIC values of the different medial and lateral compartments and subregions of the tibia and femur from baseline to one year and two-year follow-up are shown in table 2, and are generally small (on average 3.4%).

In the multilevel analysis the overall average dGEMRIC change over 2 years was non-significant (Δ-8.08; 95%-CI: -24.46 to 8.29, p=0.260). dGEMRIC changes were dependent on baseline dGEMRIC indices. Taking this into account a statistically significant effect for side was found and a possible effect of treatment was found. Table 3 shows the effect of treatment on change in dGEMRIC indices (corrected for the dGEMRIC baseline indices), for subgroups regarding side and treatment type.

Of both treatments, HTO was associated with a statistically significant reduction (cartilage worsening) in medial dGEMRIC indices (Δ-44.93, 95%-CI:-67.94 to -21.91) and increase (cartilage improvement) at the lateral side (Δ+26.36, 95%-CI:+2.71 to +50.03). For KJD, the changes over two years were not statistically significant (table 3). Relative changes compared to baseline were minimal[34] (HTO medial: -6.6% p<0.001 and lateral +3.3% p=0.023 and KJD medial: -3.2% and lateral +2.1%).

Association between change in radiographic and clinical parameters and change in dGEMRIC

Evaluating the association between change in JSW and change in dGEMRIC over two years, possible effect modification was also observed by side and treatment and thus results

Table 3: The effect of joint sparing treatments on dGEMRIC indices, linear mixed effects models

Parameter	*Estimate[$] (95%-CI: lower, upper)*	*P-value*
KJD medial	-23.07 (-49.52, 3.37)	0.087
KJD lateral	11.65 (-14.39, 37.70)	0.380
HTO medial	-44.93 (-67.94, -21.91)	**<0.001**
HTO lateral	26.36 (2.71, 50.03)	**0.029**

All models were controlled for baseline dGEMRIC indices. [$] Mean change in dGEMRIC indices per subgroup (as a result of treatment in a knee compartment).*dGEMRIC indices from baseline over all regions were analyzed using multilevel analysis (i.e. a linear mixed effects model), a random intercept at the region level was included to account for clustering of dGEMRIC indices within regions. The effect treatment (KJD or HTO), side of the knee (medial and lateral) on change in dGEMRIC indices were evaluated as fixed effect in the model. Change in dGEMRIC index was statistically significantly related to side (p<0.001), but not to treatment (p=0.8002), but the interaction term indicated that the effect of treatment may be modified by side (p=0.09). So effects per subgroup (HTO lateral / HTO medial / KJD lateral / KJD medial) were estimated in the model.

Table 4: The association of change in dGEMRIC indices with change in joint space width (JSW) and change in WOMAC evaluated using linear mixed effects models

	Parameter	*Estimate[$] (95%-CI: lower, upper)*	*P-value*
ΔdGEMRIC vs. ΔJSW*	*KJD medial*	0.49 (-23.04, 24.02)	0.968
	KJD lateral	0.01 (-18.40, 18.43)	0.999
	HTO medial	-14.84 (-41.39, 11.70)	0.276
	HTO lateral	25.73 (7.49, 43.96)	**0.007**
ΔdGEMRIC vs. ΔWOMAC#		1.59 (0.67, 2.51)	**<0.001**

All models were controlled for baseline dGEMRIC indices. [$]One unit of JSW / WOMAC change is related to this average change in dGEMRIC indices.[*]A statistically significant effect for side of the knee was found (p<0.001). Evaluating modification of the association between JSW change and dGEMRIC change by side in the regression model also indicated that effect modification may be present (regression coefficient: 14.62, p=0.20), thus all further analyses were stratified by side. Hereafter, modification of the association between JSW change with dGEMRIC change by treatment was evaluated (regression coefficient of -30.57, p=0.03), justifying additional stratification by treatment. [#]A statistically significant effect for side of the knee (p<0.001) and treatment (p<0.001) was found. Evidence for modification of the association between change in WOMAC and dGEMRIC change by side or by treatment was not found (WOMAC*side: p=0.71, and WOMAC*treatment: p=0.42), thus the group was not stratified for treatment and/or side.

were stratified by side and treatment (table 4). Only the positive association between the change in lateral JSW and change in lateral dGEMRIC indices in patients treated with HTO were observed; where one mm increase in JSW was associated with an increase of about 26 dGEMRIC ms (p=0.007, table 4). This effect was not found for the medial compartment and not found after KJD for either of the two compartments. For the association between change in WOMAC and change in dGEMRIC over two years no evidence for modification of the association by side or by treatment was found and thus results were applicable to the total group (KJD and HTO). Results indicate that one unit increase in WOMAC (clinical improvement) was associated with an increase (tissue structure improvement) in dGEMRIC indices of about 1.6 ms (p<0.0001, table 4).

Discussion

In these sub-cohorts, clear clinical improvement and radiographic cartilaginous tissue repair were found, without significant change in cartilage quality as determined by dGEMRIC at two years after KJD or HTO treatment. An increase in dGEMRIC signal, increase in cartilage GAG content, viz. quality improvement, seems to correlate with an increase in clinical benefit as determined by WOMAC.

For this study patients were included originating from two separate RCTs. There were differences in baseline characteristics (of inclusion criteria) for those two RCTs, which was reflected in the extended imaging cohort where a higher age and a more severe K&L grade

for KJD at baseline as compared to HTO was found. This can be explained by the fact that part of the included KJD patients (10 out of 20) were originally considered for TKA, and these patients generally suffer from more severe OA than patients considered for HTO. The current study might be underpowered to provide final conclusive answers due to the relative low numbers of patients included. Despite these limitations, it is the first data on comparing cartilage quality between these regenerative treatments.

One of the main reasons for patients to undergo treatment of an osteoarthritic knee is to alleviate pain and recover function. Even in this small study, both are achieved as seen in the clear decrease in VAS-Pain and increase in WOMAC scores, one year after treatment and maintained for another year, after either KJD or HTO. Interestingly, despite minor changes in dGEMRIC signal, for the overall group, change in WOMAC score was positively associated with a change in dGEMRIC indices, independent of side or of treatment, implying a clinically relevant correlation between increase in cartilage quality as determined by dGEMRIC and patients' experienced clinical benefit. The mechanism behind this inter-relation can only be speculated on.

After correction for baseline dGEMRIC indices over all ROIs, no statistically significant differences between HTO and KJD on change in dGEMRIC values were found. On average there is a decrease in medial and an increase in lateral dGEMRIC indices for HTO patients. This increase in GAG content at the lateral compartment after HTO and decrease at the medial compartment might be the result of wedging of the joint after HTO, resulting in a slight lateral compression and a slight medial decompression, and with that relative (apparent) change in GAG signal. This is supported by a study demonstrating the sensitivity of dGEMRIC values to cartilage compression and unloading.[35] Change in dGEMRIC indices are on average all quite small, representing relative small changes in cartilage quality over two years. The assumption of compression of the lateral compartment is however not supported by the observation that a significant relation between a decrease in lateral JSW and a decrease in cartilage quality (dGEMRIC indices) was found in specifically the lateral compartment of HTO patients. This positive association between change in JSW and change in dGEMRIC signal in specifically the lateral compartment indicates that in case of an increasing lateral joint space width, despite wedging of the whole joint, quality of cartilage (higher dGEMRIC score) improves in these cases, over two years. So, this might represent actual improvement of quality accompanying an increase in JSW. However, the fact that this is only found in the lateral compartment upon only HTO treatment and that absolute changes are small argues its relevance.

No statistically significant relation between structural change and dGEMRIC change in KJD patients was found. dGEMRIC values are expected to improve only if cartilage damage is at the early stage, whereas if the collagen structure is already compromised, a replenishment

of GAGs becomes more difficult, which could explain the statistically significant influence of baseline dGEMRIC values on the change over time. The lack of statistically significant or consistent change in dGEMRIC values for KJD, together with the clear increase in JSW, suggests that the tissue quality in KJD patients, on average, including the newly formed, is maintained. It might be argued whether this quality is sufficient, as baseline values are obtained from presumably impaired cartilage tissue in a severely damaged OA joint. Unfortunately, the dGEMRIC signal of the baseline condition of the treated joints was not compared with the contra-lateral healthy joint. Since dGEMRIC values are expected to decrease over time in damaged joints, although no data are available, the maintenance of cartilage quality over time could be considered a positive finding. KJD and HTO may have been useful in stopping further cartilage degeneration, indicated by minor or absent changes in dGEMRIC indices.

