Osteoarthritis: Towards a progressive APPROACH

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Towards a progressive APPROACH

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(met een samenvatting in het Nederlands)

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1 Introduction

Introduction

Previously, osteoarthritis (OA) was described as a joint disease of 'wear and tear' leading to cartilage destruction and secondary synovial inflammation[1]. However, nowadays, there is compelling evidence that OA is a disease of the whole joint, including multiple joint tissues[2], where synovial inflammation, peri-articular bone changes, and soft tissue changes play a key role in the pathogenesis[1-4]. Main symptoms of OA are pain, stiffness, and functional limitations[1], resulting in decreased quality of life. OA affects more than 300 million people worldwide[5], and an ageing population and increasing obesity will further contribute to the burden of OA, since both are strong predisposing factors in development and progression[6,7].

In general, OA patients are all treated in a similar way. The first step being non-pharmacological interventions, such as exercise programs and weight loss[8,9]. When these interventions fail, pain medication is frequently described, however their effectiveness is often limited. Moreover, OA treatment should not only reduce symptoms, but also prevent, or even better reverse, the progressive cartilage damage and reduce inflammatory responses. Therefore, an ideal disease modifying osteoarthritis drug (DMOAD) combines analgesic, chondroprotective, and anti-inflammatory effects all in one treatment. At present no drugs are approved by the Food and Drug Administration (FDA) or European medical Agency (EMA) for this indication and the quest for such disease modifying treatment has still to be considered the 'holy grail'[6].

The goal of the research described in this thesis is to contribute to the search for successful OA treatments, in two different ways:

1. Investigating possible DMOADs, including synovial inflammation as an important player in OA pathogenesis
2. Providing support for a new approach for design of OA clinical trials

Thesis outline

Part 1: Current (pre)-clinical approach

The first part of this thesis contributes to the search for a successful DMOAD. It describes a randomized clinical trial investigating the DMOAD effects of celecoxib, explores the role of granulocyte macrophage colony-stimulating factor (GM-CSF) and its receptor in OA knee pain, and evaluates pre-clinical results of the Interleukin-4 (IL-4) and IL-10 fusion protein (IL4-10 FP).

Multiple international guidelines recommend the use of topical/oral non-steroidal anti-inflammatory drugs (NSAIDS) as a first step in the treatment of OA pain[8-10]. NSAIDs are anti-inflammatory drugs with analgesic effects, suggesting an interaction between these two DMOAD properties and supporting the abovementioned idea of OA being a disease of the whole joint, with an important role for synovial inflammation in pain.

Unfortunately, the first generation NSAIDs (ibuprofen, indomethacin, diclofenac, naproxen) have been demonstrated to have adverse effects on cartilage and could add to OA progression[11-18], in particular when used chronically, as is often needed in OA. In contrast, the second generation NSAIDs, the selective COX-2 inhibitors (coxibs) were suggested to have beneficial effects on cartilage[15-17,19-24]. A systematic review described the different aspects of disease modifying properties of celecoxib[25]. Like other NSAIDs, celecoxib indeed has analgesic and anti-inflammatory effects, but it also shows chondroprotective effects and inhibits bone destruction in *in vitro* and *in vivo* in animal models. Nevertheless, good randomized controlled trials evaluating the DMOAD activity of celecoxib in humans are lacking. Therefore, based on a previous pilot study where celecoxib showed chondroprotective and anti-inflammatory activity[26], a sufficiently powered and blinded randomized clinical trial was performed to evaluate DMOAD effects of celecoxib, which is described in **chapter 2**.

Another possible treatment targeting inflammation and OA pain is anti-GM-CSF treatment. The hemopoietic growth factor GM-CSF[27] acts as pro-inflammatory cytokine[28], and is suggested to play a role in OA disease progression and pain[27,28]. In a mouse model with collagen-induced arthritis (CIA), GM-CSF deficient mice showed less development of cartilage damage, synovial inflammation, and osteophytes compared to wild-type CIA mice. Next to the effects on joint tissues, wild-type mice treated with monoclonal antibodies against GM-CSF also showed less pain[29], indicating a key role of GM-CSF in the development of joint damage, synovial inflammation and pain in OA (the three targets of DMOAD therapy). In addition, in patients with inflammatory hand OA, monoclonal antibodies against GM-CSF reduced hand pain[30]. To investigate whether GM-CSF indeed plays a role in OA pain in humans, and the possible role of synovial inflammation herein, in **chapter 3** the correlation between expression of GM-CSF in joint tissues and knee pain in knee OA patients was evaluated.

Although the concept of combining IL-4 and IL-10 to treat inflammatory joint diseases was already conceived many years ago[31], and the combination showed chondroprotective effects in rheumatoid arthritis[32], only recently this concept was further developed by combining the two molecules into one molecule, the IL4-10 FP. Our group and others have demonstrated chondroprotective, anti-inflammatory, and analgesic effects of IL4-10 FP in multiple *in vitro* an *in vivo* disease models[33-36]. As a first step to provide further insight in the pathophysiological mechanisms behind the therapeutic effects of IL4-10 FP, **chapter 4** summarizes the effects of IL-4, IL-10, and IL4-10 FP on OA cartilage, synovial tissue, and pain. **Chapter 5 and 6** describe two animal studies evaluating the effects of IL4-10 FP *in vivo.* As repetitive administration of the human IL4-10 FP in dogs causes an immune response against the IL4-10 FP, a species specific canine IL4-10 FP was developed and tested. The therapeutic effect of the canine IL4-10 FP on cartilage damage, synovial inflammation, and pain was evaluated in the canine Groove model of OA **(chapter 5)**. Preclinical testing of DMOADs is frequently performed by treating prophylactically or early in the OA process,

immediately after OA induction (mostly post-traumatic OA), in young and normal-weight animals. However, in clinical practice, OA is more age-related and frequently associated with obesity. Therefore, in **chapter 6**, a rat model was used combining surgical OA induction (Groove model) with obesity (evoked by high-fat diet)[37], to evaluate the DMOAD effect of intra-articular IL4-10 FP in this clinically (more) relevant OA model.

Part II: First steps towards a new APPROACH

This part focuses on a different approach for the design of OA clinical trials. Despite the growing OA burden, pharmaceutical companies have lost interest in the development of OA drugs because of disappointing results in long-lasting and large, and with that expensive, clinical trials. The highly heterogeneity of the disease among different patients likely contributes to the disappointing results found in clinical trials, and questions the abovementioned one-size-fits-all approach[6]. A wide range of underlying pathways ultimately lead to similar levels of joint destruction[38]. Besides, OA disease progression, and with that also tissue repair, is in general a slow process which take years, while trials generally last only two or three years. To create a window for DMOADs to show their effectiveness, ideally one would like to include patients who, if left untreated, would have shown relevant disease progression within the trial duration. Only a small amount of patients with early OA will develop severe disease progression[39]. Without pre-selection of (fast) progressive patients, clinical trials require large group sizes and long follow-up periods. This is often not feasible from a time and economical perspective. Therefore, there is a need for a different approach in DMOAD trial design. The second part of this thesis describes the first steps towards such an approach.

For such an approach, it is essential to distinguish multiple OA phenotypes, with different disease characteristics and underlying pathways. A first step is to be able to identify relatively fast progressors (at the level of structural damage but also pain). **Chapter 7** describes the design of the clinical study specifically set-up to predict OA progression and define multiple OA phenotypes: **A**pplied **P**ublic-**P**rivate **R**esearch enabling **O**steo**A**rthritis **C**linical **H**eadway (IMI-APPROACH). The unique multi-step selection process used machine learning models to select patients based on predicted progression (of structural damage and/or pain). The first model used historical data from existing OA cohorts to rank patients based on the likelihood of showing structural and/or pain progression. Then the highest ranked patients were invited for a screening visit to collect up-to-date data. This data was used in the second model to, again, rank patients based on their likelihood of showing progression. The 75% highest ranked patients were included in the IMI-APPROACH cohort study. **Chapter 8** describes in more detail the baseline characteristics of the IMI-APPROACH participants (n=297), in relation to their predicted likelihood for structural and pain progression.

Since OA pain is very complex and not always clearly related to joint tissue damage, the relevance of defining different pain phenotypes is key in the design of clinical trials. In **chapter 9** we therefore explore a possible specific OA pain phenotype; patients with a likely neuropathic pain component.

Another important difficulty in clinical DMOAD trials is the absence of robust and sensitive disease parameters. New sensitive and robust OA markers could be used to improve the design and outcome of clinical trials regarding patient selection and/or treatment evaluation.

Functional limitations, as a result of the interplay between joint damage and pain, are important for OA patients. Mostly, function is evaluated by questionnaires, by very complex, time-consuming, and expensive analysis in an optical gait lab, or by insensitive physical tests. In the IMI-APPROACH cohort study, a new measurement was added; motion analysis using the GaitSmart® system. Although analysis in an optical gait lab is sensitive and provides relevant data, it is often hard to include in clinical set-up, due to the abovementioned reasons. The GaitSmart® system is a more practical method, using six Inertial Measurement Units (IMU) which are attached to the patient's body. It can be performed anywhere and takes 10-15 minutes. In **chapter 10 and 11**, the relationship between GaitSmart® analysis and radiographic OA **(chapter 10)** and physical function **(chapter 11)** is evaluated.

In **chapter 12** two-year progression of the IMI-APPROACH participants was evaluated and a first attempt was made to distinguish progressors from non-progressors.

Finally, results of the different chapters are summarized and discussed in context in **chapter 13**, placing them in a broader perspective, and providing suggestions for future research.

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1

PART I:
CURRENT (PRE-)CLINICAL APPROACH

2 Lack of a clear disease modifying activity of celecoxib treatment of end-stage knee osteoarthritis: a randomized observer blinded clinical trial

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Abstract

Objectives

To evaluate the *in vivo* disease modifying activity of the selective COX-2 inhibitor celecoxib, compared to no treatment and naproxen, treating end-stage knee osteoarthritis, using detailed *ex vivo* tissue analyses.

Methods

Patients (n=172) with end-stage knee osteoarthritis (OA) were randomized to four groups and treated for four weeks prior to knee replacement surgery: celecoxib 2dd200mg, naproxen 3dd250mg, celecoxib 2dd200mg stopped 3 days prior to surgery, or no treatment.

Cartilage and synovial tissue collected during surgery were analyzed *ex vivo*, with cartilage proteoglycan release as primary outcome. Additionally, several markers of synovial inflammation and clinical effects were determined.

Results

Data of 138 patients could be analyzed, 34 patients were lost for several reasons. The expression of COX-2 in both cartilage and synovial tissue was statistically significantly decreased in patients treated with celecoxib (p=0.017 and p=0.001, respectively), indicating the drug has reached the knee joint within the treatment period. Nonetheless, no significant effect on proteoglycan release, retention or content was found. Synovial inflammation markers did not show any statistically significant decreases although nitric oxide levels in celecoxib treated patients suggest a beneficial effect of celecoxib compared to no treatment. WOMAC scores did not statistically significant improve after treatment, though celecoxib treated patients reported a slightly higher Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score compared to non-treated patients.

Conclusion

No direct effect on cartilage upon short-term *in vivo* treatment of knee OA patients with celecoxib could be detected, although decreased expression of COX-2 confirmed its intra-articular availability. Effects on synovial inflammatory mediators and clinical outcome seemed only limited. As such the previous reported disease modifying effects of celecoxib in *in vitro* and pilot clinical studies could not unambiguously be confirmed.

Introduction

Osteoarthritis (OA) is the most common joint disorder, frequently causing pain, loss of function, and disability in adults[1]. The unknown etiology results in deterioration of structure and function of the whole joint. Treatment of OA is initially conservative, predominantly symptomatic, and is not stopping progression of the disease. Ultimately, in end-stage disease, surgical intervention is indicated. Currently there is no indisputable curative treatment. In fact, the number of joint replacements is increasing exponentially[2,3].

After acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs) are recommended as 2nd step in the pharmacological treatment of OA[4-6]. Consequently, NSAIDs are among the most commonly used pharmacological agents to alleviate OA symptoms[7]. Although they help relieve symptoms such as pain and inflammation, the direct effects of NSAIDs on cartilage may be of importance in the treatment of joint diseases, specifically when inflammation is relatively mild, as in OA, and treatment is chronic. Direct effects of NSAIDs on cartilage cannot be studied easily in clinical trials and are generally ignored in clinical practice. Effects of NSAIDs on inflammation shade their direct effects on cartilage[8]. In addition, (intrinsic) cartilage changes, both catabolic and anabolic, are generally very slow processes in OA. Evaluation of cartilage degeneration by imaging and biomarker evaluation is hampered by the still limited sensitivity of these methods[9]. Although limited in number, different results have been published on direct effects of NSAIDs on cartilage. *In vitro* and animal *in vivo* studies reported adverse effects of commonly used NSAIDs, like indomethacin, diclofenac, and naproxen[7,10,11]. Also neutral and beneficial effects have been published, e.g. for piroxicam and aceclofenac[7,11,12]. The number of human *in vivo* studies is very limited. Indomethacin and diclofenac were reported to accelerate hip and knee osteoarthritis progression[9,13]. As such, the conventional NSAIDs are potentially harmful regarding their direct effects on cartilage.

The 2nd generation NSAIDs, the selective cyclooxygenase-2 (COX-2) inhibitors (coxibs) were suggested to have beneficial effects on cartilage volume and defects based on MRI, while non-selective COX-2 inhibitors as comparators were deleterious[14]. Several *in vitro* studies suggested that the selective COX-2 inhibitor celecoxib has beneficial effects on cartilage. Celecoxib was reported to have a favorable effect on overall metabolism of proteoglycans and hyaluronan, making a detrimental effect on articular cartilage during long-term use unlikely[15]. Moreover, reduced production of arthritis-associated mediators and increased production of anabolic indicators by celecoxib in pig chondrocytes has been demonstrated[16]. Celecoxib showed positive effects on turnover of proteoglycans in cartilage tissue explants from OA patients[17-19].

Animal *in vivo* studies showed variable effects of celecoxib on cartilage. A chondroneutral effect was found in the canine Groove model and a murine OA model[20,21]. Celecoxib reduced matrix metalloproteinase expression and delayed the progress of arthritic damage in a

posttraumatic OA mouse model[16]. In an OA model in rats celecoxib reduced the OA-like histological changes and suppressed chondrocyte apoptosis[22]. Studies with intra-articular injections with celecoxib, as an attempt to prevent cardiovascular side-effects, show promising results on cartilage protection[23,24].

By treating patients shortly before joint replacement surgery, the benefit of *in vivo* treatment of celecoxib was combined with the benefit of *ex vivo* evaluation of the joint tissues. Using this approach, it was demonstrated that a 3-month celecoxib treatment decreased COX-2 and microsomal prostaglandin E synthase-1 gene expression by the OA cartilage[25]. Moreover, cartilage proteoglycan turnover, specifically proteoglycan loss, was affected beneficially by a 4-week celecoxib treatment prior to joint replacement surgery, while indomethacin showed a tendency towards negative effects[26].

Considering the effects of celecoxib on cartilage, synovial tissue and bone *in vitro* as well as *in vivo*, celecoxib and maybe other COX-2 inhibitors were postulated to have disease modifying osteoarthritic drug (DMOAD) activity. This potential is underlined by the continuous interest in celecoxib, with more recent developments such as locally applied sustained release of celecoxib[27]. Nonetheless, randomized clinical trials (RCTs) examining DMOAD activity are lacking[29].