The question remains whether there is an increase in cartilage quality of the residual tissue with newly formed tissue of inferior quality, whether the new tissue is of similar quality as the residual unchanged tissue, or whether it is residual cartilage tissue that has decompressed and thereby showed an apparent decrease in quality (lower GAG content per volume).
It was subjectively observed that cartilage quality in the deeper layers (on to the bone) seemed to improve over two years (representative image shown in the supplementary figure 2). In the original MRI KJD studies it was demonstrated that newly formed tissue is largely filling up denuded bone areas, thus cartilaginous tissue is formed in the deep layers.[36] This is suggestive of newly formed quality tissue, filling in denuded bone area's but is far from conclusive.

With regards to the dGEMRIC imaging technique; a series of scans, acquired with different echo-times, is necessary to calculate dGEMRIC indices. Increased scanning times increase the risk of patient motion in-between sequences (repositioning), potentially decreasing the efficacy of the fitting. Repositioning effects in our study were minimized by implementing image registration.[37] Longitudinal evaluation of cartilage repair, such as represented in this explorative study, assume equal distribution of gadolinium within the joint. Although our contrast protocol is very strict, variations are inevitable, amongst others because of heterogeneous uptake of gadolinium in repair tissue over time, influenced not only by GAG content but also patient motion, water content, and permeability of tissue.[38,39] Note that it takes also quite some time for the contrast to distribute throughout the body. This variation may add to the inability to detect small changes over time.
GAG concentration is, given its substantial contribution to load-bearing, a good measure to distinguish healthy from degenerated tissue.[23] However, studies have shown that some results cannot be explained by GAG measurements alone, but might be found in a combination of several quantitative MRI techniques, morphological, and clinical evaluation.[23,40] dGEMRIC is considered a valuable tool in evaluating cartilage quality, but there are also alternative

MRI techniques available to assess cartilage quality, such as sodium MRI, T1 rho, and T2 mapping.[23]

All limitations of dGEMRIC imaging considered in general and in this specific small size study, implementation of a strict contrast administration protocol, minimized patient motion during acquisition, post-processing image registration, and minimal variation between observers should be sufficient to consider dGEMRIC indices as representative for cartilage quality with respect to GAG content/distribution in this study. Assuming this, despite the limited number of patients, it might be concluded that cartilaginous repair upon HTO and KJD is not accompanied by further decrease in GAG content. Future studies powered to elucidate differences between HTO and KJD treatment on dGEMRIC indices should be performed to support current findings, and provide conclusive answers.

Summarizing, the significant clinical benefit and increase in radiographic JSW one year after treatment of medial compartmental OA by either HTO or KJD, maintains throughout the second year of follow-up, postponing the natural OA progression rate and with that knee arthroplasty. There seems to be a clinically relevant relation between the increase in cartilage quality as determined by dGEMRIC and patients' experienced clinical benefit determined by WOMAC. Assuming natural deterioration of the cartilage tissue seen in osteoarthritis patients, is reflected in loss of GAG and therefore also applies to a decrease in dGEMRIC indices, KJD and HTO may contribute to regeneration of cartilaginous tissue with maintenance of cartilage quality, and thereby delaying the degeneration process.

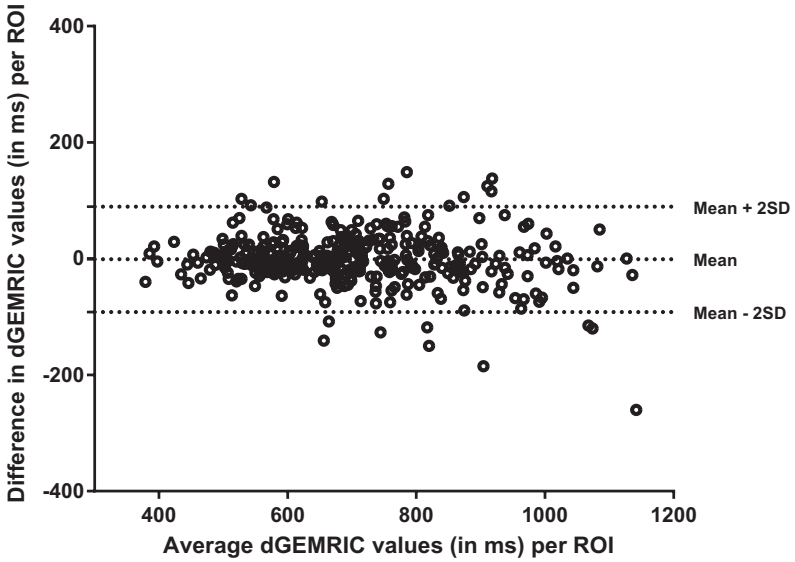
Supplementary information

Knee joint distraction

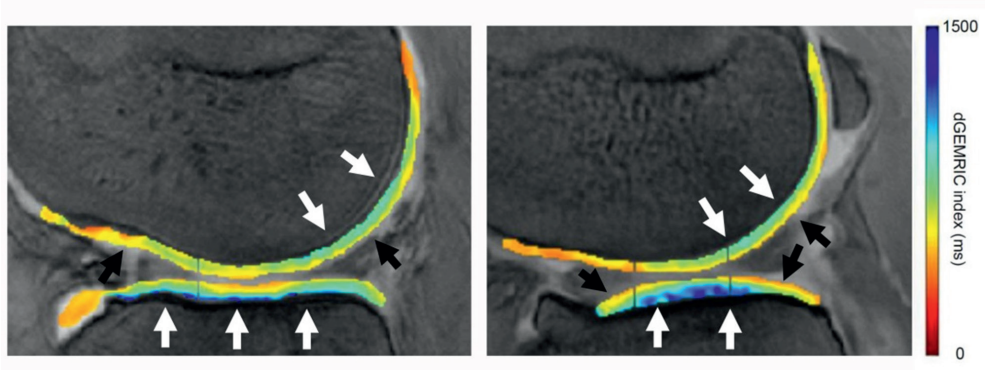
Knee joint distraction was performed by use of an external distraction device. On both the medial and lateral side of the femur and the tibia, two half-pins were placed, to be attached to an external fixation device (see figure 2). Intra-operatively 2 mm distraction was applied, and post-operatively 1 mm per day until 5 mm was reached. At day four a weight-bearing radiograph was taken, ensuring 5 mm distraction was applied with respect to the pre-operative weight-bearing radiograph. The patient was then discharged and full weight-bearing was encouraged. Three weeks after the operation, the pintracts were checked for infections and distraction distance was assessed on weight-bearing radiographs. Six weeks after the operation the distraction distance was checked for the last time prior to removal of the frame under general/spinal anesthesia. While under anesthesia, the knee was brought into flexion, stretching the fibrotic scar tissue around the pintracts. Patients were advised to gradually increase weight-bearing of the joint with 10 to 15 kg per week and physiotherapy on demand. Prophylactic low molecular weight heparine was given for nine weeks, during the distraction period and the three weeks thereafter.

High tibial osteotomy (medial opening wedge)

Medial opening wedge osteotomy aims at offloading the affected medial compartment of the knee by displacing the mechanical axis to the unaffected lateral compartment of the knee (from varus to valgus). Using weight-bearing radiographs, the method of Miniaci was utilized to determine the optimal degree of correction [19]. When performing the osteotomy the lateral cortex was left intact and the tibia was fixed with a plate. Patients were hospitalized for a maximum of three days before being discharged, followed by six weeks of limited (max 15 kg), weight-bearing. Knee flexion and extension was unrestricted. Prophylactic low molecular weight heparin was given until six weeks after the HTO. Six weeks after the HTO, stability was evaluated, and radiographs were taken to assess consolidation, if deemed sufficient, full weight-bearing was allowed and physiotherapy was recommended. At eighteen months the plate was removed to enable MR imaging.



Supplementary figure 1: Bland-Altman plot for the average dGEMRIC index of the ROIs; manually delineated by two independent, blinded, observers in corresponding slices.



Supplementary figure 2: dGEMRIC images of the medial tibiofemoral joint two years after HTO (left) and KJD (right). In the increased joint space old and newly formed cartilage is impossible to distinguish, however, in these representative images it is especially evident that the deeper cartilage layer (white arrows) has higher dGEMRIC indices than the superficial layer (black arrows).

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CHAPTER 9

Summary

General discussion

Summary

Over the past decades, rheumatoid arthritis (RA) has transformed from a debilitating chronic disorder into a much better manageable disease thanks to increased understanding of etiologic factors and the availability of a spectrum of disease-modifying drugs.[1] The current state-of-the-art treatment of RA includes early and intensive intervention.[2] In contrast, treatment of osteoarthritis (OA) is necessarily limited to conservative treatment. Eventually, surgery is to be considered if conservative therapy does not suffice anymore. A number of clinical trials support the hypothesis that the OA pathogenesis in some patient categories might involve a substantial inflammatory component.[3] It is hypothesized that disease-modifying anti-inflammatory treatment might be effective in these specific patients.[3,4]

As in RA, early detection of synovitis in hand joints of patients with (erosive) hand OA, and consecutively early therapeutic intervention might be important to retard or prevent long-term joint damage and function loss. In OA patients without synovitis, joint degeneration might be a more cartilage and bone driven process, probably initiated and perpetuated by biomechanical factors. In KJD, a temporary biomechanical intervention seems to provide an intra-articular environment for joint repair.[5] The initial boost of cartilaginous tissue regeneration potentially provides long-term structural benefit, and is able to prevent or delay the need for joint replacement.[6]

The aim of this thesis was to identify and validate tools for non-invasive assessment of tissue characteristics, in patients with RA or OA. These would be of use to better monitor disease activity, disease progression, and treatment effects. The aspects and imaging modalities as studied in this thesis are summarized below, followed by a general discussion and future perspectives.

Part I: Monitoring inflammation in hand osteoarthritis and rheumatoid arthritis
The first part of this thesis described the validation and application of optical spectral transmission (OST) as an imaging technique to assess synovitis. The first two chapters showed that OST might facilitate tight-control and treat-to-target treatment of RA. In the next two chapters the potential relevance of synovitis in OA was discussed and a first OST model for imaging synovitis in (inflammatory) hand OA was presented.

Chapter 2 showed that a new OST model might be suitable as a disease activity monitoring tool in RA patients with moderate disease activity. Implementation of a novel light source yielded significant improvement over a previous OST model, and was found to be a reliable tool for assessing hand and wrist synovitis. OST correlated better to ultrasound (US) than the 28-joint disease activity score (DAS28). Especially osteophytes appeared to affect performance of OST.

Chapter 3 described the protocol of a randomized clinical trial, in which a specifically designed software application created the opportunity to compare conventional DAS guided and novel OST guided DMARD treatment of early RA, in a double-blind and safe, yet patient-tailored, tight-control setting. The trial will evaluate clinical efficacy and cost-effectiveness of both treatment strategies. The specifically designed decision-making software application can also be used in other randomized controlled trials (RCTs) testing novel methods of guiding the tight-control treatment in RA.

In **chapter 4** the role of inflammation in OA was discussed extensively, with special interest in the synovium and capsule. Chronic as well as recurrent low-grade synovial inflammation definitely contributes to progression and symptoms of particular OA patient categories. Low-grade inflammation may even initiate the disease. This may provide a rationale for the use of anti-inflammatory drugs to modify the disease course. It might, on the other hand, suggest that in addition to the inflammatory component perpetuating the disease, a degenerative biomechanically driven component is able to drive the disease independently of inflammation.

Chapter 5 showed a new OST model that performs well in the assessment of synovitis in the proximal interphalangeal (PIP) joints of OA patients, but less so in wrist and distal interphalangeal (DIP) joints. Reliability of OST measurements was high. Possibly, optimal development of the OA OST model was hampered by disease characteristics in the patient population: the prevalence and severity of synovitis in hand and wrist joints were low.

Part II: Monitoring tissue damage and repair in knee osteoarthritis
The second part of this thesis showed that knee joint distraction (KJD) was a valuable option for joint-sparing treatment of knee OA patients. The first chapter showed how clinical and radiographic changes after KJD related to high tibial osteotomy (HTO) and total knee arthroplasty (TKA). The following chapters demonstrated how imaging techniques can help to characterize the regenerative process that is initiated by KJD and HTO.

In **chapter 6**, results from the combination of two RCTs (KJD vs HTO and KJD vs TKA) provide evidence of sustained structural benefit in addition to clinical benefit up to two years after KJD, HTO, and TKA. Clinical results of KJD were slightly inferior to TKA, however, patients report the importance of retaining their own knee. Cartilage repair as demonstrated by radiographic joint space widening was more pronounced in KJD than in HTO. Net synthesis of collagen type II was increased two years after KJD.

The efficacy of HTO relies on the successful shift of the mechanical axis from the most affected tibiofemoral compartment towards the contralateral compartment. **Chapter 7** showed that KJD, not necessarily directed at changing axial knee alignment, also improves

axial alignment in the successful treatment of medial compartment knee OA. Improvement of axial alignment by KJD seemed to be caused by the increased medial compartment joint space, as seen on weight-bearing radiographs, i.e. an increase in cartilaginous tissue thickness. A more neutral axial alignment before surgery and more change in axial alignment after surgery were each associated with more reduction in pain over one year.

Articular cartilage quality will decrease in the course of OA. However, disease-modifying treatment might be able to maintain or restore cartilage quality. **Chapter 8** described the maintenance in delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) values after KJD, and the minimal decrease in dGEMRIC values after HTO, in the treatment of medial compartment knee OA. HTO and KJD showed similar effects on dGEMRIC values over time. The change in dGEMRIC values and the observed increase in radiographic joint space width (JSW) suggested that KJD and HTO were able to modify the natural course of OA. There was a clinically relevant relation between the increase in cartilage quality as determined by dGEMRIC and patients' experienced clinical benefit determined by WOMAC.

General discussion

Part I: Monitoring inflammation in hand osteoarthritis and rheumatoid arthritis
Nowadays, treat-to-target and tight-control treatment strategies are known to be both clinically effective and cost-effective in the treatment of RA.[1,7] Various definitions of low disease activity have served as treatment targets over time. Older (based on DAS28) or newer (American College of Rheumatology/European League Against Rheumatism: ACR/EULAR remission criteria) definitions of remission all have in common that they include at least tender and swollen joint counts together with an acute phase reactant.[2,8] There are however, shortcomings to these definitions. Firstly, they represent global disease activity, generally only validated at group level.[9] Secondly, even subclinical inflammation is undesirable. The only component able to potentially reflect such subclinical activity is the acute phase reactant. In OA, there is growing evidence for the involvement of an inflammatory component. Synovitis can be very hard to assess and quantify clinically, hampering identification and treatment of potential inflammatory OA subtypes.

Therefore, early, accurate, and objective methods for assessing synovitis in these rheumatic diseases are warranted, and multiple imaging methods are available to assist in this, see table 1.

Over the past few years, Optical Spectral Transmission (OST) has been implemented as an imaging technique to assess hand synovitis. The potential of OST for measuring synovitis in PIP joints of RA patients was first published in 2012.[16] Major revisions to the OST device had to be made to also be able to assess other hand joints, eventually resulting in the

Table 1: Imaging methods to assess inflammation.

Modality	*Pros*	*Cons*
Ultrasound (US)[9, 10]	- More sensitive and accurate than clinical examination - Readily available - Additional data on bone erosions, synovitis, and tendinitis	- Scanning multiple joints is time-consuming - Relatively expensive - Performance depends on the physician's skills
Magnetic resonance imaging (MRI)[9,10]	- More sensitive and accurate than clinical examination - Readily available - Additional data on bone erosions, synovitis, and tendinitis - 3 dimensional (3D) images	- Expensive - Subjective assessment of radiographs
Nuclear imaging techniques (scintigraphy, positron emission tomography (PET), single-photon emission computed tomography	- 3 dimensional (3D) images - Sensitive for inflammation	- Radioactive tracers - Quite expensive
Optical imaging techniques		
Bioluminescence[11]	- Visualization of biological processes in vivo	- Administration of foreign enzymes
Fluorescence[12]	- Potential for molecular imaging of the complete hands	- Administration of a fluorescent agent.
Near-infrared imaging		
The Lightscan[13]	- More sensitive than clinical examination	- Only evaluated in PIP joints - Scans one joint at a time - Development and valorisation lag behind to that of the HandScan
Indocyanine green (ICG)-based fluorescence optical imaging (FOI)[14]	- Capable of detecting inflammatory arthritis in humans (both RA and OA)	- Administration of a fluorescent dye - Blood screening

HandScan. OST (HandScan) proved its worth for sensitive assessment of synovitis in RA patients with low to moderate disease activity. Its performance was non-inferior to the reference standard, ultrasonography.[17–19] OST also outperformed US in assessing subclinical inflammation defined by MRI.[17] In 2015, the HandScan obtained CE certification.

The simple user-friendly interface, mobility of the device, automatic region-of-interest-placement, and automatic generation of optical scores at both patient level and joint level, make the device easy to use. OST assessment of synovitis is not limited by age, gender,

or skin colour and not all joints have to be available for analysis. Hands with significant deformations cannot be assessed properly though. As such, OST has surplus value as compared to currently available imaging methods.