Therefore, a RCT was warranted. The aim was to evaluate *in vivo* disease modifying activity of short-term celecoxib treatment, with the attempt to modulate proteolytic activity (proteoglycan release) as an early indicator for cartilage repair activity, by treating patients with end-stage knee OA with celecoxib shortly before joint replacement surgery followed by a detailed *ex vivo* assessment of joint tissues with observer blinded evaluation.

Methods

Patients

Patients with severe knee OA (n=172), on the waiting list for total knee replacement (TKR) surgery at the Sint Franciscus Gasthuis Rotterdam, were included between December 2007 and June 2009. Group size was determined based on the difference in cartilage proteoglycan loss over three days *ex vivo* incubation between no treatment and celecoxib treated patients in a previous pilot study[26], using α=0.05 and β=0.8.

Exclusion criteria were: TKR for other reasons than OA, history of gastrointestinal bleeding or perforation, increased risk for cardiovascular disease (history of cardiovascular disease like myocardium infarct, heart failure, cerebrovascular accident and transient ischemic attack; untreated/insufficiently treated hypertension; angina pectoris and use of oral anticoagulants), serious liver and/or kidney dysfunction and known intolerance for naproxen. Patients already on NSAIDs had to stop their medication at least seven days prior to start of the study medication.

The study was conducted according to the declaration of Helsinki and received ethics approval of the hospital. A written informed consent was given by each patient before participating in the study.

Study design

Patients were randomized to one of four treatment groups four weeks prior to TKR surgery. Patients were randomized to either celecoxib 2dd200mg (until surgery), celecoxib 2dd200mg until three days before surgery (to control for the obligated stop of naproxen treatment three days before surgery), naproxen 3dd250mg until 3 days before surgery, or no treatment (controls). Because of its platelet-inhibiting effect, the use of naproxen had to be stopped three days prior to surgery. All four groups included 43 patients. Because of the increased risk for gastrointestinal adverse effects with the use of naproxen, all patients also received omeprazol 20mg.

The no treatment group used no NSAIDs during the study period. To check for treatment compliance patients were interviewed whether medication was taken.

Four weeks of treatment was considered sufficient to alter cellular (proteolytic) activity in cartilage based on previous *in vitro* experiments[17], where changes in proteoglycan release, the primary outcome of this study, are enabled within one week. Clearly a 4-week treatment was anticipated to be too short to alter cartilage tissue structure significantly.

Tissue collection

After four weeks of treatment, at TKR surgery, femoral cartilage (both medial and lateral) with underlying bone and synovial tissue was obtained. Tissue was kept in phosphate buffered saline for maximum four hours during transport. At the University Medical Center Utrecht tissue was processed under laminar flow conditions. Investigators performing experiments and analysis were fully blinded to patients' clinical data and medication use. Blinding was guaranteed until all data of all patients were gathered.

From weight bearing areas of the femoral condyles all cartilage was cut aseptically at full thickness from the underlying bone. These slices were cut into full thickness squares. From each donor 24 samples were taken randomly. Twenty samples were weighted (range 5-15mg, accuracy 0.1mg) and incubated *ex vivo* for three days in culture medium for biochemical analyses (37°C, 5% CO2). Four samples were fixed for histochemistry. Additionally, four synovial tissue samples (range 50-150mg, accuracy 0.1mg) from each donor were taken randomly. Two were incubated *ex vivo* for three days in 4ml culture medium for biochemical analyses (37°C, 5% CO2). The supernatants of these cultures were harvested and rendered cell-free by centrifugation (1300g). Two samples were fixed for histochemistry.

Remaining cartilage and synovial tissue of the control group and the full celecoxib group was used for COX-2 expression analyses. The limited amount of tissue available, limited the analyses of COX-2 for cartilage to eighteen treated patients and 25 non-treated patients and for synovial tissue to 31 treated patients and sixteen non-treated patients.

Macroscopy and histochemistry

Macroscopic cartilage damage and synovial tissue inflammation were evaluated on digital high-resolution photographs of femur surface parts and synovial tissue, by two observers blinded for source of photographs (scores adapted from Mastbergen et al.[20]). Severity of cartilage damage was graded from 0-3: 0=fibrillation or focal degeneration, 1=degeneration at multiple locations, 2=degeneration at multiple locations with focal lesions, and 3=degeneration throughout the tissue with severe focal lesions and focally full cartilage abrasion. Synovial tissue inflammation was graded from 0 to 2 for color, angiogenesis and fibrillation: 0=none, 1=slightly, 2=strong. For histochemistry, cartilage and synovial tissue samples were fixed in 4% phosphate buffered formalin with 2% sucrose. Cartilage was stained with Safranin-O fast green-iron haematoxylin. Synovial tissue samples were stained with Haematoxylin-Eosin. Both tissues were sliced and from each sample three slices were used for histological scoring. Cartilage damage was scored using modified Mankin score[30,31]. Synovial tissue inflammation was graded using modified Goldenberg and Cohen score[32,33].

Two observers blinded to the source of the samples, scored all samples and the averages of observers and samples were taken as representative score of each donor. When observers scored >1 point difference, consensus was sought.

Intra-articular COX-2 expression

As a control whether the drug had actually reached the joint, COX-2 expression in cartilage and synovial tissue was evaluated by Western Blot analysis. Total protein was extracted by crushing cartilage and synovial tissue samples using sonification and RIPA lysis buffer. Supernatant was collected and the concentration of each sample was determined using a bicinchoninic acid protein assay kit. Proteins were denatured, separated by 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane 0.45µm. Membranes were incubated overnight with primary antibodies against COX-2 (polyclonal rabbit anti-human, 1:1500) and marker protein glyceraldeyde 3-phosphate dehydrogenase (GAPDH) (monoclonal mouse anti-human, 1:500). Membranes were washed and incubated with secondary antibodies. For GAPDH a rabbit anti-mouse Immunoglobulin G (IgG) horseradish peroxidase (HRP)-labelled antibody (1:1000) was used and for COX-2 a goat anti-rabbit IgG HRP-labelled antibody (1:10.000). Protein bands of GAPDH (37kDa) and COX-2 (69-72kDa) were visualized using the Proxima C16 Phi+. Protein expression was analyzed by TotalLab 1D software, comparing COX-2 band volumes to GAPDH (marker protein) band volumes.

Proteoglycan turnover

Twenty randomly taken cartilage samples of each donor were used for biochemical analysis. Proteoglycan release, release of newly formed proteoglycans (as a measure of retention of proteoglycans) and protecoglycan content were determined as described before[26,31,34]. Values for total proteoglycan release were normalized to proteoglycan content of explants and expressed as percentage glycosaminoglycan (GAG) release. Values for cartilage proteoglycan content were normalized to wet weight of the cartilage sample and expressed as milligrams of GAG per gram wet weight of cartilage tissue (mg/g). Values for release of newly formed proteoglycan were normalized to proteoglycan synthesis rate, and expressed as percentage of newly formed proteoglycans.

Synovial tissue inflammation

Interleukin-1β (IL-1β), tumor necrosis factor-α (TNFα) and nitric oxide (NO) levels were determined as described before[26]. IL-1β and TNFα were determined by enzyme linked immunosorbent assay and expressed as pg/ml per mg synovial tissue and NO levels were determined using the standard Griess reaction and expressed as µM per mg (wet weight) synovial tissue.

Clinical outcome

Patients were asked to fill out a Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire to evaluate pain, stiffness, and function, before and after medical treatment[35].

Statistical analysis

Patients were not blinded for medication. As such clinical outcome, based on patient report was not blinded. For all other parameters, the trial was fully blinded until all assays were performed and all data were collected.

In all cases, multiple data from each patient (e.g. the results of twenty cartilage samples per patient obtained at random and handled individually) were averaged and taken as a representative value for that patient. This is done to compensate for focal differences in composition and bioactivity of the tissue (especially in case of severe OA).

Because of a drop-out of 34 patients, intention to treat and per protocol analyses were performed. Data from per protocol analyses have been given in figures and tables and did not differ from those of intention to treat analyses. When assumptions for parametric testing were not met a log transformation of the variables was performed. Statistical evaluation of effects of treatment was performed using MANOVA tests followed by Dunnett's post-hoc tests for further analysis of differences and Independent Sample T-tests for analysis of COX-2

expression. P-values <0.05 were considered statistically significant. All analyses were done using SPSS Statistics 21.0.

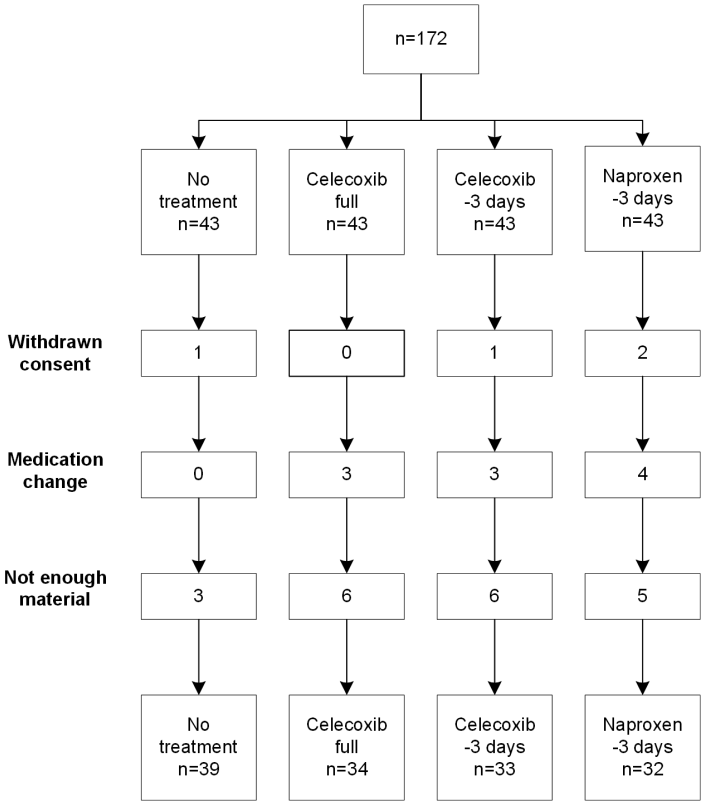
The primary hypothesis was that four weeks *in vivo* treatment of knee OA patients with celecoxib can alter proteoglycan release of cartilage (beneficially) via direct or indirect *in vivo* effects of celecoxib.

Results

Patient characteristics

All 172 patients were included and randomized to four treatment groups. After randomization, a total of 34 patients were lost to follow-up due to several reasons, summarized in figure 1. Statistically, there was no selective drop-out in either of the treatment groups. In total, 138 patients could be evaluated.

Figure 1. Patient flow



Patient characteristics for each of the treatment groups (per protocol analyses) are provided in table 1. For none of the four groups demographic data differed significantly from all randomized patients (data not shown). None of the characteristics was significantly different between the four groups.

Table 1. Patient characteristics

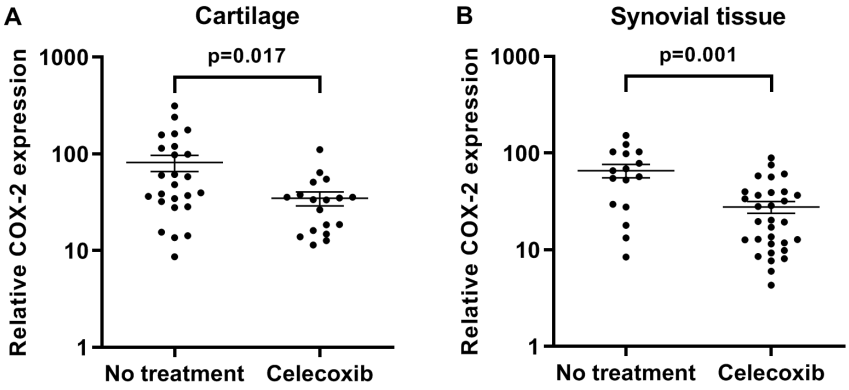
	No treatment n=39	***Celecoxib full n=34***	***Celecoxib -3 days n=33***	***Naproxen -3 days n=32***
DEMOGRAPHICS				
Age (year)	70.0 (1.4)	66.8 (1.3)	65.7 (1.3)	66.4 (1.5)
Female/male*	25/14	22/12	20/13	25/7
Height (cm)	168 (1)	171 (1)	170 (2)	167 (1)
BMI (kg/m^2)	28.5 (0.9)	28.3 (0.7)	30.4 (0.9)	29.5 (0.9)
RADIOLOGY				
KL grade	3.2 (0.6)	2.8 (0.7)	3.1 (0.6)	3.1 (0.7)
Joint space narrowing (0-3)*	2.4 (0.6)	1.9 (0.7)	2.1 (0.7)	2.1 (0.7)
Osteophytes (0-3)*	1.9 (0.7)	1.6 (0.8)	1.6 (0.9)	1.6 (0.8)
CARTILAGE				
Macroscopic score	2.4 (0.1)	2.2 (0.1)	2.3 (0.1)	2.2 (0.1)
Histological score	4.8 (0.2)	4.8 (0.2)	4.5 (0.1)	4.6 (0.1)
SYNOVIUM				
Macroscopic score	3.1 (0.2)	3.0 (0.2)	3.1 (0.2)	3.2 (0.2)
Histological score	4.8 (0.2)	5.1 (0.2)	4.9 (0.1)	5.0 (0.2)
WOMAC				
Pain	47.7 (3.5)	40.0 (3.2)	44.2 (3.4)	42.7 (3.2)
Stiffness	40.0 (4.5)	37.9 (4.2)	35.9 (3.9)	36.7 (3.7)
Function	45.0 (3.5)	37.6 (3.2)	45.1 (3.5)	44.7 (2.7)
Total	45.0 (3.3)	38.1 (3.1)	44.1 (3.4)	43.7 (2.8)

Patient characteristics presented as means (SEM) (except for gender* which is presented as female/male ratio).
BMI: Body Mass Index, KL: Kellgren and Lawrence grade, WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

Expression of COX-2

In order to evaluate whether treatment led to a decrease in the main target of NSAIDs, COX-2 activity, *ex vivo* COX-2 levels in cartilage and synovial tissue were determined by Western Blot (fig. 2). The no treatment group and celecoxib until surgery group were considered as most extremes and evaluated. A statistically significant decrease in COX-2 expression in both cartilage (35%, p=0.017) as well as synovial tissue (41%, p=0.001) was found, indicating that the drug had reached the knee joint in amounts able to decrease COX-2 amount in both tissues.

Figure 2. Intra-articular COX-2 expression



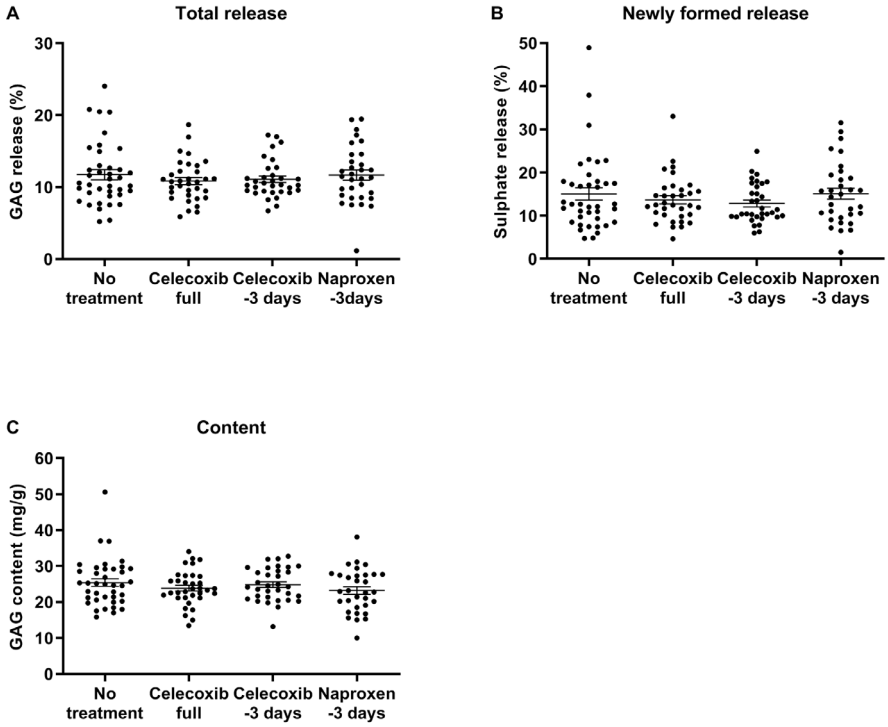
The COX-2 levels in cartilage (A) and synovial tissue (B) of the no treatment group and celecoxib until surgery group. Results are presented for individual patients (dots) and mean±SEM (dash with whiskers).