The work done in this thesis started by replacing the light-emitting-diode (LED) light-source, that was implemented in previous OST studies, and was found insufficiently powerful in assessing wrist synovitis. Higher penetrability and a more patient-tailored approach was possible by changing from a LED light to a scanning laser light source, and the resulting increased performance was shown in **chapter 2**. Hitherto, the influence of disease-related (patho-)physiological findings on the assessment of synovitis by OST was unknown, but necessary for determining clinical applicability of OST. Bone and tendon pathology increase the risk of misclassification, but considering the prevalence of these pathological findings, only osteophytes are clinically relevant.
OST assessment of synovitis in hands over time is optimized by standardisation through software recording previous joint positioning with respect to hand contours, automatic region-of-interest (ROI) placement, a wedge and plate to standardize hand position, and objective and automated generation of optical scores. Altogether, OST might be a promising candidate for predicting treatment efficacy. Three new OST studies are currently underway. In two of these studies, the assessment of disease activity and the prediction of treatment response by OST is compared with the DAS28, in either dMARD-naïve patients or patients treated with a first biological dMARD. The third study, the RCT evaluating the efficacy and cost-effectiveness of OST-guided treatment described in **chapter 3**, has started in April 2017. The results of these studies will help decide whether OST assessment of synovitis could direct DMARD treatment on its own or could have a supplementary role. The first ever OST OA model showed fair performance for assessing synovitis in PIP joints in inflammatory hand OA. Further research is warranted to improve its performance, especially in the more frequently affected DIP joints. In hand OA, however, the high prevalence of osteophytes might be more important than in RA, as these are known to be a potential confounding factor. Despite the difference in performance of OST in RA and hand OA, reproducibility appeared to be excellent in both.
The continuous development, validation, CE certification, and relatively low costs of the HandScan have led to international publicity and business (Hemics BV), making it a potentially successful product.

In the future, the objective measurements of the HandScan can help define subpopulations of hand OA patients with synovitis and compare these between centres. The HandScan is currently being used in multiple centres in the Netherlands, Norway, Germany, and Belgium. The metatarsophalangeal (MTP) joints are also frequently affected in the course of RA,[20] and are among the most difficult to assess for synovitis clinically. The limited penetrability of laser light used in the HandScan (660 and 808 nm) is sufficient for assessing synovitis in

the MTP joints. A 'foot scanner' as a plugin device for the available HandScan is currently under development.

(Relatively) expensive and time-consuming imaging techniques like MRI and US are impractical for tight-control treatment strategies. Yet, MRI and US are the reference standard of choice in clinical trials for assessing synovitis. US and MRI, but also imaging techniques like fluorescence imaging, and OST each quantify and score different aspects of synovitis. Therefore, head-to-head comparison of these techniques in patient populations with a variety of synovitis severity in different joints would be of relevance. These populations could also be patients with other inflammatory diseases affecting the hand and wrist joint, such as psoriatic arthritis, systemic lupus erythematosus, and juvenile idiopathic arthritis.

OST measures of synovitis cannot be expected to ever perfectly resemble US measures of synovitis, owing to the differences in technique, scoring, and operator dependency. In this thesis, US has served as the reference standard in assessing hand and wrist synovitis, and discrepancies between OST and US scores are seen as a shortcoming of OST. However, this might actually be inappropriate, as previous research showed higher correlations between OST measures of subclinical synovitis with MRI than with US.[17] This raises the question whether US is the ideal reference standard for assessing (subclinical) synovitis. Therefore, future studies should include MRI, at least for a subpopulation of patients with low disease activity. Both US and MRI have the advantage over OST that they allow assessment of other articular and periarticular tissues as well, e.g. cartilage, bone, tendons and muscles. Therefore, optical imaging tools will rather complement than replace current imaging modalities used in RA and OA.[13]

The OST models for RA and OA are both developed and validated within, for each, the same cohort, and US as a reference standard. Future research must include an external validation cohort for each of these OST models. Additionally, these models should be evaluated in healthy subjects without synovitis.

In clinical practice, treatment decisions depend on patient preferences, physician expertise and preferences, and the tools used for assessing disease activity. Rheumatologists should be aware that treatment decisions made with tools capable of assessing subclinical inflammation, might contradict their personal preference or the disease activity as reported by patients. While subclinical inflammation might explain structural deterioration in early RA patients in clinical remission,[21] guiding treatment in early RA patients using sensitive imaging tools (e.g. an US tight-control strategy[22]) have not (yet) been proven to result in more clinical improvement as compared to conventional tight-control strategy.

Summarizing, the improved OST RA model led to an increased performance in assessing synovitis in the hands of RA patients. External validation of this model, and the results of the ongoing RA studies will elucidate whether OST is a tool to sensitively assess synovitis, to predict treatment response, or to guide tight-control treatment. OST in OA seems promising, but the performance needs to be improved before considering clinical applicability.

Part II: Monitoring tissue damage and repair in knee osteoarthritis

Nowadays, potential disease-modifying OA treatments and drugs are under investigation or offered to selected patient populations in the treatment of knee OA.[23] Some of these aim to not only be analgesic, but simultaneously be chondroprotective and anti-inflammatory, such as the interleukin-4 and interleukin-10 (IL4-10) fusion protein.[24,25] In order for such developments to become successful, it is essential to have sensitive, objective, and accurate tools to evaluate effectiveness. Clinical outcomes are important, but are subjective and correlate poorly with disease activity.[26] Currently used radiographic monitoring of cartilage is indirect, not sensitive enough to capture subtle changes, and does not provide information on cartilage quality.[27]

Currently, established imaging techniques to assess the quality of knee cartilage in clinical trials are lacking, although guidelines for OA research do recommend implementing imaging biomarkers in intervention trials.[28] Articular cartilage tissue changes are the main outcome in evaluating disease-modifying Osteoarthritis drug (DMOAD) treatment,[28] in which changes in cartilage morphology play a crucial role.

Histological assessment of GAG and collagen content after joint sparing treatment is infeasible, but is set as the gold standard for validating imaging techniques that quantify cartilage composition. One study by *van Tiel et al.* compared two MRI techniques (dGEMRIC and T1ρ Mapping), quantifying cartilage biochemical composition in knee OA patients prior to TKA, to cartilage histology in post-operatively harvested cartilage.[29] They concluded that only dGEMRIC is a robust method for quantifying GAG content, and both imaging techniques showed a weak correlation with collagen content. Development and validation of other imaging techniques capable of (indirectly) assessing cartilage composition and characteristics is ongoing. For GAG content in particular, T1rho-mapping, sodium imaging, and GAG specific chemical exchange saturation transfer (gagCEST) are novel alternatives to dGEMRIC.[30] Still, dGEMRIC remains indispensable, being referred to as the reference technique for developing new imaging techniques for cartilage quality assessment.[31] A head-to-head comparison of different imaging techniques assessing cartilage in vivo, biochemical markers (preferably taken from the joint[32]) and in vitro reference standards (preferably within the same patient) will show what the best technique is for assessing cartilage quality.

Bone and synovium are also subject to changes from OA (**chapter 4**) and are generally assumed to be primarily responsible for pain.[33] DMOAD treatment should not only be chondroprotective, but also be analgesic, and ideally be anti-inflammatory and capable of reversing degenerative bone changes (e.g. bone marrow lesions). Imaging techniques capable of simultaneously assessing these tissues, might be more representative in evaluating joint-sparing treatment.

The predominant pathologic processes that affect joint tissue, in the course of OA, are known to not (necessarily) occur simultaneously. Imaging techniques visualizing changes in tissue (characteristics) should be applied accordingly.

In early stage OA, proteoglycans are lost from the articular cartilage (quantifiable by: *dGEMRIC*), collagen fibres change in size and orientation (*T2-mapping*), without macroscopic alterations in cartilage morphology (lack of structural changes: *radiographs, CT, and MRI*), but with initiation of synovitis (*US, MRI; and for the hand OST*) and increased inflammatory biochemical markers. Further progression of the disease causes cartilage loss and enhanced inflammation (*x-ray, CT, OST, and MRI*) which leads to pain and swelling of the joint. In late OA, the collagen matrix is severely compromised, with so much thinning and defects,[34] that quantitative MRI analysis of cartilage becomes increasingly more difficult. Regenerative processes of joint tissue from joint-sparing treatment probably happen unsynchronized as well. Knee joint distraction (KJD) temporarily creates a beneficial intra-articular environment, in which short-term changes in cartilage morphology probably precede structural and biomechanical changes, considered necessary to maintain long(er) term clinical improvement.

Work from this thesis brings the field a step closer to understanding the changes in tissue characteristics in the regenerative process after KJD and HTO, and also pinpoints the missing information that novel imaging techniques might provide in future studies.
The chapters of **part II** of this thesis substantiated previous findings of clinical and structural benefit of KJD as an alternative to HTO in the treatment of medial tibiofemoral knee OA.[6,35–37] Structural changes are primarily assessed on weight-bearing, semi-flexed radiographs, currently still the gold standard,[38] and quantified using joint space width (JSW). The evident increase in medial JSW (**chapters 6-8**), is believed to be the result of increased cartilage thickness. As knee radiographs are typically obtained under weight-bearing conditions, the JSW increase, probably reflects an increase in cartilage volume as well as sufficient resilience to bear the weight. These changes are to the extent that they lead to a change in axial alignment, under those weight-bearing conditions (**chapter 7**). Previous studies on the effect of KJD have demonstrated that the increased JSW on radiographs is also evident in cartilage volume on MRI.[39] The lack of correlation between JSW on weight-bearing radiographs and 3D-volumetric cartilage assessment on non-weight-bearing MRI or (indirectly by) non-weight-bearing CT, is a well-known problem.[40] To partially explain the lack of a correlation between the different imaging techniques to quantify the JSW, also with respect to the influence of weight-bearing, a study is designed to perform weight-bearing MRIs using a tiltable MRI table.
KJD is also known to effect other tissues in the knee, including bone.[39] Bone (shape) changes probably play an important role in redistribution of joint loading and could thereby contribute in alleviating pain. Imaging tissue characteristics after KJD in the future will include, bone shape analysis on MRI[41] (data acquired), changes in 3D joint space distribution and bone density on CT (data acquired).
KJD undoubtedly has an effect on the composition of the cartilage in the knee. GAG content, as reflected by dGEMRIC values, remained equal after treatment (**chapter 8**). This suggests

that the cartilaginous tissue, which has increased in thickness, is replenished with GAGs. Whether this is still far from normalisation of GAG content remains to be established as healthy controls were not available. Net collagen type II synthesis (expressed using serum PIIANP and urinary CTX-II: **chapter 6**) is decreased directly after KJD treatment and thereafter increases over time up till 2 years. T2-mapping (data acquired) quantifies collagen content, and has been shown capable of differentiating hyaline cartilage from fibrocartilage. These collagen markers combined (imaging and net collagen type II synthesis) should provide credible proof of changes in collagen content in cartilage over time. Taking into account the findings from previous studies and data presented in this thesis, as well as results from other cartilage repair surgeries,[42,43] it seems that KJD might indeed cause regeneration of functional cartilaginous tissue.

Future studies of potential disease-modifying interventions can benefit from the findings in this thesis. Imaging results from any joint-sparing treatment should ideally be compared to a control OA population of similar age, gender and severity of knee OA (Kellgren and Lawrence grade). Observational studies, like the OsteoArthritis Initiative (OAI), longitudinally collect clinical data, biological samples, and joint images of knee OA patients at risk of progression and patient with knee pain at risk of developing knee OA.[44] Unfortunately these data were not available for comparison at the time of analysing the dGEMRIC data (**chapter 8**).They were also not used for the radiographic evaluation of the data of the **chapters 6-7**, but were available for the 5 year follow-up of KJD in an open prospective cohort study.[6]
A common practice in OA trial research is that imaging outcomes are only determined in particular subpopulations (as was done in **chapter 6** and **8**) or post-hoc analyses (**chapter 7**). This might lead to underpowered studies[45] and/or introduce bias. However, financial aspects, the current lack of effective DMOADs, and delayed implementation of novel techniques in clinical practice, make it hard to include qualitative imaging methods on a large scale in RCTs.
Future research should also focus on the optimal timing for the assessment of tissue changes by quantitative imaging techniques. This will help optimize study designs, increase chances of finding meaningful relations between clinical and structural outcomes, and predict long-term clinical response.

Summarizing, the structural changes seen on weight-bearing radiographs after KJD, suggest resilient cartilage capable of improving axial alignment. dGEMRIC imaging, used to quantify cartilage composition, demonstrated maintenance of GAG content in the increased medial joint space after KJD. T2 mapping will provide information on collagen distribution and be combined with biomarkers to further investigate the nature of the cartilaginous tissue formed after KJD and HTO.

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ADDENDUM

Nederlandse samenvatting

Dankwoord

List of publications

Curriculum vitae

Nederlandse samenvatting

Het houdings- en bewegingsapparaat bestaat uit botten, kraakbeen, spieren, pezen en ligamenten. Het maakt lichaamsbeweging mogelijk en zorgt tegelijkertijd voor de vorm, stevigheid en stabiliteit van het lichaam. Een gewricht verbindt twee (of meer) botten als scharnier. Het meest bewegelijke en meest voorkomende soort gewricht in het menselijk lichaam is het synoviale gewricht. Kenmerkend voor dit soort gewricht is het gewrichtskapsel, dat bestaat uit sterk bindweefsel, dat om de gewrichtsholte ligt en aan de binnenkant bekleed is met een speciaal slijmvlies, het synovium. Dit produceert vloeistof, het synoviale vocht, dat zorgt voor "smering" en "voeding" van het gewricht. De boteinden in het synoviale gewricht zijn bekleed met kraakbeen. Dit zorgt voor een glad oppervlak, waardoor de botten zonder veel weerstand kunnen scharnieren. Bij gezonde volwassenen bevat kraakbeen geen zenuwen of bloedvaten; kraakbeen is daardoor afhankelijk van het synoviale vocht voor bouwstoffen. Afwezigheid van zenuwen betekent dat kraakbeen zelf geen pijn kan doen. Bot, daarentegen, is zeer goed doorbloed en bevat veel (pijn)zenuwen. Bot dient als geraamte voor aanhechting van pezen, maar bevat ook een voorraad van mineralen zoals calcium. Daarnaast bevat het beenmerg, dat bloedcellen produceert. Bot wordt continu afgebroken en aangemaakt, aangepast aan trek-, druk- en schuifkrachten die ontstaan door beweging en belasting. Onder normale omstandigheden is er een evenwicht (homeostase) tussen afbraak en aanmaak van kraakbeencellen en andere cellen in het gewricht. Om gewrichten gezond te houden, zijn beweging en belasting essentieel, maar (langdurig) verkeerde belasting of relatieve overbelasting bij gewrichtsaandoeningen, zoals ontsteking van het gewricht, kan leiden tot onherstelbare gewichtsschade. Er zijn aandoeningen die het gewricht beschadigen, zoals artrose (in de volksmond ook wel "gewrichtsslijtage" genoemd) en reumatoïde artritis (RA).

Reumatoïde artritis

RA is een relatief veel voorkomende, chronische auto-immuunziekte, gekenmerkt door gewrichtsontstekingen en algemene verschijnselen van ontsteking, zoals verhoogde ontstekingswaarden in het bloed en ochtendstijfheid. In de afgelopen tientallen jaren is RA veranderd van een invaliderende chronische ziekte, doordat er veel gewrichtsbeschadiging ontstond, in een beter behandelbare ziekte. Dit komt doordat er in de beginfase van de ziekte effectiever behandeld wordt, met effectieve geneesmiddelen (“*disease modifying antirheumatic drugs”*: DMARD's). Vooral in het beginstadium van RA wordt de patient frequent beoordeeld en wordt de medicatie zo nodig aangepast; we spreken van "*patient-tailored* (op de individuele patiënt toegesneden) *tight-control"*. Het doel van de behandeling is volledige afwezigheid van ziekteactiviteit ("*remissie*"); werken naar dit behandelingsdoel wordt "*treat-to-target"* genoemd. Met de verscheidenheid aan beschikbare medicatie is er, tot op zekere hoogte, gepersonaliseerde behandeling van de individuele patiënt mogelijk en zijn remissie en het voorkómen van gewrichtsschade haalbare doelen van behandeling geworden.

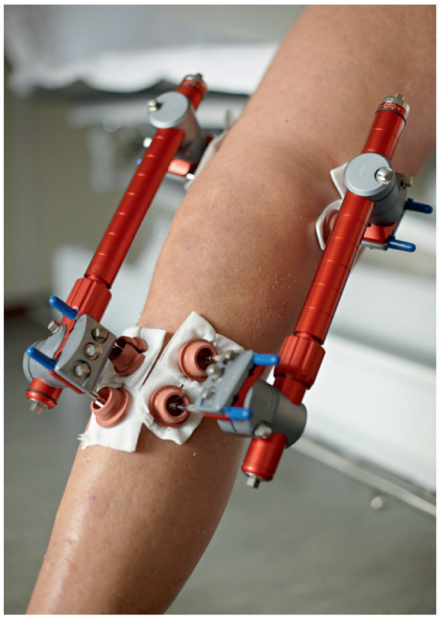
Het blijft echter een uitdaging om de behandeling van RA verder te verbeteren, waarbij overbehandeling, bijwerkingen en hoge kosten worden vermeden.