Proteoglycan turnover

Despite the fact that COX-2 was reduced in joint tissue of the celecoxib treated patients, this was not reflected in a changed *ex vivo* total proteoglycan release (fig. 3a), the primary outcome parameter of this study. No statistically significant effects on proteoglycan loss could be found (-7.5%, p=0.878 and -5.6%, p=0.990 for treatment until surgery and stopped three days in advance, respectively). The naproxen group also showed no change in proteoglycan loss (-0.5%, p=0.978).

Similar results were seen in the *ex vivo* retention of newly formed proteoglycans in the tissue; +9.0% for celecoxib until surgery, +14.7% for celecoxib stopped three days in advance and -0.4% for naproxen treated patients (all not statistically significant, fig. 3b). As anticipated for only a 4-week treatment period, *ex vivo* proteoglycan content was not statistically significant different between the four treatment groups (fig. 3c).

Figure 3. Proteoglycan turnover

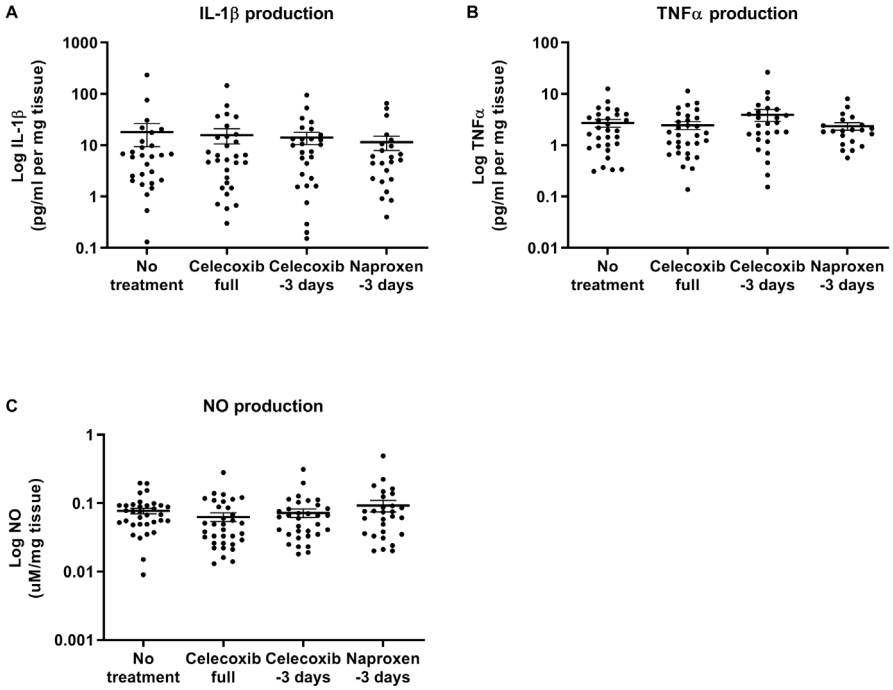


Proteoglycan release (A), release of newly formed proteoglycans (B) and proteoglycan content (C) of the four treatment groups. Results are expressed for individual patients (dots) and mean±SEM (dash with whiskers).
GAG: Glycosaminoglycans.

Synovial tissue inflammation

In order to evaluate inflammatory activity of synovial tissue, *ex vivo* IL-1β, TNFα, and NO production of synovial tissue were measured (fig. 4). NO levels of celecoxib treated patients suggested a decrease but were not statistically significant changed (-18.3%, p=0.164). For IL-1β and TNFα production by synovial tissue no (statistical) differences between groups were found either. As such, no clear anti-inflammatory effects could be demonstrated upon treatment with celecoxib.

Figure 4. Synovial inflammation



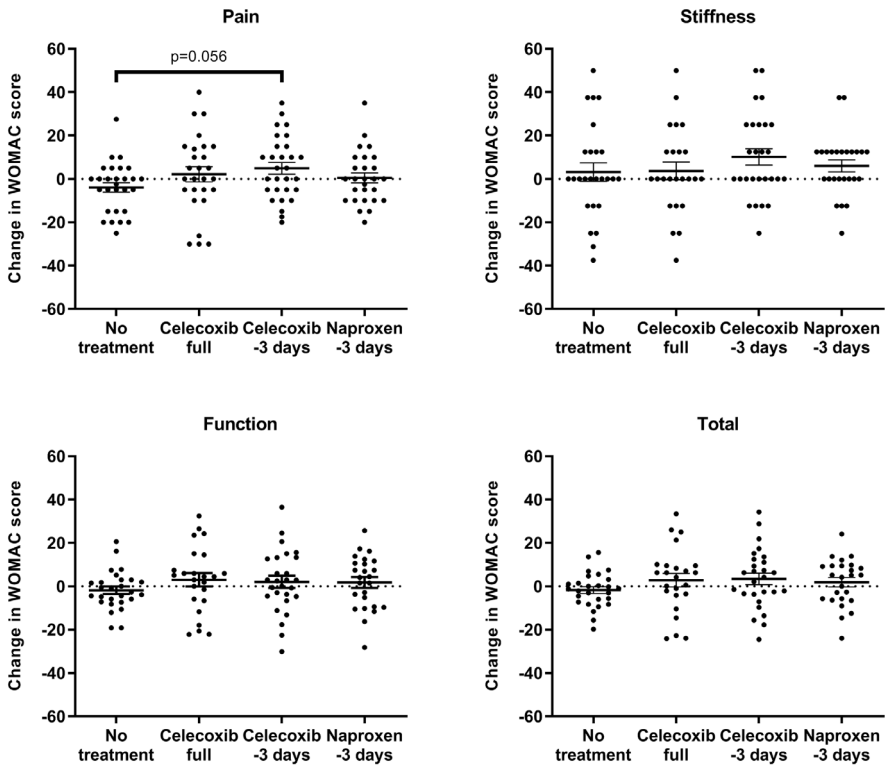
Ex vivo release of inflammatory cytokines IL-1β (A), TNFα (B) and NO (C) in 3-day synovial tissue culture media of the four treatment groups. Results are expressed for individual patients (dots) and mean±SEM (dash with whiskers). IL-1β: Interleukin-1β, TNFα: Tumor necrosis factor-α, NO: Nitric oxide.

WOMAC score

Although this study was not designed to evaluate clinical efficacy, changes in WOMAC scores before and after treatment were evaluated. One of the celecoxib treatment groups reported an improvement in WOMAC pain score, compared to the no treatment group (p=0.056 for celecoxib stopped three days in advance). The other celecoxib group showed a comparable trend (p=0.275). One can argue whether these changes were clinically relevant as actual changes are very small and patients were not blinded. Naproxen treatment did not show a change in WOMAC pain score (fig. 5a).

Comparable trends were obtained for the other WOMAC subscales (fig. 5b, 5c) and the total WOMAC score (fig. 5d).

Figure 5. WOMAC scores



Changes in WOMAC pain (A), stiffness (B), function (C) and total score (D) in the four treatment groups. Changes are calculated as scores after four weeks of treatment minus baseline scores. Results are expressed for individual patients (dots) and mean±SEM (dash with whiskers).

WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

Discussion

The present randomized controlled observer blinded trial could not confirm the previously reported DMOAD effect of a 4-week celecoxib treatment of severe OA prior to TKR surgery, despite using good powered group sizes (based on the pilot study[26]). The celecoxib group showed the most profound effect on proteoglycan release, the primary endpoint. Nonetheless, this effect was minor and not statistically significant. Similarly, the decreased NO production upon celecoxib treatment appeared not statistically significant. Very limited clinical benefit was reported by the patients receiving celecoxib. Taken together, the reported disease modifying effects of celecoxib *in vitro* and effects in small size clinical studies could not unambiguously be confirmed, however these findings are based on end-stage knee OA and might not hold for early OA.

No altered cartilage proteoglycan loss (via a direct effect on chondrocytes or indirectly via changing synovial cell activity) upon short-term treatment in this RCT could be demonstrated. This despite a decreased COX-2 expression in cartilage and synovial tissue demonstrating drug bioavailability within this short-term RCT, although this evaluation was limited to the patients who received full celecoxib treatment or no treatment for reasons mentioned previously. Extrapolating this to the other treatment groups these data suggest that the absence of effect on proteoglycan turnover cannot simply be explained by a lack of drug bioavailability in the joint during the 4-week treatment.

A great advantage of the set-up of this RCT is the opportunity to perform a full detailed biochemical analysis of the articular cartilage and other joint tissues while the treatment was given *in vivo*. A drawback is that in addition to the direct effects on cartilage (as evaluated thus far *in vitro*), there will be an effect on synovial tissue inflammatory activity, with expectedly indirect effects on cartilage. Moreover, the cartilage tissue studied involves severely damaged (end-stage disease) tissue.

Patients that received no treatment showed proteoglycan retention, - release, and - content typical for osteoarthritic cartilage[26,31]. But unanticipated, proteoglycan release was not statistically significant changed by treatment. This suggests that earlier studies using a similar protocol may have been biased by group size[26]. However, the present RCT, blinded for the primary outcome evaluation, has also its limitations: the condition of cartilage before start of treatment is unknown, enabling only unpaired evaluation. In case values were already different before start of medication despite randomization, which could be the case in patients on long-term NSAID treatment before inclusion for example, changes due to treatment might not have become apparent. It is expected that histochemical and radiographic evaluation of cartilage after treatment is representative of cartilage condition before start of treatment, as it is unlikely that histochemical grade and X-ray (Altman) score will change significantly in a relative short period of four weeks. It should be kept in mind

that cartilage tissue studied involves severely damaged (end-stage disease) tissue. Only hyaline cartilage that could be cut from the joint surface after replacement surgery was used. This is the limitation of the approach using *in vivo* treatment with extensive detailed *ex vivo* evaluation of tissues obtained after replacement surgery. It could be interesting to evaluate similar parameters in healthy or less severely damaged cartilage, as in earlier stages of OA, when treatment with NSAIDs is considered. But in such an approach biopsies need to be taken.

Others reported on *in vivo* studies in which celecoxib did seem to have a chondroprotective effect. An explanation for differences between those studies and ours could be duration of celecoxib treatment: four weeks prior to TKR surgery (based on our pilot study[26]) *vs* three months up to three years in other studies[25,36]. Although patients were told not to use any NSAIDs beside their treatment regime it cannot be ruled out that patients may have used NSAIDs (occasionally) on top of their prescribed dosage. When this happened more often in the no treatment group, because they were without pain medication compared to the treatment groups, this could have caused the lack of significant differences in the clinical outcome.

Another possible explanation of lack of clear statistically significant effects could be the number of patients who dropped out of the study. Based on a previous pilot study we included 172 patients with a power of 0.86. The loss of 34 patients led to a power of 0.82, a minimal decrease, not expected to be the sole cause of the absence of an effect. This is supported by the fact that there are no differences in outcome when the study is evaluated by intention to treat analyses *vs* per protocol analyses.

Both celecoxib treated groups show a tendency towards a lower percentage proteoglycan release compared to the no treatment group. Nonetheless these effect sizes noticed were not in the magnitude of the results shown in previous work, both *in vitro* as *in vivo*[17-19,26]. However, it should be kept in mind that a decrease of 5% or more in proteoglycan loss in four weeks can be of clinical relevance in case of long term treatment. Another (indirect) measure of treatment efficacy is the inhibition of proinflammatory cytokine release; in addition to analgesic effects, NSAIDs are known to have anti-inflammatory effects. This is best seen in the synovial lining of the affected knee. Both, celecoxib and naproxen treatment where unable to lower the release of IL-1β and TNFα by synovial tissue, but NO production could be diminished. A decrease in IL-1β and TNFα might have been expected, however in end-stage disease inflammation might be limited.

Corroborating the very mild effects of celecoxib on biochemical level, analgesic effects of celecoxib treatment evaluated by WOMAC scores were also limited. It might be that four weeks of medication is critical, and a minimum (and maybe too short) duration to observe clinical effects, although in clinical practice sufficient.

In conclusion, the present randomized controlled observer blinded clinical trial demonstrated no clear decrease in cartilage tissue proteoglycan release as surrogate for cartilage repair activity (via DMOAD activity) by a 4-week celecoxib treatment in end-stage disease, despite sufficient intra-articular availability of the drug. No adverse effects were found either.

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3 Expression of GM-CSF and its receptor in the synovium of osteoarthritis patients is negatively correlated with pain

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Abstract

Objectives

The crosstalk between the immune and nervous system in the regulation of osteoarthritis (OA) pain is increasingly becoming evident. Granulocyte macrophage-colony stimulating factor (GM-CSF) signals in both systems and might be a new treatment target to control OA pain. Anti GM-CSF treatment has analgesic effects in OA without affecting synovitis scores, suggesting that treatment effects are not caused by local anti-inflammatory effects. This study evaluates whether expression of GM-CSF and its receptor GM-CSFrα in synovial tissue is linked to synovial inflammation and/or knee pain in knee OA patients.

Methods

Cartilage and synovial tissue of knee OA patients (n=20) were collected during total knee replacement. Cartilage damage was evaluated by histology and *ex vivo* matrix proteoglycan turnover. Synovial inflammation was evaluated by histology and *ex vivo* synovial production of tumor necrosis factor-α (TNFα), prostaglandin E_2 (PGE_2), and nitric oxide (NO). Numbers of synovial tissue cells expressing GM-CSF or GM-CSFrα were determined by immunohistochemistry. Pain was evaluated using Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire and Visual Analogue Scale (VAS) knee pain.

Results

Collected cartilage and synovial tissue had a typical OA phenotype with enhanced cartilage damage and synovial inflammation. GM-CSF and GM-CSFrα expressing cells in the synovial sublining correlated negatively with knee pain. Cartilage damage and synovial inflammation did not correlate with knee pain.

Conclusion

Unanticipated, the negative correlation between synovial tissue cells expressing GM-CSF and/or GM-CSFrα and OA knee pain suggests that local presence of these molecules does not promote pain, and that the role of GM-CSF in OA pain is unrelated to local inflammation.

Introduction

Increasing evidence suggest a bidirectional crosstalk between the immune system and nervous system as a regulator of osteoarthritis (OA) pain, broadening the search for possible new treatment targets in OA pain. In a recently published review, Conaghan and colleagues focused on inflammation and peripheral nociception and suggested granulocyte macrophage colony-stimulating factor (GM-CSF) as one of the possible targets[1].