Artrose

Artrose is een aandoening van vooral het kraakbeen in gewrichten. Artrose komt bij oudere mensen vaak voor en treft voornamelijk, maar zeker niet alleen, lichaamsgewicht-dragende gewrichten (zoals knie, heup en gewrichten van de wervelkolom). Artrose is de meest voorkomende chronische gewrichtsaandoening wereldwijd en treft alleen al in Nederland 1,1 miljoen mensen. Meer mensen zullen de komende tientallen jaren artrose krijgen, als gevolg van vergrijzing, de langere levensverwachting en door toenemend overgewicht van de bevolking, want overgewicht is een risicofactor voor artrose.

Tot op heden is er geen behandeling die artrose kan tegenhouden. De behandeling is allereerst gericht op voorlichting (leefregels, hoe met artrose om te gaan), pijnstilling met pijnstillers en het voorkómen van functionele beperkingen, met oefeningen en fysiotherapie. Als dit ontoereikend is, kan een operatie met het plaatsen van een kunstgewricht worden overwogen. Het ontbreken van een effectieve behandeling voor artrose kan verschillende oorzaken hebben. Eén verklaring is dat artrose verschillende oorzaken kan hebben, die elk verschillende behandelmethoden vereisen. Artrosepatiënten met ontstekingsverschijnselen van gewrichten zouden bijvoorbeeld vooral baat kunnen hebben van ontstekingsremmende geneesmiddelen. Patiënten met artrose zonder ontstekingsverschijnselen, door bijvoorbeeld overbelasting, zouden vooral baat kunnen hebben van behandelingen die de mechanische belasting veranderen. Voortbordurend op het idee van verandering van belasting van het artrotische gewricht is een nieuwe therapie ontworpen in het UMC Utrecht: gewrichtsdistractie, toegepast voor de behandeling van artrose van het kniegewricht. Hierbij wordt de knie met artrose gedurende zes tot zeven weken enkele millimeters uit elkaar wordt getrokken met behulp van een distractieframe met pinnen door het bot, zie figuur 1. In de periode na distractie kan herstel van het gewricht plaatsvinden. Voorgaand onderzoek heeft uitgewezen dat deze ingreep effectief is en het plaatsen van een kunstgewricht kan uitstellen of zelfs voorkómen.

Om de ernst en het beloop van een ziekte, en het effect van behandeling te beoordelen, zijn doelmatige meetresultaten (uitkomstmaten) nodig. Klinische uitkomstmaten, zoals ernst van pijn of beperkingen, zeggen iets over een patiënt. Structurele uitkomstmaten, zoals de wijdte van de gewrichtsspleet op röntgenfoto's, die de dikte van gewrichtskraakbeen weergeeft, zeggen iets over een gewricht. Hoewel beide soorten uitkomstmaten essentieel zijn, komen de resultaten ervan in de praktijk niet altijd overeen. Verdere informatie zou kunnen worden verkregen met meer ingrijpende technieken, zoals het nemen van biopsieën (stukjes weefsel uit het gewricht voor onderzoek). Een biopsie kan het gewricht echter beschadigen. Een andere mogelijkheid is laboratoriumonderzoek van bepaalde



Figuur 1: Kniedistractie.

stofjes ("*markers*"), bijvoorbeeld in bloed of synoviale vloeistof. Zij kunnen inzicht geven in verschillende ziektemechanismen, maar zijn alleen vaak niet representatief voor het gewricht. Kortom, er is behoefte aan niet-belastende uitkomstmaten, die kunnen helpen bij de beoordeling van gewrichtsweefsels van patiënten met RA of artrose. Beeldvorming zou zo'n uitkomstmaat kunnen zijn. Het doel van dit proefschrift is om nieuwe beeldvormende technieken te onderzoeken als uitkomstmaten voor RA en artrose.

Deel I: Meten van gewrichtsontsteking in handen van patiënten met artrose of reumatoïde artritis

Het eerste deel van dit proefschrift beschrijft onderzoek van een beeldvormende techniek, te weten optische spectrale transmissie (OST), om gewrichtsontsteking te meten. OST wordt hier toegepast in de gebruiksvriendelijke HandScan, zie figuur 2. De patiënt met artrose in de handen of met reumatoïde artritis, steekt de handen in de HandScan. In dit apparaat worden de handen verlicht, waardoor de omvang en snelheid van de bloedstroom in gewrichten gemeten kunnen worden. Deze weerspiegelen de mate van gewrichtsontsteking: des te meer en snellere doorbloeding, des te meer gewichtsontsteking. Uit het onderzoek beschreven in de eerste twee hoofdstukken blijkt dat het meten van gewrichtsontsteking met OST toegepast zou kunnen worden bij de intensieve en op remissie gestuurde behandeling van RA. In de daaropvolgende twee hoofstukken wordt het toepassen van OST bij patiënten met ontsteking van handgewrichten door artrose besproken.

Hoofdstuk 2 toont aan dat een vernieuwd OST apparaat geschikt kan zijn om ziekteactiviteit van RA patiënten te monitoren. Toepassen van een nieuwe lichtbron in het nieuwe

Figuur 2: De HandScan.

OST-apparaat laat verbetering zien, in vergelijking met een eerder OST apparaat. OST blijkt betrouwbaar ontstekingen in handgewrichten te meten. Om in de praktijk ontstekingsactiviteit van gewrichten bij RA patiënten te meten wordt gebruik gemaakt van de zogeheten DAS28 ("*Disease Activity Score",* met onder andere het scoren van zwelling en pijn van 28 gewrichten). De gouden standaard voor het vaststellen van gewrichtsontstekingen is echter echografie, waarmee gewrichtsontsteking betrouwbaar kan worden weergegeven. De meetresultaten van OST komen beter overeen met die van echografie dan die van de DAS28. Wel blijken botuitsteeksels in gewrichten ("*osteofyten*", die ontstaan door langdurige gewrichtsbeschadiging) de betrouwbaarheid van meetresultaten van OST te verminderen.

Hoofdstuk 3 beschrijft het protocol van een onderzoek met RA patiënten, om de effectiviteit en kosteneffectiviteit van twee behandelstrategieën met elkaar te vergelijken. Deze behandelstrategieën zijn beide op de individuele RA-patiënt toegesneden behandelingen, maar worden verschillend gestuurd. Dit sturen geschiedt met meten van gewrichtsontsteking met de DAS28 of OST. Om OST voor dit doel in te zetten is specifieke software ontwikkeld. In beide behandelingsgroepen wordt het *tight-control* principe aangehouden, worden dezelfde medicijnen gebruikt en is de behandeling er op gericht, om zo spoedig mogelijk remissie te behalen bij patiënten bij wie kort tevoren de diagnose RA is gesteld. Het onderzoek betreft dus alleen het sturen van de behandeling. De software om OST hiervoor te gebruiken is ook toepasbaar in ander onderzoek met RA-patiënten.

In **hoofdstuk 4** wordt gewrichtsontsteking bij artrose besproken, met speciale aandacht voor synovium en gewrichtskapsel. Chronische en/of terugkerende lichte gewrichtsontsteking

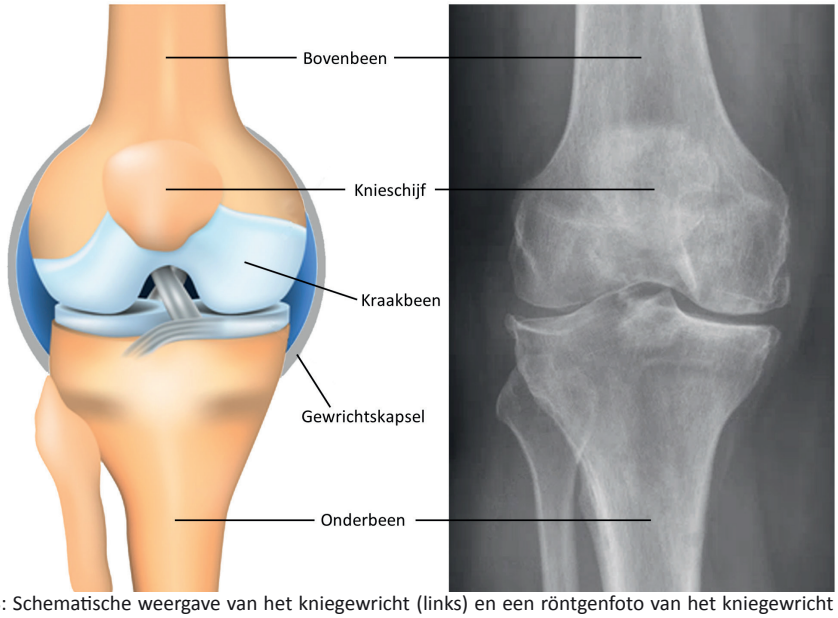
die voorkomt bij sommige patiënten met artrose veroorzaakt zeer waarschijnlijk een deel van de klachten en kan het ziektebeloop negatief beïnvloeden. Dit zijn argumenten om ontstekingsremmende geneesmiddelen in te zetten bij deze patiënten met artrose; hopelijk komt dat het ziektebeloop van artrose ten goede.