GM-CSF is a hematopoietic growth factor[2], but also has an important role in maintaining autoimmunity (e.g. after chemotherapy) and in inducing inflammation by activating various cell types[3]. GM-CSF also has direct effects on neurons, increasing mechanical hypersensitivity, as well as indirect effects via cyclooxygenase or chemokine (C-C motif) ligand 17 (CCL17)- and eicosanoid-dependent pathways[4]. GM-CSF is expressed in synovial membranes of rheumatoid arthritis (RA) as well as OA patients[5]. Several clinical trials with drugs targeting GM-CSF or its receptor (GM-CSFr) show beneficial effects in the treatment of RA. Mavrilimumab, a fully human monoclonal antibody (mAb) targeting GM-CSFr, improved the Disease Activity Score 28 score after 12 weeks, and resulted in a greater percentage of American College of Rheumatology-20 responders at week 24 in a phase IIb randomized placebo-controlled trial in patients with moderate-severe RA[6]. Two human mAbs blocking GM-CSF, MOR103 and namilumab, also had beneficial effects and were well tolerated in RA[7,8].

In OA, GM-CSF plays a role in disease and pain progression as well. In GM-CSF deficient mice the development of joint damage and OA pain in a collagenase-induced instability mouse model of OA was significantly lower compared to wild-type mice[9]. In a phase IIa study in patients with inflammatory hand OA, anti-GM-CSF mAb induced larger reductions in hand pain, compared to placebo, although little differences were found in numbers of tender and swollen joints or MRI inflammation related endpoints[10]. The discrepancy between improvement in clinical pain scores and unaltered synovitis supports the idea that OA pain is not just a result of local inflammation but that other mechanisms of action, possible systemic or related to the nervous system, play a role as well[1]. To investigate whether GM-CSF and its receptor are related to knee pain in knee OA patients and, if so, the role of synovial inflammation therein, we evaluate the correlation between the number of cells expressing GM-CSF or GM-CSFrα in the synovial tissue, inflammation, and clinical knee pain in OA patients.

Methods

Patient selection

This study is a post hoc evaluation of data of the control group of a study on the disease modifying effects of celecoxib in end-stage OA, registered under number NL18274.101.0711.

Twenty patients from the no treatment trial arm, that did not receive medication in the year before total knee replacement (TKR), were included for the present evaluation and additional analyses.

The trial was performed in accordance with the Declaration of Helsinki and approved by the Dutch Central Committee on research Involving Human Subjects (CCMO). All patients signed informed consent before inclusion.

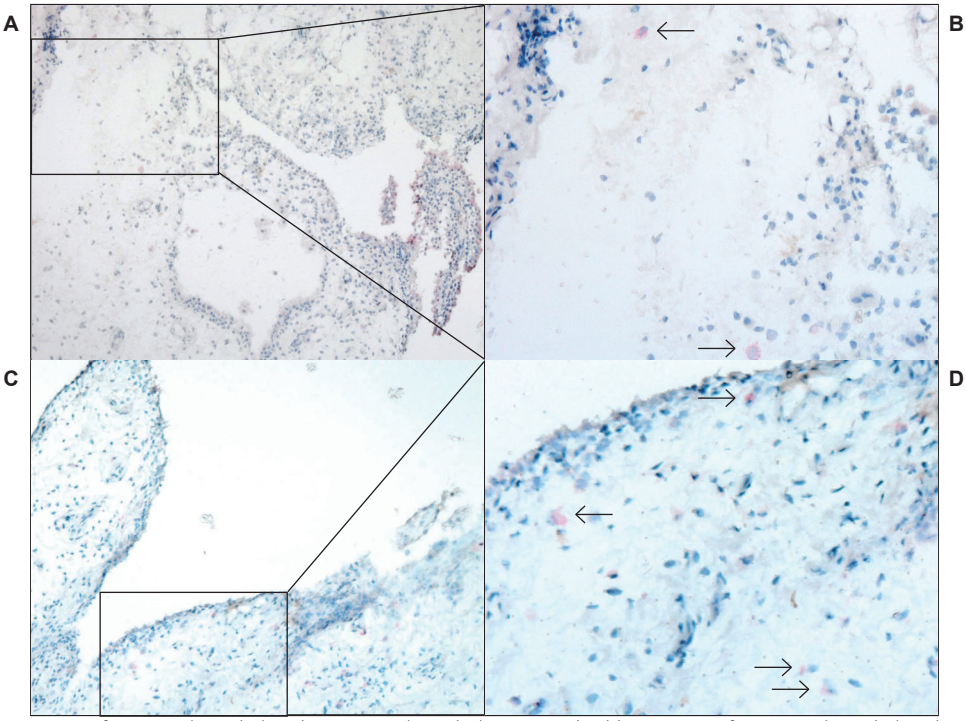
Clinical outcome parameters for pain

Pain was evaluated using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire pain subscale (0=maximum pain, 100=no pain) and the Visual Analogue Scale (VAS) for pain (mean of VAS night, VAS morning and VAS general; 0=no pain, 100=maximum pain). Both were collected twice (six weeks before surgery and just before surgery) and the average value of both time points was used.

Immunohistochemistry of GM-CSF(r)

At TKR surgery, synovial tissue samples were collected and kept in phosphate buffered saline for transport. Two samples of each donor were used for immunohistochemistry. Samples were embedded in Tissue Tek and stored at -80°C. For the present study 6µm slices were cut with a cryotome and incubated with primary antibodies for GM-CSF (LS-C41956; LSBio) or GM-CSFrα (CD116, alpha chain of GM-CSFr; LS-C40980, LSBio) for one hour at room temperature. Subsequently, sections of both stainings were incubated with a secondary antibody (BrightVision Poly-AP-Anti Ms/Rb/Ra) for 30min. Sections were counterstained with Alkaline Phosphate substrate and scored microscopically (magnification 40x) by one observer. The number of positive cells per mm^2 synovial sublining was determined for GM-CSF as well as GM-CSFrα. Figure 1 shows an example of the IHC staining.

Figure 1. Expression of GM-CSF and GM-CSFrα in synovial sublining



Expression of GM-CSF (A and B) and GM-CSFrα (C and D) in synovial sublining. Magnification 4x (A and C) and 10x (B and D). Arrows indicate cells positive for target.

Evaluation of synovial inflammation

Two representative synovial tissue samples of each donor were embedded in paraffin, cut in 5µm sections and stained with Haematoxylin-Eosin to evaluate synovial inflammation using modified Goldenberg and Cohen score (range 0-10).

Two additional samples of each patient were cultured for three days in Dulbecco's modified Eagle medium (DMEM) supplemented with glutamine (2mM), penicillin (100 IU/mL), streptomycin sulphate (100µg/mL), ascorbic acid (0.85 mM) and 10% human male AB+ serum to determine *ex vivo* produced inflammatory mediators. Supernatants were collected and centrifuged (1300xg). To determine prostaglandin E_2 (PGE_2) enzyme immuno assay was used. Tumor necrosis factor-α (TNFα) was determined using enzyme linked immunosorbent assay. Nitric oxide (NO) was determined using the standard Griess reaction. Detailed procedures were published previously[11].

Evaluation of structural cartilage damage

Cartilage samples were collected simultaneously with the synovial tissue. Two representative samples of each donor were embedded in paraffin, cut in 5µm sections and stained with

Safranin-O-fast-green to evaluate microscopic cartilage damage using modified Mankin score (range 0-11).

Eight cartilage samples were cultured *ex vivo* at 37°C and proteoglycan (PG) synthesis and release were determined as measures of chondrocyte activity as described before[12]. In short, after one hour of pre-culture, 20µCI Na235SO4 in DMEM was added to each sample. After four hours of labeling, samples were washed 3x 45 minutes in medium and subsequently cultured for another three days in order to determine *ex vivo* release. Next, samples were digested with papain for two hours at 65°C. Glycosaminoglycans (GAGs) were precipitated by addition of cetylpyridium chloride. $^{35}SO^{42-}$-labelled GAGs were measured by liquid scintillation on a TRI-CARB 2800TR. Values were normalized to specific activity of the pulse medium, pulse time, and wet weight of the explants and expressed as nmoles of sulphate incorporated per hour per gram wet weight of the cartilage (nmol/h/g). For the total release of PGs, Alcian Blue staining of the medium was quantified photometrically with chondroitin sulphate as a reference. The total amount of GAGs released (blue staining) is expressed as a percentage of the original tissue content (%GAG release).

Statistical analysis

WOMAC scores were converted to 100-WOMAC. By doing that, for WOMAC as well as for VAS pain a higher value on the x-axis reflects more pain. All statistical tests were performed using IBM SPSS Statistics version 25.0.0.2. Shapiro-Wilk tests were used to see whether parameters were normally distributed. In case parameters were not normally distributed, a log transformation was performed (NO and PG release). Pearson's correlation coefficient was determined to evaluate correlations between all parameters. For PGE_2 log transformation did not result in a normal distribution, therefore a Spearman correlation coefficient was determined instead. P-values <0.05 were considered statistically significant.

Results

Patient characteristics

Patients represented a typical OA population, comparable to the original cohort[11]; age 69.3 (5.7) years, 13/20 female (65%), BMI 26.7 (4.7) and Kellgren and Lawrence (KL) grade ≥2 (table 1).

Table 1. Patient characteristics

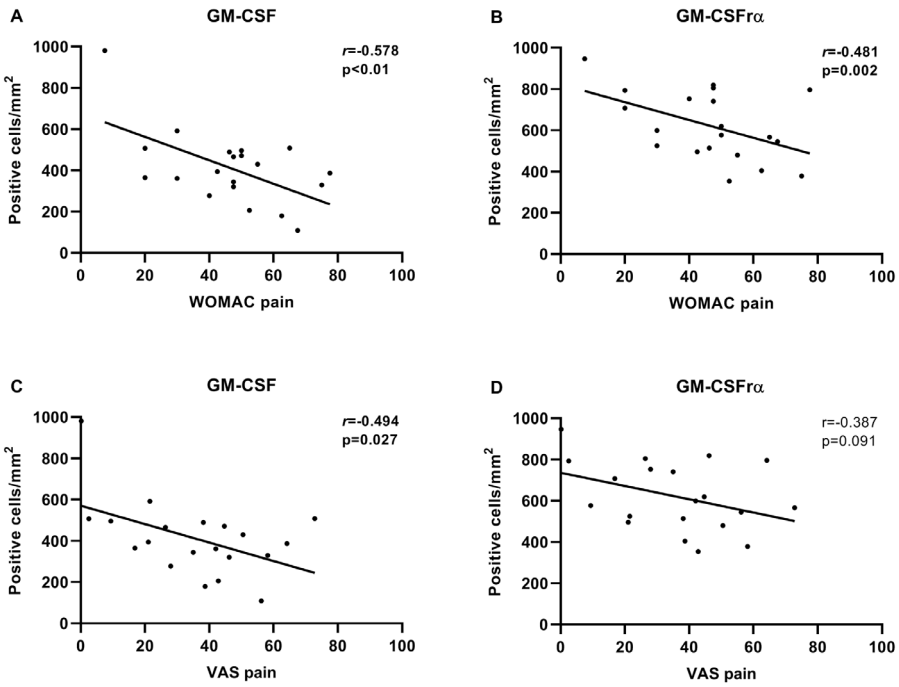
	Gender	*Age (years)*	*BMI*	*KL grade*	*Safranin O*	*HE*
1.	F	64	24.1	3	3.6	4.7
2.	F	76	33.2	3	5.7	5.0
3.	F	61	20.8	2	4.3	3.3
4.	F	73	26.4	4	4.7	3.3
5.	M	79	28.4	3	4.1	4.3
6.	F	71	35.4	3	8.9	3.8
7.	F	70	29.8	3.5	3.2	4.0
8.	F	74	22.5	3	4.0	6.0
9.	M	68	30.3	4	7.4	6.8
10.	M	61	24.4	3	7.3	4.8
11.	M	64	24.5	4	3.7	4.0
12.	M	62	22.2	3	3.7	3.3
13.	F	78		4	4.8	5.5
14.	M	63	23.3	3	5.8	6.3
15.	F	61	22.8	4	4.5	5.0
16.	M	68	37.9	3	4.7	5.0
17.	F	73	24.4	3	5.3	4.7
18.	F	69	26.0	3	5.0	6.0
19.	F	69	26.2			6.3
20.	F	71	23.9	3	5.2	
Mean	F: 13/20	69.3	26.7	3.2	5.0	4.8
SD	(65%)	5.7	4.7	0.5	1.5	1.1
ORIGINAL COHORT (n=172)						
Mean	F: 119/172	67.5	29.5	3.1	4.7	5.0
SD	(69%)	8.4	5.3	0.7	1.0	1.2

BMI: Body Mass Index, KL: Kellgren and Lawrence grade, Saf O: Safranin-O fast green-iron haematoxylin staining of cartilage samples, HE: Hematoxylin-Eosin staining of synovial tissue samples.

GM-CSF and GM-CSFrα expressing cells in synovial sublining correlated with clinical OA pain and inflammation

Correlations between GM-CSF and GM-CSFrα expressing cells in synovial sublining and clinical pain are shown in fig. 2. Number of GM-CSF expressing cells in the synovial sublining was negatively correlated with WOMAC knee pain; *r*=-0.578, p<0.01, and VAS knee pain; *r*=-0.494, p=0.027. For GM-CSFrα expressing cells in the synovial sublining negative correlations were found as well; *r*=-0.481, p=0.032 for WOMAC pain and *r*=-0.387, p=0.091 for VAS pain.

Figure 2. Correlation between GM-CSF and GM-CSFrα expression and WOMAC and VAS knee pain



Negative correlations were found between number of cells expressing GM-CSF or GM-CSFrα in the synovial sublining and clinical knee pain measured by WOMAC and VAS pain score (mean of VAS night, VAS morning and VAS general). Data is represented as individual values (dots) and a correlation (continuous line).
WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index, VAS: Visual analogue scale.

Ex vivo production of the inflammatory synovial mediators TNFα, PGE_2, and NO was negatively correlated with GM-CSF expressing cells, but not GM-CSFrα expressing cells. No relations between number of cells expressing GM-CSF or its receptor and cartilage damage or chondrocyte activity were detected (table 2).

Table 2. Correlations between GM-CSF and GM-CSFrα and markers for inflammation and cartilage damage

	GM-CSF		*GM-CSFrα*	
	r	***p-value***	***r***	***p-value***
INFLAMMATION				
Synovium histology	-0.165	0.499	-0.083	0.737
TNFα	**-0.487**	**0.029**	-0.144	0.545
PGE_2*	**-0.482**	**0.037**	-0.212	0.382
Ln(NO)	**-0.603**	**0.006**	-0.285	0.237
STRUCTURAL DAMAGE				
Cartilage histology	-0.139	0.571	-0.216	0.374
Proteoglycan synthesis	-0.005	0.984	0.242	0.305
Ln(Proteoglycan release)	-0.015	0.951	-0.277	0.237

Results are given as Pearson's correlation coefficients, except for PGE_2. Spearman correlation coefficient is given instead*.

GM-CSF: Granylocyte macrophage-colony stimulating factor, GM-CSFrα: Granylocyte macrophage-colony stimulating factor receptor-α, TNFα: Tumor necrosis factor-α, PGE_2: Prostaglandin E_2, Ln: Natural logarithm, NO: Nitric oxide.

There was no statistically significant correlation between clinical pain and inflammation or cartilage damage and chondrocyte activity (table 3).

Table 3. Correlations between markers for inflammation and cartilage damage and clinical pain

	WOMAC pain		*VAS pain*	
	r	***p-value***	***r***	***p-value***
INFLAMMATION				
Synovium histology	0.117	0.634	0.058	0.814
TNFα	0.216	0.361	0.180	0.449
PGE_2*	0.165	0.500	0.181	0.459
Ln(NO)	0.200	0.411	0.225	0.354
STRUCTURAL DAMAGE				
Cartilage histology	0.010	0.969	0.138	0.572
Proteoglycan synthesis	-0.246	0.269	-0.214	0.364
Ln(Proteoglycan release)	0.150	0.527	0.234	0.322

Results are given as Pearson's correlation coefficients, except for PGE_2. Spearman correlation coefficient is given instead*.

WOMAC: Western Ontario and McMaster Universities Index, VAS: Visual analogue scale, TNFα: Tumor necrosis factor-α, PGE_2: Prostaglandin E_2, Ln: Natural logarithm, NO: Nitric oxide.

Discussion

In this study we identified that the number of cells expressing GM-CSF and GM-CSFrα in the sublining of the synovial tissue was negatively correlated with OA pain. It is known that in human[13] and equine[14] OA joints, nerve fiber density is decreased in superficial layers of synovial tissue, closer to the synovial surface. Therefore, in this study, only the deeper layer (sublining) of the synovial tissue was examined.

Negative correlations between synovial GM-CSF and GM-CSFrα expression and WOMAC and VAS knee pain were found. The correlations between GM-CSF and pain might be skewed by one data point. Sensitivity analysis leaving out this value resulted in negative correlations with p-values of 0.293 and 0.210 for VAS and WOMAC pain, respectively. This would mean a tendency towards the negative correlation (or even absence of a correlation) between GM-CSF and pain, but not a positive correlation. We therefore considered our general conclusion valid. Taken the limited number of patients available into account, it was chosen to include all values of all subjects in the analyses.

The negative correlation suggests that local GM-CSF at the synovium likely does not promote pain in advanced OA. Intriguingly, systemic neutralization of GM-CSF with neutralizing antibodies reduces pain and disease progression in an experimental model of OA[9] and inflammatory hand OA in humans[10], indicating that GM-CSF promotes pain. Possibly the stage of OA affects whether GM-CSF contributes to OA pain locally and/or systemically. Structural changes (e.g. cartilage degeneration and/or osteophyte formation) are more prominent in advanced OA, whilst in early OA more features of synovial inflammation can be present[15]. Otherwise, differences in tissue-dependent contributions of GM-CSF may be at the core of these differences in the contribution of GM-CSF in OA pain.

The absence of a correlation between inflammatory markers and OA pain suggests that local synovitis is not the only mechanism contributing to OA pain. The lack of a strong correlation between radiographic damage and knee pain[16] also suggests involvement of other pain mechanisms than that caused directly by damage of the joint. Indeed intrathecally administered mAb against GM-CSF reduced pain in a mouse model for neuropathic pain, indicating that GM-CSF in the central nervous system contributes to pain[17]. Neuropathic pain mechanisms and the central nervous system are also involved in OA pain[18,19] indicating OA pain is not only the result of sensitization/activation of peripheral sensory neurons by damage/inflammation. For example, significant relationships were found between peripheral sensitization of local nociceptors (knee) and central sensitization of dorsal horn neurons (leg, arm), detected by lower pressure pain thresholds, and pain intensity in OA patients. Moreover, OA patients have enhanced evoked temporal summation and reduced descending inhibition pointing to abberant central pain processing[18]. Future research needs to demonstrate whether the analgesic effects of peripheral anti GM-CSF treatments are caused through an effect at the central nervous system, locally at the joint, or otherwise.

In our study population, central sensitization most likely plays a dominant role in pain as patients had advanced knee OA, which might explain the absence of a positive correlation between local factors including GM-CSF and GM-CSFrα and knee pain parameters found in this study.

In the EULAR recommendations for pain management in inflammatory arthritis and osteoarthritis a biopsychosocial model of pain is recommended[20]. It is important to realize that OA pain never only encompasses biological factors, whether local or systemic, but also psychological and social factors.

OA is a very heterogeneous disease, consisting of multiple OA phenotypes (subtypes) with distinct underlying pathobiological and pain mechanisms and structural and functional consequences. The correlations between GM-CSF and GM-CSFrα and local and systemic pain parameters most likely vary between these OA phenotypes. Future research should focus on selecting OA patients with specific OA phenotypes, to see whether the role of GM-CSF differs between those groups and evaluate if one of these phenotypes might be more susceptible for beneficial effects of treatment targeting GM-CSF or its receptor.

In conclusion, the negative correlation between local GM-CSF and GM-CSFrα expression and knee OA pain suggests these molecules not to be locally involved in OA knee pain development and supports a more systemic role of GM-CSF in OA pain, possibly related to the nervous system.

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4 The role of interleukin-4 and interleukin-10 in osteoarthritic joint disease: a systematic narrative review

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Abstract

Objectives

A fusion protein of Interleukin-4 and -10 (IL4-10 FP) was developed as disease modifying osteoarthritis drug (DMOAD), and chondroprotection, anti-inflammation and analgesia have been suggested. To better understand the mechanisms behind its potential as a DMOAD, this systematic narrative review aims to assess the potential of IL-4, IL-10 and the combination of IL-4 and IL-10 for treatment of osteoarthritis. It describes the chondroprotective, anti-inflammatory and analgesic effects of IL-4, IL-10, and the IL4-10 FP.

Methods

Pubmed and Embase were searched for publications published from 1990 until May 21th 2021 (moment of search). Key search terms were: Osteoarthritis, Interleukin-4, and Interleukin-10. This yielded 2479 hits, of which 43 were included in this review.

Results

IL-4 and IL-10 showed mainly protective effects on osteoarthritis (OA) cartilage *in vitro* and *in vivo*, as did the IL4-10 FP. Both cytokines showed anti-inflammatory effects, but also pro-inflammatory effects. Although only *in vitro,* the IL4-10 FP showed purely anti-inflammatory effects, indicating pro-inflammatory effects of one cytokine can be counteracted by the other when given as a combination. Only a few studies investigated the analgesic effects of IL-4, IL-10 or the IL4-10 FP. *In vitro,* IL-4 and the IL4-10 FP were able to decrease pain mediators. *In vivo,* IL-4, IL-10 and the IL4-10 FP were able to reduce pain.

Conclusion

In conclusion, this review describes overlapping, but also different modes of action for the DMOAD effects of IL-4 and IL-10, giving an explanation for the synergistic effects found when applied as combination, as is the case for IL4-10 FP.

Introduction

Osteoarthritis (OA) is a progressive joint disease characterized by changes in multiple joint tissues, leading to pain, stiffness, and loss of function[1]. Cartilage, bone, and synovial tissue show prominent structural changes in OA, and pain is the most important symptom and the reason for OA patients to seek medical assistance. An ideal OA treatment reduces symptoms, but also prevents further structural damage by combining chondroprotective, anti-inflammatory, and analgesic effects all in one disease modifying OA drug (DMOAD). None of the current potential DMOADs have yet been approved for the treatment of OA by regulatory authorities worldwide. As criteria for approval of a DMOAD, a drug needs to provide both structural and clinical improvement[2,3]. The persisting effort over the past years into development of new DMOADs has generated promising leads and candidate therapies. One of these promising approaches is the usage of anabolic stimuli.

Regulatory cytokines such as interleukin-4 (IL-4) and IL-10, well known for their anti-inflammatory activity, are also anabolic stimulating cytokines, that are produced by a variety of immune cells. IL-4 acts via two types of heterodimeric IL-4 receptors (IL-4R), expressed on numerous cell types, both immune and non-immune cells. Type 1 consists of the IL-4Rα subunit and the common γ chain (γc), while Type 2 consists of IL-4Rα and IL-13Rα1 chains[4,5]. The IL-10 receptor is composed of two subunits, IL-10R1 and IL-10R2[5] and also expressed on immune and non-immune cells. In the osteoarthritic joint increased expression of both IL-4 and IL-10 receptors has been demonstrated. For example chondrocytes express the IL-10R[6,7], and both types of the IL-4R[4,7]. Importantly, IL-4 and IL-10 have chondroprotective effects. *In vivo,* IL-4 influences proteoglycan metabolism by inhibition of matrix metalloproteinases (MMPs), and prevents apoptosis of chondrocytes and fibroblast like synoviocytes (FLS)[5]. IL-10 stimulates synthesis of collagen type II and aggrecan, two important proteins in the extracellular matrix (ECM) of cartilage, affects proteoglycan metabolism, reduces MMPs, and, like IL-4, prevents apoptosis of chondrocytes[5].

IL-4 and IL-10 have been tested for their anti-inflammatory and chondroprotective effects for treatment of rheumatoid arthritis (RA), however results were inconsistent. IL-10 has some proinflammatory properties, including stimulation of B cell activity and upregulation of Fc receptors on antigen presenting cells that in certain conditions could counteract its strong anti-inflammatory properties. This along with the short half-life of IL-10 has been suggested as potential explanations for the somewhat disappointing results. Both *in vitro* and *in vivo* IL-10 was shown to increase Fc receptors on myeloid cells in the circulation of RA patients treated with IL-10[8]. Nonetheless, in experimental *in vitro* an *in vivo* models IL-10 (and IL-4) strongly prevented inflammation-induced cartilage degeneration, with combining both cytokines having additive effects. IL-10 also directly stimulated proteoglycan synthesis in cartilage explants[9]. Moreover, in psoriatic arthritis, significant immune modulation was found after subcutaneous administered IL-10, however no beneficial effects were found on clinical manifestations[10].

Recently, the interest in IL-4 and IL-10 as therapeutics has been fueled by the development of a fusion protein of IL-4 and IL-10 (IL4-10 FP) to promote efficacy of the individual cytokines by facilitating synergy, promoting unique signaling[11] and enhanced bioavailability[12].

Since its development, the effects of the IL4-10 FP have been evaluated in multiple studies and joint diseases. IL4-10 FP reversed persistent inflammatory pain in multiple mouse models[12], protected against blood-induced cartilage damage and inhibited production of pro-inflammatory cytokines *in vitro*[13,14], attenuated cartilage damage but not synovial inflammation in a mice model of hemophilic arthropathy[13], and reduced disease severity in established experimental arthritis in mice[14].

Compared to healthy cartilage, OA cartilage expresses increased levels of IL-4R and IL-10[7], indicating OA cartilage may become more responsive for the effects of IL4-10 FP. Studies evaluating the efficacy of IL4-10 FP in OA indeed show promising results. IL4-10 FP has chondroprotective and anti-inflammatory effects in OA cartilage explants[7], and chondroprotective and analgesic effects in a canine OA model, as well as in a rat OA model[15,16].

This systematic narrative review aims to assess the potential of IL-4, IL-10 and the combination of IL-4 and IL-10 for treatment of osteoarthritis. It describes the chondroprotective, anti-inflammatory and analgesic effects of IL-4, IL-10, and the IL4-10 FP to better understand the mechanisms behind its potential as a DMOAD.

Methods

Study selection

Pubmed and Embase were searched for publications published from 1990 until May 21th 2021 (moment of search). Key search terms were: Osteoarthritis, Interleukin-4, and Interleukin-10. Duplicates were removed and the remaining articles were screened based on title and abstract by two reviewers (EH and EMH). Disagreements between reviewers were resolved by consensus including a third reviewer (SCM). Eligibility criteria were English language and availability of full text. All study types, describing direct effects of IL-4, IL-10 or the IL4-10 FP on OA joint tissues or patient reported outcome measures were included. Reviews were excluded, but their reference lists were checked for additional articles.

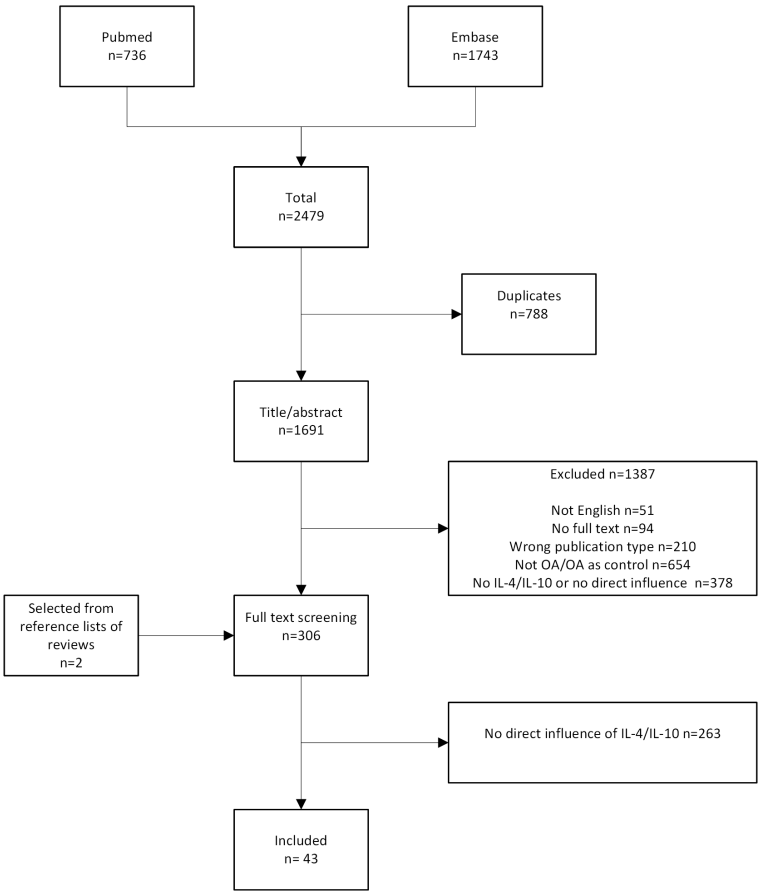
Data collection

Full texts of included articles were screened by two authors (EH and EMH) to extract information on: (1) cytokine of interest, (2) experimental set-up, and (3) chondroprotective, anti-inflammatory, and/or analgesic effects described. Remaining articles were grouped and described per DMOAD characteristic (chondroprotective, anti-inflammatory, and analgesic).

Results

The initial search yielded 2479 results. After removal of duplicates (n=788), 1691 articles remained. Selection on title/abstract led to an additional 1387 exclusions for several reasons; not written in English (n=51), no full text available (n=94), wrong publication type (e.g. reviews, letter to the editor, commentary on original articles, etc., n=210), not about OA or OA only used as control group (n=654), and IL-4, IL-10, or IL4-10 FP not used as intervention (only as outcome measurement) (n=378). Reference lists of excluded reviews fetched two more articles eligible for inclusion, which led to a total of 306 articles for screening of full text. 263 out of 306 articles did not describe an effect of IL-4 or IL-10 on OA joint tissues or patient reported outcome measures and were excluded. The remaining 43 articles were included in this review (fig. 1).

Figure 1. Selection process



OA: osteoarthritis, IL-4: Interleukin-4, IL-10: Interleukin-10.

Chondroprotective effects

A total of 26 articles investigated chondroprotective effects of either IL-4 or IL-10 or a combination of both.

In unstimulated human OA cartilage explants, IL-4 had no effect on glycosaminoglycan (GAG) release, or on expression of enzymes that can induce GAG release (A desintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 and -5)[17]. Similarly, macrophage conditioned medium (MCM) of human monocyte derived macrophages stimulated with IL-4 (M(IL-4)) did not affect GAG release and expression of ADAMTS-4 and -5, collagen (COL) 2A1, MMP-1, or MMP-13[18].