Vingers hebben drie gewrichten, één aan het uiteinde (DIP gewricht), één in het midden (PIP gewricht) en één aan de basis (MCP gewricht). Duimen hebben twee gewrichten, één halverwege (IP gewricht) en één aan de basis (MCP gewricht). **Hoofdstuk 5** beschrijft hoe het nieuwe OST apparaat presteert bij het meten van gewrichtsontsteking bij mensen met handartrose. Het blijkt dat de resultaten van OST in de PIP gewrichten redelijk overeenkomen met echografische tekenen van ontsteking, maar dat dit minder het geval is voor polsen en DIP gewrichten. Mogelijk komt dit doordat er in DIP- en polsgewrichten weinig ontsteking was. De betrouwbaarheid van de OST-resultaten was wel hoog, met andere woorden, herhaaldelijke metingen gaven vrijwel gelijke waardes.

Deel II: Meten van kraakbeenschade en -herstel bij behandelingen van knieartrose
Het tweede deel van dit proefschrift bevestigt dat kniedistractie een waardevolle therapie kan zijn voor knieartrose. In het eerste hoofdstuk worden de klinische en röntgenologische veranderingen na kniedistractie vergeleken met die na andere vaak toegepaste behandelingen van knieartrose; standscorrectie van het onderbeen (*hoge tibiakop osteotomie*) en inbrengen van een kunstknie (*kniearthroplastiek*). Verder wordt beschreven dat beeldvorming kraakbeenherstel door kniedistractie en standscorrectie van het onderbeen kan weergeven.

In **hoofdstuk 6** laten twee onderzoeken (kniedistractie versus hoge tibiakop osteotomie en kniedistractie versus kniearthroplastiek) aanhoudende vermindering van klachten tot twee jaar na kniedistractie, hoge tibiakop osteotomie en kniearthroplastiek zien en verbetering van kraakbeen tot twee jaar na kniedistractie en hoge tibiakop osteotomie. Hoewel de vermindering van klachten bij kniedistractie iets minder uitgesproken was dan na kniearthroplastiek, geven patiënten aan dat zij het belangrijk vinden om hun eigen knie te behouden, d.w.z. dat zij geen kunstknie hoeven te krijgen. Herstel van kraakbeen, zichtbaar door toename van de wijdte van de gewrichtsspleet op röntgenfoto's (zie figuur 3), is meer uitgesproken na kniedistractie dan na hoge tibiakop osteotomie. De mate van verwijding van de gewrichtsspleet als maat voor genezing van kraakbeen correspondeert met toename van een marker in het bloed, die aanmaak van bepaald kraakbeenweefsel weerspiegelt (*PIIANP*, dat netto synthese van collageen type II weergeeft).

Bij hoge tibiakop osteotomie wordt de stand (de mechanische belastingsas, of beenas) van het onderbeen zodanig veranderd, dat de belasting van het kraakbeen aan de meest artrotische zijde van de knie wordt verminderd. In **Hoofdstuk 7** wordt beschreven dat



Figuur 3: Schematische weergave van het kniegewricht (links) en een röntgenfoto van het kniegewricht (rechts). Kraakbeen is op röntgenfoto's niet direct zichtbaar, maar wel indirect, doordat het kraakbeen de afstand tussen de twee botuiteinden in het gewricht bepaalt.

kniedistractie, niet in de eerste plaats gericht op het veranderen van de beenas, deze wel verbetert. Dit komt doordat daar waar het meeste artrose in het gewricht is en het dunste kraakbeen, de kraakbeendikte toeneemt als gevolg van het genezingsproces, waardoor de gewrichtsspleet wijder wordt en de beenas verandert (verbetering). Een goede beenas vóór de operatie en verbetering van de beenas na operatie zijn beide geassocieerd met grotere afname van pijn één jaar na de operatie.

Bij knieartrose neemt niet alleen de dikte, maar ook de kwaliteit van gewrichtskraakbeen af. Een behandeling die het ziektebeloop van artrose positief beïnvloedt zou bij voorkeur ook de afname in kwaliteit van artrotisch gewrichtskraakbeen moeten tegenhouden. Kraakbeenkwaliteit kan worden beoordeeld met een specifieke beeldvormende techniek genaamd dGEMRIC ("*Delayed gadolinium-enhanced MRI of cartilage*"), een speciale vorm van MRI (magneetscan). In **hoofdstuk 8** wordt beschreven hoe deze techniek gebruikt is om het effect van kniedistractie en hoge tibiakop osteotomie op kraakbeenkwaliteit te vergelijken. Kniedistractie en hoge tibiakop osteotomie geven beide een duidelijke verwijding van de gewrichtsspleet, wijzend op toegenomen kraakbeendikte. Kniedistractie gaf geen, en hoge tibiakop osteotomie gaf minimale afname van kraakbeenkwaliteit. Beide behandelingen hebben een positieve invloed op het beloop van knieartrose. Het welbevinden van patiënten neemt na beide operaties in het algemeen toe, maar meer bij patiënten met toegenomen kraakbeenkwaliteit.

Dankwoord

Dit proefschrift is tot stand gekomen met de hulp van een heleboel mensen, mijn dank hiervoor! Hopelijk ben ik in dit dankwoord niemand vergeten.

Allereerst wil ik de patiënten bedanken; de bereidheid om (meermalen) deel te nemen aan onderzoeken en de getoonde interesse in het onderzoek, zowel persoonlijk als voor toekomstige patiënten, zijn motiverend voor iedere onderzoeker.

Dan gaat mijn dank uit naar mijn promotoren, copromotoren en dagelijkse begeleiders. Professor Lafeber, beste **Floris**, bedankt voor je visie en begeleiding de afgelopen jaren. Als 'jonge hond' heb ik van je geleerd wat het inhoud om een onderzoeker te zijn en hopelijk heb jij ook nog iets gehad aan mijn slides vol met complexe beeldverwerking. Ik vond het fijn hoe je deur altijd open stond, om even kort over iets te sparren. Bedankt voor alle mogelijkheden die je mij hebt geboden en ik ga er volledig vanuit dat ArthroSave een groot succes zal worden. Professor van Laar, beste **Jaap**, je bent nog niet zo lang mijn promotor, maar wel vanaf het begin betrokken bij de HandScan studies. Mede dankzij je kennis en ervaring in klinische studies en de efficiëntie van onze meetings, was er niet veel voor nodig om de klinische studies in goede banen te leiden. Dokter Jacobs, beste **Hans**, bedankt voor je begeleiding en je bereidbaarheid om 'er even voor te gaan zitten'. Als ik dan een aantal uur later opstond van het krukje, was een artikel van voor naar achter doorgelopen en (vrijwel) klaar voor indiening. Bedankt voor de bijzondere en creatieve metaforen, in een poging mij orde te laten scheppen in chaotische teksten. Ps. Ik krijg nog een biertje van onze weddenschap. Dokter van Spil, beste **Erwin**. Je werd mijn copromotor toen je in Sydney zat. Hoewel de afstand en het tijdsverschil zo groot waren, heeft de samenwerking daar absoluut niet onder geleden. Dit is in mijn ogen tekenend voor hoe jij bent, als persoon en als begeleider. Als je besluit ergens voor te gaan, dan doe je dat met volle overtuiging. Bedankt voor het te allen tijden beschikbaar zijn, je creatieve maar ook altijd politiek correcte oplossingen en je aanstekelijke vrolijkheid. **Anne Karien** en **Simon**, hoewel jullie niet officieel als begeleiders zijn bestempeld, zijn jullie dit de afgelopen jaren zeker wel geweest! Bedankt voor de gezelligheid; de uitjes, de koffiemomenten, de ritten naar Twente en naar de deelnemende centra, en natuurlijk bedankt voor de levenswijsheden en wetenschappelijke adviezen tijdens de vele meetings die we hebben gehad!

Verder wil ik alle reumatologen, AIOS, verpleegkundigen en researchverpleegkundigen uit het UMC Utrecht bedanken voor jullie bijdrage aan alle onderzoeken. In het bijzonder **Karin**, **Joke** en **Annemieke** voor jullie harde werk binnen de HandScan onderzoeken. Het Clinical Study Team, **Janneke**, **Anne Karien** en **Marjolein** voor de adviezen en hulp bij het indienen van onderzoeken bij de METC. En natuurlijk ook dank voor iedereen buiten het UMC Utrecht, die een steentje heeft bijgedragen aan de verschillende klinische studies;

de reumatologen en reumaconsulenten van het Máxima Medisch Centrum Eindhoven, Meander Medisch Centrum Amersfoort, Noordwest Ziekenhuisgroep Alkmaar en het Gelre Ziekenhuizen Apeldoorn. In het bijzonder **Toon**, "dokter HandScan", bedankt voor je inzet in alle HandScan onderzoeken en je gemotiveerde en altijd vrolijke, maar nuchtere instelling.