In chondrocytes stimulated with inflammatory cytokines, IL-4 treatment had positive effects on collagen levels. IL-4 reduced collagenase production[19,20], increased the mRNA expression of type II collagen[21-23], and lowered the release of total collagen[22]. This was confirmed by immunohistochemistry[21]. In addition, IL-4 increased expression of proteoglycans[21], by enhancing synthesis[24], and reducing release[22]. More specifically, IL-4 normalized the IL-1β- and Tumor necrosis factor-α (TNFα)-induced reduction of aggrecan mRNA expression[21,22], reduced mRNA expression of Cathepsin B after cyclic tensile stress[25], and reduced ADAMTS-4 mRNA expression, but without effect on protein expression[26]. IL-4 also reduced the synthesis of MMPs at mRNA and protein levels[19,21,23,26-30], and induced CBP/P300-interacting transactivator 2 (CITED2), which downregulates MMP-13[29]. Knockdown of IL-4 in IL-1β-stimulated chondrocytes decreased cell viability and increased apoptosis[31] indicating an essential role of IL-4 in regulating survival of chondrocytes. IL-4 reduced IL-1β-induced proliferation of dedifferentiated chondrocytes, but not primary chondrocytes[19,24].

In human OA cartilage explants that were exposed to mechanical compression, IL-4 protected the explants against histological degeneration and it increased the number of transcription factor SOX9-expressing chondrocytes compared to uncompressed cartilage explants. IL-4 did not affect SOX9 mRNA expression[17]. This protective effect of IL-4 was confirmed in a rat study, where IL-4 decreased the MMP-13 synthesis after mechanical loading[25]. Furthermore, neutralizing IL-4 antibodies blocked the positive effects of mechanical stimulation (increased aggrecan and decreased MMP-13) in normal chondrocytes but not in OA chondrocytes, suggesting an important role of IL-4 in the anabolic response to mechanical stimulation in healthy cartilage, but not in diseased cartilage[32].

In an *in vivo* rat model for OA, induced by anterior cruciate ligament tear and medical meniscectomy, IL-4 transfected spheroids of mesenchymal stem cells (MSCs) reduced chondrocyte apoptosis, signs of histological cartilage degeneration, and MMP-13 expression[23]. In an IL-4 knock-out mice model, exposed to treadmill running, CITED2 mRNA and protein levels were lower compared to wild-type mice, whilst MMP-13 levels were slightly higher[29], confirming that CITED2 is a pivotal downstream molecule in IL-4 mediated

MMP-13 reduction. Lastly, intra-articular injection with IL-4 inhibited cartilage destruction in two surgically induced OA models[29,33].

In unstimulated chondrocytes, IL-10 did not affect collagenase 3 (which cleaves collagen II) levels[20], but an adenoviral vector overexpressing IL-10 slightly increased MMP-13 expression[34]. In addition, in unstimulated human OA cartilage explants, M(IL-10) MCM had no effect on COL2A1 expression[18]. In line with this, overexpression of IL-10 in bone marrow mesenchymal stem cells (BM-MSCs) with Adeno Associated Virus (AAV) IL-10 did not affect GAG release or content[30].

In contrast, the BM-MSCs reduced MMP-13 expression in IL-1β/TNFα-stimulated cartilage explants, but BM-MSCs transduced with AAV null had the same effect, suggesting the positive effect is not related to IL-10 expression[30]. Nevertheless, in isolated chondrocytes stimulated with inflammatory cytokines, IL-10 overexpression (using an adenovirus or lentivirus, respectively) upregulated type II collagen and aggrecan[34,35], and downregulated type X collagen[35] mRNA and MMP-13 expression[34]. Recombinant IL-10 also impaired MMP-13 expression, but had no effect on TNFα-mediated aggrecan expression[34].

IL-10 reduced injury induced chondrocyte apoptosis, COL10A1 and COL1A1 expression, GAG release, MMP-13 synthesis and ADAMTS-4 mRNA expression[36,37]. In addition, IL-10 restored COL2A1 expression, and increased GAG content, and mRNA expression of aggrecan and transcription factor SOX9[37].

In vivo, in a murine collagenase-induced OA model, human MSCs overexpressing viral IL-10, a product of Epstein-Barr virus that exhibits 84% amino acid sequence homology with human IL-10, reduced the percentage of CD4+ and CD8+ T cells in popliteal lymph nodes, however histologically, no effects on cartilage were found[38]. In a rabbit model, the combination of IL-10 and IL-1Ra gene therapy markedly reduced cartilage pathology and decreased proteoglycan loss, and had greater chondroprotective effects than either of these cytokines alone[39].

Studies in which the fusion protein of both cytokines (IL4-10 FP) was used revealed that IL4-10 FP normalized the Osteoarthritis Research Society International cartilage structural damage score that was increased in the canine or rat OA Groove model[15]. Moreover, IL4-10 FP increased proteoglycan synthesis and reduced proteoglycan release in OA cartilage explants[7,15], and normalized the reduced proteoglycan content in an *in vivo* canine OA model[15,16]. IL4-10 FP reduced MMP-3 expression, but slightly increased MMP-1 expression in OA chondrocytes, whereas in synovial fibroblasts both were reduced. The release of tissue inhibitor of metalloproteinases (TIMP-1, which inhibits MMPs) was not affected by IL4-10 FP[7].

Anti-inflammatory effects

Even more articles (n=35) reported on anti-inflammatory effects of both cytokines in OA models on multiple levels.

IL-4 may exert anti-inflammatory action because it is able to compromise the binding of TNFα to its cell-surface receptors in synovial fibroblasts. IL-4 mildly upregulated cell-surface TNFR, but in addition increases the level of soluble TNFR-75, competing with cell-surface TNFR for binding of TNFα[40]. IL-4 increased IL-6[41] and Chemokine (C-C motif) ligand (CCL2) production[42] in OA synovial fibroblasts, indicating some pro-inflammatory effects in OA tissue. However, IL-4 also antagonized interferon-γ (IFNγ)-induced expression of Intercellular Adhesion Molecule 1[41], and promoted expression of the anti-inflammatory cytokine IL-1Ra[42]. Besides, IL-4 inhibited expression of cyclooxygenase-2 (COX-2), the main enzyme for PGE_2 production after TNFα stimulation[40], and IL-1β-induced proliferation[19], and Prostaglandin (PGE) production[42] of synoviocytes. IL-4 reduced IL-1β and TNFα production in lipopolysaccharide (LPS)[43]-, or Leukotriene B_4 (LTB_4)-stimulated OA synovium[44], but had no effect on these cytokines in unstimulated tissue[44].

Next to cells from the synovial tissue, chondrocytes are able to produce inflammatory mediators as well. In unstimulated human OA chondrocytes, IL-4 had no effect on levels of arachidonic acid, phospholipase A2 (PLA2), COX-2, mPGES-1, or PGE_2 expression[45-47], suggesting a PLA2 and eicosanoid-independent mechanism of action[46]. Indeed, addition of the Nitric Oxide Synthesis (NOS) inhibitor ι-NIO abolished the effects of IL-4, suggesting a NO dependent mechanism of IL-4 in the downregulation of IL-1β-induced PGE_2[24], but no direct effect of IL-4 on NO production was found in primary human OA chondrocytes[19].

IL-4 inhibits NO production in chondrocytes stimulated with pro-inflammatory cytokines[19,21-24,28,48], although no effect was found on the IL-17-induced NO production in human OA chondrocytes[49]. IL-4 inhibited CCL5, CCL3, and CCL4 mRNA and protein expression, but did not affect C-X-C motif chemokine 1 (CXCL)1 and CXCL8 expression[26]. Knockdown of IL-4 led to an increase in TNFα, IFNγ, and IL-6 in IL-1β-stimulated chondrocytes[31].

Pre-treatment of rat chondrocytes with IL-4 reduced mechanical stress induced expression of IL-1β[25]. Likewise, rIL-4 suppressed the mechanical stress induced iNOS mRNA, and NO expression in rat chondrocytes[33].

Transfection of IL-1β/TNFα-stimulated canine chondrocytes with IL-4 gene therapy using a COX-2 or Cytomegalovirus (CMV) promotor reduced COX-2 and mPGES-1 expression, and with that downregulated PGE_2 release[22,28], and reduced production of IL-1β, TNFα, IL-6, and IL-8[21,22,27,28]. Furthermore, IL-4 upregulated IL-1Ra[22,28] and insulin-like growth factor 1 (IGF-1; responsible for de novo synthesis of collagen and upregulation of proteoglycan production)[22,28], and reduced the expression of binding protein and receptors for IGF-1[21]. Besides, neutralizing IL-4 prevented downregulation of NO[22].

Intra-articular injection with rIL-4 in a rat OA model decreased the population of NT-positive chondrocytes (a measurement for NO mediated tissue damage)[33]. Intra-articular administered IL-4 MSC spheroids reduced the expression of the inflammatory mediators Traf6 and Tlr4[23].

Two studies evaluated effects of IL-10 on histological synovitis. One study reported no effect of IL-10 gene therapy on histological synovitis in a rabbit OA model[39], another study reported less synovial changes in a murine collagenase-induced OA model after injection with humans MSCs overexpressing vIL-10[38].

Like IL-4, IL-10 is also able to compromise the binding of TNFα to its cell-surface receptors in synovial fibroblasts. IL-10 increases the level of sTNFR-75, and reduces the expression of cell-surface TNFR[40]. In synovial fibroblasts, IL-10 increased expression of the anti-inflammatory human leukocyte antigen G[50], and inhibited expression of COX-2[40]. In contrast, IL-10 had an opposing stimulatory effect on the expression of pro-inflammatory cytokines IL-1β and TNFα in LPS[43]-, or LTB_4-stimulated OA synovium[44]. In 3D synovial micromasses generated from primary synovial cells from OA patients that were stimulated with LPS, TNFα, or IL-1β, rIL-10 induced suppressor of cytokine signaling (SOCS) 3, and reduced LPS-induced IL-1β and TNFα expression[51]. In synovial fluid, IL-10 suppressed proliferation and IFNγ expression of autologous T cells[52].

In unstimulated OA cartilage samples, M(IL-10) MCM increased IL-1β and Suppressor Of Cytokine Signaling (SOCS)1[18]. Likewise, in cartilage explants stimulated with IFNγ and TNFα, to simulate inflammation, M(IL-10) MCM increased NO production[18]. The BM-MSCs overexpressing IL-10 (using adenovirus or lentivirus) reduced IL-1β and IL-6 expression in IL-1β/TNFα-stimulated cartilage explants[30]. Again, BM-MSCs transfected with AAV null had the same effects, suggesting the anti-inflammatory effect is also not mediated by IL-10[30]. Indeed, overexpression of IL-10 using an adenoviral vector did not influence IL-1β or IL-6 production, but did however increase TNFα production[34]. Similar to IL-4, IL-10 had also no effect on levels of arachidonic acid, PLA2, COX-2, mPGES-1, or PGE_2 expression in unstimulated human OA chondrocytes[45-47].

In contrast to unstimulated chondrocytes, overexpression of IL-10 reduced IL-1β and IL-6 expression in IL-1β-stimulated chondrocytes[34,35]. IL-10 had no effect on IL-1β[48]-, or IL-17-induced NO production in chondrocytes[49].

In bovine cartilage explants, IL-10 did not affect basal NO expression, but it did reverse the increase in NO and NOS2 mRNA expression after mechanical injury[36].

IL-10 gene therapy using a CXCL10 promoter reduced IL-1β and IL-6 expression in the previously mentioned synovial micromasses[51].

The fusion protein of IL-4 and IL-10 reduced the release of IL-6 and IL-8 by OA synovial tissue and cartilage explants *in vitro*[7], and canine IL4-10 FP reduced TNFα production in

LPS-stimulated canine whole blood cultures[15]. In *in vivo* OA models these effects were less clear, mainly due to the usage of non-inflammatory OA models[15,16].

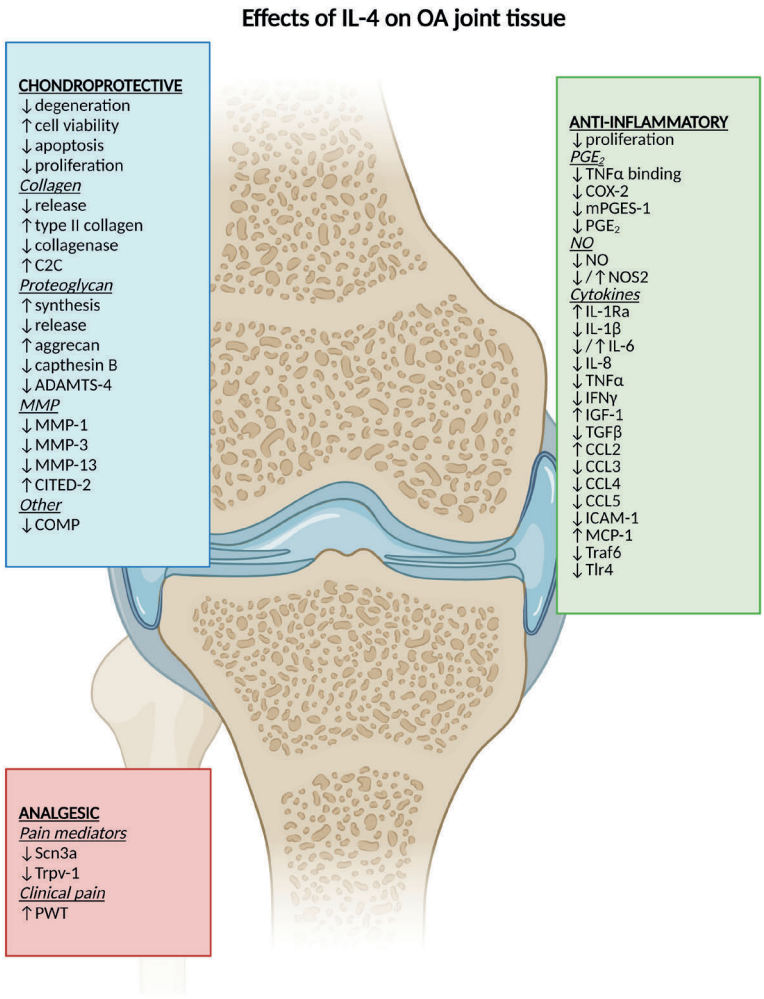
Analgesic effects

Only three studies reported on the effects of IL-4 and/or IL-10 on pain mediators. IL-4 could not restore vascular endothelial growth factor (VEGF) expression in human cartilage explants[17] which was increased after compression. In a rat OA model, intra-articular implantation of IL-4 MSC spheroids reduced the expression of Scn3a and Trpv-1, two pain related ion channels, in the spinal cord[23]. *In vitro,* IL4-10 FP inhibited the release of VEGF and nerve growth factor, two pain related mediators, from OA synovial tissue. In OA cartilage only VEGF was significantly inhibited[7].

In an *in vivo* rat model for OA induced by anterior cruciate ligament tear and medical meniscectomy IL-4 MSC spheroids decreased mechanical allodynia[23]. IL-10 knockout (IL-$10^{-/-}$) mice develop stronger signs of pain such as thermal hyperalgesia and mechanical allodynia after chemically induced OA (monoiodoacetate model (MIA)) compared to control mice. Moreover, dorsal root ganglia were destructed in MIA injected IL-$10^{-/-}$ mice while normal morphology was maintained in MIA injected control mice. These results suggest that IL-10 deficiency exacerbated pain progression[53]. In companion dogs with naturally occurring OA intra-articular injection with IL-10 encoding plasmid DNA decreased pain as measured by a visual analogue scale[54].

In animal models, joint loading is often used as a surrogate for pain, where more joint loading indicates less joint pain. In the canine Groove model, intra-articular injections with IL4-10 FP led to increased joint loading (i.e. less pain), which lasted for approximately one day[7,15]. In the rat Groove model, a transient analgesic effect of IL4-10 FP was seen as well[16].

Figure 2. Effects of IL-4 on OA joint tissue



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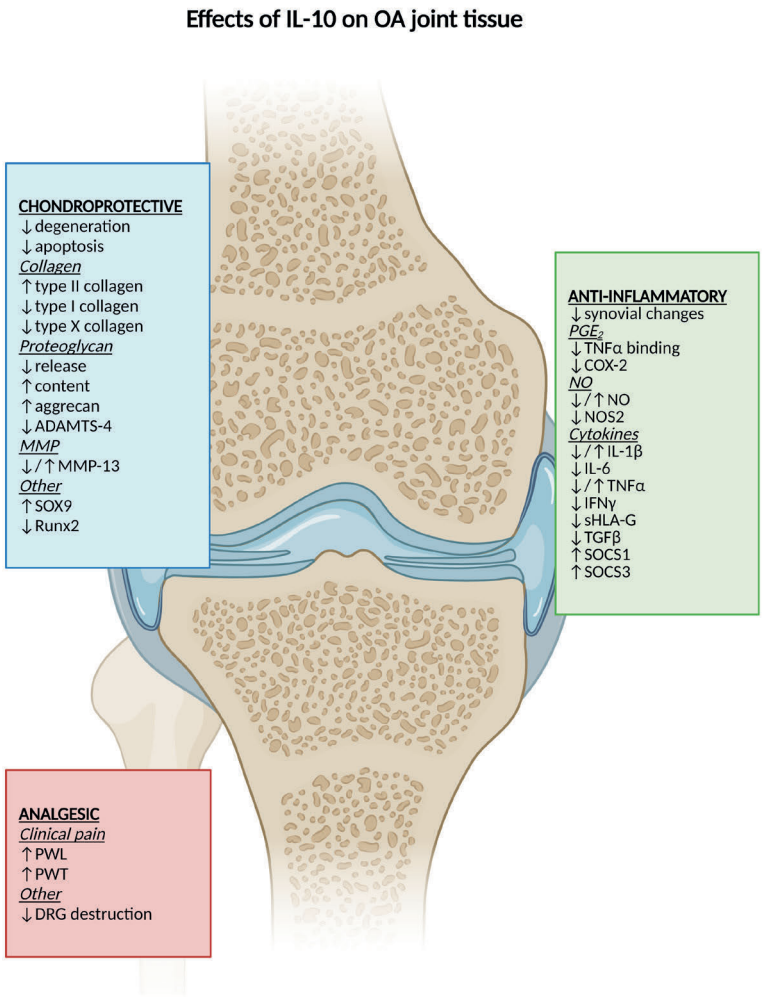
↓: reduction, ↑: increase, ↓/↑: different results

ADAMTS: A desintegrin and metalloproteinase with thrombospondin motifs, C2C: Collagen type II C-terminal cleavage neoepitope, CCL: Chemokine (C-C motif) ligand, CITED2: Cbp/P300 Interacting transactivator with Glu/Asp rich carboxy terminal domain 2, COMP: Cartilage oligomeric matrix protein, COX-2: Cyclooxygenase-2, ICAM-1: Intercellular adhesion molecule-1, IFNγ: Interferon-γ, IGF-1: Insulin growth factor-1, IL: Interleukin, MCP-1: Monocyte chemoattractant protein-1, MMP: Matrix metalloproteinase, mPGES-1: Microsomal prostaglandin E synthase-1, NO: Nitric oxide, NOS2: Nitric oxide synthase-2, PGE_2: Prostaglandin E2, PWT: Paw withdrawal threshold, Scn3a: Sodium voltage-gated channel alpha subunit 3 coding gene, TGFβ: Tumor growth factor-β, TNFα: Tumor necrosis factor-α, Trpv-1: Transient receptor potential cation channel subfamily V member 1 coding gene.

Figure 3. Effects of IL-10 on OA joint tissue



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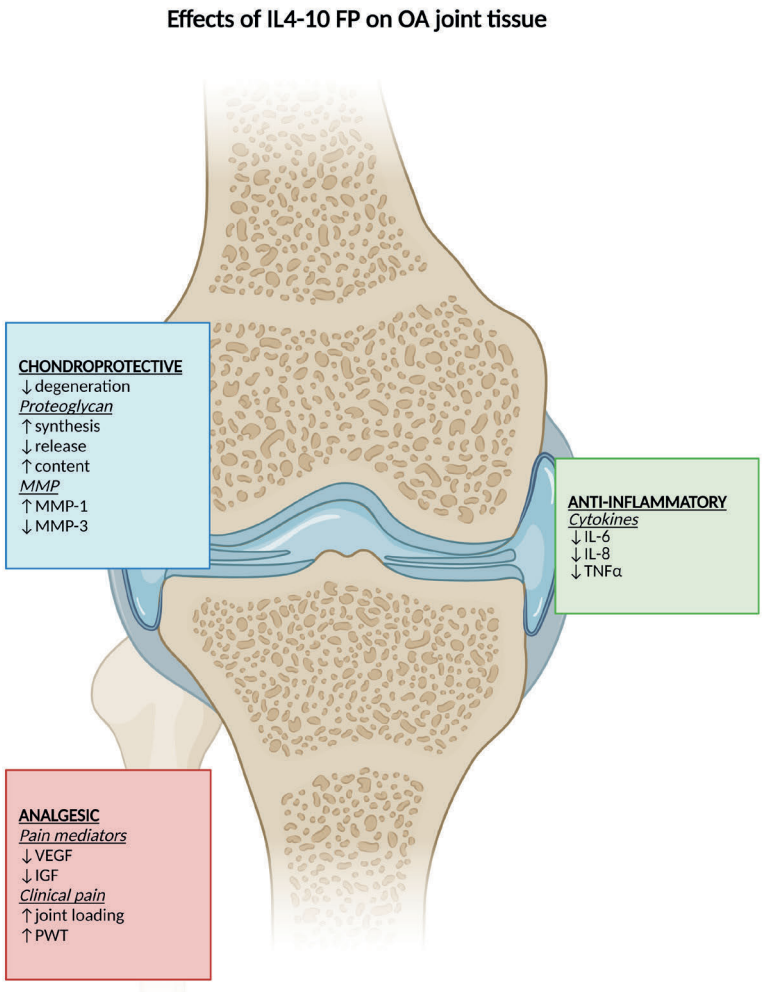
↓: reduction, ↑: increase, ↓/↑: different results

ADAMTS: A desintegrin and metalloproteinase with thrombospondin motifs, COX-2: Cyclooxygenase-2, DRG: Dorsal root ganglia, (s)HLA-G: (soluble) Human leukocyte antigen G, IFNγ: Interferon-γ, IL: Interleukin , MMP: Matrix metalloproteinase, NO: Nitric oxide, NOS2: Nitric oxide synthase-2, PGE_2: Prostaglandin E2, PWL: Paw withdrawal latency, PWT: Paw withdrawal threshold, SOCS: Suppressor of cytokine signaling, SOX9: Transcription factor sox 9 coding gene, TGFβ: Tumor growth factor-β, TNFα: Tumor necrosis factor-α.

Figure 4. Effects of IL4-10 FP on OA joint tissue



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↓: reduction, ↑: increase, ↓/↑: different results

IGF: Insulin growth factor, IL: Interleukin, MMP: Matrix metalloproteinase, PWT: Paw withdrawal threshold, TNFα: Tumor necrosis factor-α, VEGF: Vascular endothelial growth factor.

Discussion

This systematic narrative review describes the chondroprotective, anti-inflammatory, and analgesic effects, the three pillars of a successful DMOAD, of the anti-inflammatory cytokines IL-4 and IL-10, and the IL4-10 FP. In general, both cytokines show promising effects on all three outcomes, as did the IL4-10 FP. Regarding chondroprotection, multiple studies describe a lack of effect of the separate cytokines, and one study reported negative effects of IL-10 (increased MMP-13)[34]. In addition, some studies reported pro-inflammatory effects, despite IL-4 and IL-10 being anti-inflammatory cytokines. However, the IL4-10 FP showed purely anti-inflammatory effects, suggesting that by combining both cytokines into one treatment, the anti-inflammatory effects of one cytokine can counteract the possible pro-inflammatory effects of the other, and vice versa. It was shown previously that by using the IL4-10 FP, indeed the adverse effects of IL-10 are prevented by IL-4[14]. Also, in blood-induced cartilage damage the combination of IL-4 and IL-10 has advantages over IL-10 mono therapy[55,56], confirming the beneficial effects of combining both cytokines.

Next to the advantage of counteracting each others possible adverse effects, the IL4-10 FP also provides the possibility of synergy between IL-4 and IL-10. Both cytokines act via a different intracellular pathway, leading to additive anti-inflammatory effects[56,57]. Indeed, the IL4-10 FP was more effective in treating persistent inflammatory hyperalgesia in an *in vivo* mice model, compared to IL-4 or IL-10, as well as to the combination of both separate cytokines[12]. Besides, by combining both cytokines into one molecule, the molecular weight increases, leading to improved bioavailability. Sialylation of the IL4-10 FP increased the molecular weight even further, and resulted in higher half-life compared to the half-life of both cytokines alone[58].

The Food and Drug Administration and European Medicines Agency require a DMOAD to slow down joint space narrowing on x-rays and relieve clinical symptoms[2,3]. Despite many attempts, leading to promising results in phase II and III trials[59], there is still no approved DMOAD available. TissueGene-C (a mixture of human allogeneic chondrocytes and irradiated cells engineered to overexpress transforming growth factor-β1) showed chondroprotective and analgesic effects in a rat OA model, accompanied by increased IL-10 expression, suggestive for anti-inflammatory effects[60]. In humans, intra-articular injection with TissueGene-C led to less structural progression[61]. In addition, in a phase III trial symptomatic benefits were found[62]. A non-inferiority trial comparing diacerein (inhibitor of IL-1β) and celecoxib (a selective COX-2 inhibitor), showed that diacerein was non-inferior to celecoxib regarding symptomatic benefit and showed a good safety profile[63]. A phase III trial evaluating the effects of Tocilizumab in hand OA showed similar pain relief in the Tocilizumab group compared to the placebo group[64].

Multiple fusion proteins are developed in the search for a DMOAD as well. The OSCAR-Fc protein (fusion between osteoclast associated receptor and the Fc part of human IgG1) reduced cartilage damage in two *in vivo* mice models[65], but other pillars of a DMOAD therapy still need to be investigated. A fusion protein of tumor growth factor-β and latency associated peptide (LAP-MMP-mTGF-β3) showed promising results in a rat OA model[66]. This LAP has also been used for TIMP-3[67]. These fusion proteins are developed to reduce side effects of the active component, rather than combining two active components (they are biologically inactive until cleavage of the LAP to release the therapy). This also accounts for the HB-NC4 (heparin binding domain-N-terminal non-collagenous domain 4) fusion protein, which had been developed to overcome the main limitation of NC4 therapy, namely targeting ability[68]. In contrast, the IL4-10 FP is the only fusion protein designed to combine the effects of two active signaling components.

The search for a successful DMOAD is continuing, and the Wnt/β-catenin signallig pathway inhibitor Lorecivivint, the bisphosphonate zoledronic acid, and multiple anti-inflammatory agents successful in the treatment of RA are tested in phase III trials in OA patients[59], and for Vitamin D a phase IV trial is being conducted in OA[59]. For now, the IL4-10 FP is one of the few compounds which has shown promising results on all three aspects of ideal DMOAD therapy in *in vitro* and *in vivo* animal models, although not in one model. Still, a lot of work is needed to develop the IL4-10 FP as a DMOAD, suitable for clinical practice. At present, in OA the IL4-10 FP is envisioned to be used as intra-articular treatment, to reduce the possibility of systemic side effects[15,16], and with that is not usable for OA in smaller joints or in patients with polyarthritis. A major drawback of direct intra-articular application as applied so far is the rapid clearance out of the joint cavity due to the relatively low molecular weight, leading to only relative short transient effects and the necessity of weekly injections. More sustained effects on one injection, or other delivery routes need to become key in future studies to ensure clinical application.

At the same time, the concept of OA being a heterogeneous disease existing of multiple phenotypes is getting more and more attention, and future studies are needed to decide which patients should form the ideal target population for IL4-10 FP treatment (but also for other possible DMOADs). The canine and rat Groove model of OA mimic early post-traumatic OA, and inflammation was too mild to evaluate anti-inflammatory effects[15,16]. The IL4-10 FP consists of two anti-inflammatory cytokines, and in that line of thought, it seems reasonable to assume that patients with an important inflammatory component as underlying mechanism for OA will most likely benefit from IL4-10 FP treatment.

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5 Canine IL4-10 fusion protein provides disease modifying activity in a canine model of osteoarthritis: an exploratory study

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Abstract

Objectives

An ideal disease modifying osteoarthritis drug (DMOAD) has chondroprotective, anti-inflammatory, and analgesic effects. This study describes the production and characterization of a canine IL4-10 fusion protein (IL4-10 FP) and evaluates its *in vivo* DMOAD activity in a canine model of osteoarthritis (OA).

Methods

The canine Groove model was used as an *in vivo* model of degenerative knee OA. Six weeks after OA induction dogs were intra-articularly injected weekly, for ten weeks, with either IL4-10 FP or phosphate buffered saline (PBS). In addition to the use of human IL4-10 FP, canine IL4-10 FP was developed and characterized *in vitro,* and tested *in vivo*. Force plate analysis (FPA) was performed to analyze joint loading as a proxy measure for pain. After ten weeks dogs were euthanized and cartilage and synovial tissue samples were analyzed by histochemistry (Osteoarthritis Research Society International scores) and biochemistry (cartilage proteoglycan (PG) turnover).

Results

Repetitive intra-articular injections with human IL4-10 FP led to antibody formation, that blocked its functional activity. Therefore, a canine IL4-10 FP was developed, which completely inhibited lipopolysaccharides-induced tumor necrosis factor-α production by canine blood cells, and increased PG synthesis of canine cartilage *in vitro* (p=0.043). *In vivo*, canine IL4-10 FP restored the, by OA impaired, joint loading (p=0.002) and increased cartilage PG content (p=0.029).

Conclusion

This first study on the potential DMOAD activity upon prolonged repeated treatment with IL4-10 FP demonstrates that a species specific variant has anti-inflammatory and chondroprotective effects *in vitro* and chondroprotective and analgesic effects *in vivo.* These data warrant further research on the DMOAD potential of the IL4-10 FP.

Introduction

Osteoarthritis (OA) is characterized by changes in all (peri-)articular tissues, such as cartilage, (subchondral) bone, synovial tissue, ligaments, and muscles[1,2]. Currently, OA treatment is mostly symptomatic and mainly focused on pain reduction. If conservative treatments (e.g. physical therapy), analgesic drugs (e.g. acetaminophen), and/or anti-inflammatory drugs (e.g. non-steroidal anti-inflammatory drugs) fail, limited joint preserving surgical options are available (e.g. joint distraction), and finally a total joint replacement becomes inevitable[2]. Therefore, there is still a great need for joint preserving conservative drug treatments. Such treatments should ideally be chondroprotective (regenerative), anti-inflammatory, and analgesic, and should be preferably combined in one drug. At present such treatments do not exist, although multiple drugs are studied as potential disease modifying OA drug (DMOAD). Intra-articular administration of fibroblast growth factor-18 has demonstrated chondroprotective effects in a bovine *in vitro* model[3], in an *in vivo* rat model[4] and in a randomized, double-blind, placebo-controlled trial, although pain relief was not achieved[5,6]. Transforming growth factor-β1 producing human chondrocytes were able to generate cartilage with hyaline-like characteristics in two different animal models[7] and demonstrated clinical benefits in multiple clinical trials[8-12] with small, not statistically significant but encouraging chondroprotective activity[8,12,13], and anti-inflammatory activity[13]. SM04690, a Wnt pathway inhibitor, induced chondrocyte differentiation, reduced cartilage catabolism *in vitro,* and showed chondroprotective activity in a rat model of OA[14], and in humans[15]. None of these studies demonstrated chondroprotective, anti-inflammatory, and analgesic effects in combination.