Bedankt iedereen van Hemics, met name **Wouter**, **Koen**, **Michiel**, **Petra** en **Peter**. Bedankt voor de technische uitleg als ik in Eindhoven langskwam, voor de urenlange overleggen aan de telefoon, de gigantische spreadsheets, de gemeenschappelijke trainingen die we gaven in de deelnemende centra, het aan mijn zijde staan bij belangrijke presentaties en natuurlijk voor het auteurschap bij artikelen. Veel succes met de HandScan, dat jullie nog maar vele internationale successen zullen behalen (en vooral de patiëntenzorg zullen verbeteren).

Alle coauteurs die hierboven niet genoemd zijn, bedankt voor jullie bijdrage aan het onderzoek: **Arno**, **Karen**, **Koen**, **Paco**, **Patricia**, **Pieter**, **Roel**, **Ronald**, **Sander** en **Wilbert**.

AIOs, OIO's en lab-mensen, bedankt voor de gezelligheid, jullie vormden voor mij een belangrijke motivatie om naar mijn werk te komen, naast het onderzoek natuurlijk. **Thijmen**, "Poofie", van begeleider tot collega, je was er eigenlijk altijd, bedankt voor de ontelbare hilarische momenten en succes met je toekomstige werkzaamheden, wat die ook mogen zijn. **Huub**, bedankt voor de wijze levenslessen in de *Mancave*. **Eefje**, gaan we ooit nog een keer samen rijden? **Astrid**, hoe moet ik nu verder zonder je 'burgerlijke adviezen'. **Jelena**, '*what's new?*' I will miss your contagious laughter, and your ability to spill anything. **Paco**, **paco paco paco**, bedankt voor je significante bijdrage aan de artikelen. **Mylène**, leuk om je te hebben zien groeien de afgelopen jaren, van student naar collega naar begeleider. *È stato un vero piacere*. **Nadia** controleer even of je artikel al geaccepteerd is. Zowel met je proefschrift als met je "pannenkoekplantfamilie" gaat het helemaal goedkomen. **Tammo**, dankzij jou zal H3 nog lang voorzien worden van interessante feitjes en nuttige discussies nadat ik weg ben. **Maxime**, dankzij jou weet ik welke jurk ik bij welke gelegenheid moet dragen, veel succes/sterkte met de HandScan studies. **Mary**, gezellig dat je dan toch eindelijk mee bent gaan lunchen. **Xavier**, Chef, blijf je af en toe nog wat goede muziek doorsturen? **Lize, Maud, Sandhya, Jan-Ton** bedankt voor de introductie in de onderzoekswereld. **Anne** en **Willem-Paul**, ik hoop dat jullie mooie proefschriften tot stand gaan brengen. En natuurlijk 'het lab', **Arno**, **Katja** en **Marion**, bedankt. Ik kwam graag bij jullie langs om stikstof te brengen, over pijnlijke knieën te klagen, advies voor de groentetuin in te winnen of om Sinterklaas te plannen.

Ik wil ook mijn vrienden bedanken voor de support, voor de afleidingen en voor het altijd voor mij klaarstaan. In de vorm van een bak koffie, een (kleine) borrel, een feestje, een festival, boulderen, wielrennen, spelletjesavond, dineren en MannenWeekend.

A

Een speciaal woordje voor mijn paranimfen, **Erwin** en **Thijs**. Sinds het begin van mijn studie Technische Geneeskunde zijn jullie mijn beste vrienden. Jullie weten alles van wat ik de afgelopen jaren heb doorgemaakt, sterker nog, jullie waren er vrijwel altijd bij. Bedankt voor alle steun zowel in dit proefschrift als in het leven en ik hoop dat ik nog lang de wederdienst kan bewijzen. Ik heb er het volste vertrouwen in dat jullie beiden een fantastisch proefschrift gaan afleveren.

Pap en **mam**, bedankt voor het altijd blijven pushen om ervoor te zorgen dat ik het uiterste van mijzelf verlang. Bedankt voor de leuke jeugd en dat jullie altijd voor me klaar staan. Ik hoop dat jullie, nu jullie de smaak te pakken hebben, nog vele mooie reizen samen zullen maken. **Sharon**, klein zusje, ik ken weinig mensen die zo gedreven zijn als jij, zoals je naast je baan als fysiotherapeut nog een master doet. Je gedrevenheid is aanstekelijk, bedankt daarvoor. **Sjoerd**, zwager, bedankt dat je onze familie nog gezelliger (en chaotischer) maakt.

Jessica, bedankt voor de afleiding de afgelopen jaren. Je bent avontuurlijk, lief en vrolijk. We toeren op onze motoren, boulderen en gaan op verre reizen, maar door jou heb ik ook altijd een fijne thuisbasis om weer naar terug te komen. Je bent ook intelligent en motiverend. Als ik iemand nodig had om te sparren over onderzoek, kon dat gewoon met jou aan de eettafel. Mede dankzij jou is dit proefschrift er gekomen!

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List of presentations

Optical spectral transmission measurements (OST) for assessment of synovitis might be useful for hand osteoarthritis. Poster presentation at IWOAI congress 2014, Reykjavik, Iceland.

Changes in cartilage quality (dGEMRIC) following knee joint distraction or high tibial osteotomy; a two-year follow-up. Poster presentation at IWOAI congress 2016, Oulu, Finland.

Automated image registration increases the efficacy of human knee T1 mapping using dGEMRIC. Poster presentation at IWOAI congress 2016, Oulu, Finland.

Influence of joint pathology on optical spectral transmission imaging, assessing inflammation in hand and wrist joints of rheumatoid arthritis patients. Poster presentation at EULAR congress 2017, Amsterdam, the Netherlands.

Axial alignment of the knee – importance in cartilage repair? High tibial osteotomy vs. distraction. Poster presentation at EULAR congress 2017, Amsterdam, the Netherlands.

Changes in cartilage quality (dGEMRIC) following knee joint distraction or high tibial osteotomy; a two-year follow-up. Poster presentation at EULAR congress 2017, Amsterdam, the Netherlands.

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Changes in cartilage quality (dGEMRIC) following Knee Joint Distraction or High Tibial Osteotomy; a two-year follow-up. Oral presentation at ICRS congress 2017, Sorrento, Italy.

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Is standscorrectie van de knie belangrijk voor kraakbeenherstel? Osteotomie vs. kniedistractie. Oral presentation at NVR congress 2017, Arnhem, the Netherlands.

Veranderingen in kraakbeenkwaliteit (dGEMRIC) na kniedistractie en hoge tibiakop osteotomie: twee jaar follow-up. Oral presentation at NVR congress 2017, Arnhem, the Netherlands.

Changes in Cartilage Quality (dGEMRIC) Following Knee Joint Distraction or High Tibial Osteotomy: A Two-Year Follow-Up. Oral presentation at RSNA congress 2017, Chicago, IL, United States.

Curriculum vitae

Nick Jurriën Besselink was born on March 3rd 1989 in Arnhem, the Netherlands and lived in Elst with his sister Sharon and his parents, Anita and Michaël. In 2007 he finished secondary school at the Lorentz College in Arnhem and started Technical Medicine at the University of Twente. During his bachelor he became fascinated with imaging techniques, and consequently chose the master track 'Robotics and Imaging'. During this Master's program, he followed rotations at the Academic Medical Centre Amsterdam (AMC), the Netherlands Cancer Institute (NKI-AVL), the Medical Spectrum Twente, and Guys Hospital in London. These four internships all involved implementation of non-invasive imaging techniques to optimize patient care. Aspiring an academic path, he also acquired certifications for working with ionizing radiation (Radiation Safety level 3) and performing animal studies (Laboratory Animal Sciences). In 2014 he conducted his graduation research at the department of Rheumatology and Clinical Immunology at the University Medical Center Utrecht. His master's thesis aimed at digitization of knee movement allowing for the patient-specific joint sparing treatment by knee joint distraction. After successfully graduating on March 3rd 2014, he started as a PhD candidate at that very department, under the supervision of dr. WE van Spil, dr. JWG Jacobs, professor JM van Laar, and professor FPJG Lafeber. As a clinical researcher he was responsible for designing, coordinating, and performing studies, monitoring tissue characteristics in rheumatic diseases using non-invasive imaging techniques.

Currently he is living in Utrecht, The Netherlands.