Interleukin-4 (IL-4) and IL-10, two anti-inflammatory cytokines, are suggested to have therapeutic potential in OA: synovial cells and chondrocytes express IL-4 and IL-10 receptors (IL-4R, IL-10R)[16-18]. IL-4R signaling changes mechano-transduction in chondrocytes linked to matrix turnover in OA[19] and variants in the IL-4Rα gene are associated with susceptibility to OA[20]. Besides, IL-10 and IL-4 inhibit chondrocyte apoptosis and cartilage breakdown[21,22] and reduce synovial inflammation by reversing prostaglandin E_2 production by OA synovial fibroblasts[23].

IL-4 and IL-10 do have overlapping and complementary activities[24] and combined administration showed promising effects in experimental models of arthritis[25,26]. Recently, we developed a human fusion protein of IL-4 and IL-10 (hIL4-10 FP)[27] that has DMOAD properties in multiple models of OA[28]. Human OA cartilage tissue explants were demonstrated to express elevated levels of IL-4R and IL-10R, rendering OA cartilage more sensitive to the IL4-10 FP.

Moreover, two intra-articular injections of IL4-10 FP in dogs with experimental OA were analgesic and *in vitro* IL4-10 FP provided chondroprotective and anti-inflammatory activity

in human cartilage and synovial tissue explants. These findings warranted further *in vivo* evaluation on the potential disease modifying properties of this novel IL-4-10 fusion molecule. As such we designed an *in vivo* study using the canine Groove model of OA to study the chondroprotective, anti-inflammatory, and analgesic activity of the IL4-10 FP upon repeated intra-articular injections.

Methods

Production and characterization of IL4-10 FP

hIL4-10 FP was produced as previously published[29]. Canine IL4-10 FP (cIL4-10 FP) was produced by transient transfection of Human Embryonic Kidney 293F cells with pcDNA3.1-neo expression vector with dual cytomegalovirus promotor. The vector contained two transgenes: cDNA coding for cIL4-10 FP and cDNA coding beta-galactoside-2, 3-sialyl-transferase to optimize glycan capping with sialic acid. To enable purification, a hexa-histidine affinity tag was cloned at the N-terminus of cIL4-10 FP. Cells were cultured in GIBCO FreeStyle™ 293 Expression Medium. Cells were subcultured three to four times prior to transfection and transfected with 293fectin™ at the cell viability of at least 90%.

The cIL4-10 FP was purified using Nickel-nitrilotriacetic agarose according to manufacturer's protocol. Purified canine protein was dialyzed overnight against 2L of phosphate buffered saline (PBS; pH=7.4), sterile filtered and stored at -80°C. Purity of cIL4-10 FP batches was evaluated by Coomassie-stained 12% sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and high performance size-exclusion chromatography analysis (HP-SEC).

For SDS-PAGE samples were diluted 1:1 in 2x Leammli Sample Buffer containing 100mM dithiotreitol, incubated ten minutes at 95°C, and loaded on a 12% polyacrylamide gel. Electrophoresis was performed at 150V for 1,5 hours, under reducing conditions (Tris/glycine/SDS buffer). Protein bands were visualized by InstantBlue protein stain.

After electrophoresis, proteins were transferred to a nitrocellulose membrane. Membranes were blocked in 5% milk in PBS with 0.1% Tween-20 (PBST), and incubated overnight with a monoclonal mouse anti-canine IL-4 or monoclonal mouse anti-canine IL-10 antibody in PBST containing 1% milk. Membranes were subsequently incubated with goat anti-mouse Immunoglobulin G (IgG) horseradish peroxidase (HRP)-labelled anitbody for one hour at room temperature. To visualize the bands enhanced chemiluminescence Western Blotting Substrate was added according to manufacturer's protocol.

Bioactivity check of cIL4-10 FP in vitro

To assess biological activity of the cIL4-10 FP two *in vitro* experiments were performed, before *in vivo* testing. To evaluate anti-inflammatory activity of cIL4-10 FP, heparinized canine blood was diluted 1:10 in Roswell Park Memorial Institute 1640 medium supplemented

with 1% penicillin/streptomycin, and incubated with lipopolysaccharide (LPS) at 100ng/ml and cIL4-10 FP, recombinant canine IL-4 or recombinant canine IL-10, both as controls, at 0.001-3nM for eighteen hours at 37°C, 5% CO_2. Culture supernatants were then assayed for canine tumor necrosis factor-α (TNFα) using enzyme-linked immunosorbent assay (ELISA). The inhibition of TNFα production (by cIL4-10 FP, cIL-4 or cIL-10) was calculated according to the formula: Inhibition (%) = (1-(A-B)/(C-B)) x 100, where A = TNFα levels in LPS-stimulated cultures treated with cIL4-10 FP, cIL-4 or cIL-10; B = TNFα levels in unstimulated culture; and C = TNFα levels in LPS-stimulated culture.

To assess chondroprotective activity *in vitro*, surplus cartilage of five healthy dogs used for unrelated studies was used to determine cartilage proteoglycan (PG) synthesis. Per dog, sixteen cartilage explants were harvested and cultured in a 96-wells plate in culture medium containing TNFα (10ng/mL) to impair chondrocyte activity with or without cIL4-10 FP (100ng/mL) (n=8 for each condition). Samples were cultured for four days at 37°C and chondrocyte PG synthesis was determined as described before[30]. In short, after one hour of pre-culture, 20 µCI sodium sulphate ($Na_2{}^{35}SO_4$) in Dulbecco's modified eagle medium was added to each sample. After four hours of labeling, samples were washed with PBS and digested with papain for two hours at 65°C. Glycosaminoglycans (GAGs) were precipitated by addition of cetylpyridium chloride. $^{35}SO_4{}^{2-}$-labelled GAGs were measured by liquid scintillation on a TRI-CARB 2800TR. Values were normalized to specific activity of the pulse medium, pulse time, and wet weight of the explants and expressed as nmoles of $^{35}SO_4{}^{2-}$ incorporated per hour per gram wet weight of the cartilage ($nmol.h^{-1}.g^{-1}$). Data of eight samples were averaged for each cartilage donor (dog).

In vivo canine model of OA

The study was approved by the animal ethical committee of the Utrecht University (DEC 2014.III.09.081). For logistical reasons the experiment was split in two sets of eight dogs (four treated with IL4-10 FP and four with PBS).

Skeletally matured female mixed breed dogs (46 ± 31 months, 30.4 ± 17.4 kg) were matched on weight and walking pattern and then split into two comparable groups. Dogs were housed in pairs and let out on a patio in large groups for at least two hours a day. They were fed a standard diet and had water *ad libitum.* OA was induced in the right leg according to the Groove model[31]. Dexdomitor (0.02 mg/kg i.m.) and ketamin (0.5 mg/kg i.m.) were used as pre-medication before induction with thiopental (20mg/kg i.v.). Anesthesia was done with sufentanil (0.0225 mg/kg/h i.m.) and isofluran. Six to ten grooves of 2-3mm depth were made in the femoral cartilage as described in detail previously. Dogs were treated with painkillers (carprofen; 4mg/kg p.o.) and antibiotics (cefazolin; 35mg/kg i.v. pre-surgery and synulox; 15mg/kg p.o. twice a day post-surgery) one day before surgery and during five days after surgery. During the first week after surgery regular wound checks were performed. Starting two days after surgery dogs were let out on the patio again. General control of

animals and housing were performed during the whole study period. Based on previous experiments it was not expected that clinical symptoms (e.g. infection due to OA induction) would be of such degree that the human endpoint would be reached and euthanasia would be inevitable.

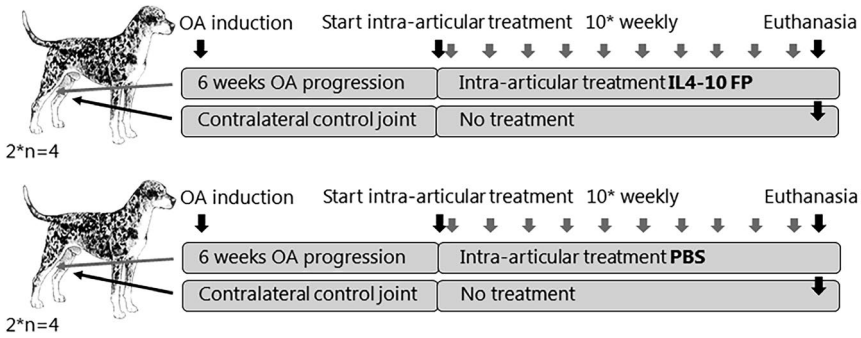
The Groove model is known to be an intrinsic cartilage degenerative model demonstrating progressive cartilage degradation[30,32]. The model has been specifically designed to test chondroprotective treatments because treatment is not counteracted by a permanent trigger (like joint instability) or by a dominating inflammatory component (like chemically induced models). As such this model is explicitly suitable for testing DMOAD activity but not mainly anti-inflammatory activity. After six weeks of OA development, dogs were treated weekly with intra-articular injections in their affected knee for ten successive weeks. Dogs in the treatment group were injected with IL4-10 FP (10µg in 500µL) whilst dogs in the control group were injected with PBS (500µL). The left contralateral knee of each dog served as an internal control. Periodically during treatment blood was drawn and force plate analyses (FPA) were performed providing data on joint loading as a proxy for pain. After the treatment period of ten weeks, dogs were euthanized and cartilage and synovial tissue samples were harvested. Natriumpentobarbital i.v. was used as euthesate. Ten minutes before euthanasia, dogs were injected with Dexdomitor (0.02 mg/kg i.m.) and ketamin (0.5 mg/kg i.m.)

Experimental set-up

The first part of the canine *in vivo* experiment was performed treating four dogs intra-articular with human IL4-10 FP and four dogs with intra-articular PBS as a control group.

As human IL4-10 FP turned out to be immunogenic in dogs after multiple intra-articular injections, the canine IL4-10 FP was produced, characterized and tested for *in vitro* bioactivity as described above. Subsequently, the second part of the canine *in vivo* experiment was performed using this canine IL4-10 FP, treating four dogs intra-articular with canine IL4-10 FP and four with intra-articular PBS as a control group (fig. 1)

Figure 1. Experimental set-up of the *in vivo* experiment



The first part of the canine *in vivo* experiment was performed treating four dogs with intra-articular human IL4-10 FP and four dogs with intra-articular PBS as a control group. Because of IL4-10 FP neutralizing antibody formation the second part was performed treating four dogs with intra-articular canine IL4-10 FP and four dogs with intra-articular PBS.

Immunogenicity assessment for IL4-10 FP

ELISA was used to determine IgG titers in serum of injected dogs. Serum was drawn before start of treatment and after every intra-articular injection. In case of antibody detection after the tenth injection, intermediate time points were analyzed as well to identify when antibody formation was initiated.

After demonstrating the formation of IgGs in the hIL4-10 FP injected dogs, whole blood assays were performed to evaluate the neutralizing effects of these antibodies. Two 96-wells plate were coated with hIL4-10 FP. One plate was covered with dilutions of serum containing possible IgGs against hIL4-10 FP (after ten injections) and one plate was covered with dilutions of serum without IgGs against hIL4-10 FP (before ten injections). Subsequently TNFα inhibiting capacity of the hIL4-10 FP was determined.

Force plate analysis

In order to evaluate pain, FPA was performed to determine joint (un-)loading as a proxy for pain. Vertical peak force (Fz), braking force (Fy+), and propulsive force (Fy-) were determined. The ratio of the affected hind leg over the contralateral control hind leg was calculated for each dog for each time point. FPA was performed before Groove surgery to determine baseline values and during the treatment period before and 24 hours after the 1st, 6th and 9th injection.

Assessment of synovial inflammation

Macroscopic scoring of synovial tissue samples was done on digital photographs of the synovial fat pad according to Osteoarthritis Research Society International (OARSI; range 0-5)[33]. Knee synovial tissue samples were embedded in Tissue Tek and stored at -80°C. Sections of 5µm were cut and stained with hematoxylin-eosin and number of cell layers, lining characteristics and cell infiltration were scored from 0-6 (total score ranged from 0-18) according to OARSI[33]. Sections were analyzed by two observers blinded for treatment, and an average of six sections (three sections per observer) was calculated for each knee.

Assessment of cartilage degeneration

Macroscopic scoring of cartilage tissue samples was done on digital photographs of the tibial plateau according to OARSI[33]. Histologic cartilage degeneration was evaluated in tibial cartilage only, because grooves were applied in the femoral cartilage. Four samples of tibial cartilage were used. Cartilage samples were fixed in formalin and embedded in paraffin. Sections of 5µm were cut and stained with Safranin-O-fast-green (Saf-O). Sections were scored for cartilage structure, chondrocyte pathology and PG staining from 0-12 (total score ranged from 0-36) according to OARSI[33]. All sections were stained in the same Saf-O baths. Each sample (four per knee) was scored by two observers blinded for treatment. An average of eight scores was used for statistical evaluation. For one dog in the cIL4-10 FP group all four pieces of the right tibia could not be evaluated due to technical issues. Data was imputated by taking the mean change of the remaining three dogs and adding this value to the value of the contralateral knee.

Cartilage proteoglycan turnover

The surgically untouched tibial cartilage was used for determining *ex vivo* PG turnover, as described before[30]. For determining PG content the GAGs in a papain digest of the tissue samples were precipitated and stained with Alcian Blue dye solution[34]. This staining was quantified photometrically by change in absorbance at 620nm. Chondroitin sulphate was used as a reference. Values were normalized to wet weight of the cartilage explants (mg/g). For total release of PGs, GAGs in the culture medium were precipitated from the medium, stained with Alcian blue dye solution and quantified photometrically with chondroitin sulphate as a reference. The total amount of GAGs released (blue staining) is expressed as a percentage of the original tissue content (% total PG release). Determination of PG synthesis is described above (under *in vitro* bioactivity experiments). Release of newly formed PGs was determined as a measurement of retention of newly formed PGs. The release of $^{35}SO_4^{2-}$-labelled PGs in the medium during three days was determined. After labelling, the samples were rinsed three times for 45 minutes in 1.5mL culture medium and then incubated in 200µL fresh culture medium without $^{35}SO_4^{2-}$-label. After three days, released GAGs in the culture medium were precipitated by Alcian Blue dye solution. $^{35}SO_4^{2-}$-labelled

GAGs were measured by liquid scintillation analysis and values were normalized to wet weight of the explants. Release of newly formed PGs is normalized to total PG synthesis rate and expressed as percentage release of newly formed PGs (% release newly formed PGs). An average of eight samples taken from predefined locations of the tibial cartilage, identical for contralateral control and experimental joints, was used as representative value for each joint. Previous research shows that values of contralateral (left) knees are comparable to baseline values of experimental (right) knees.

Statistical analysis

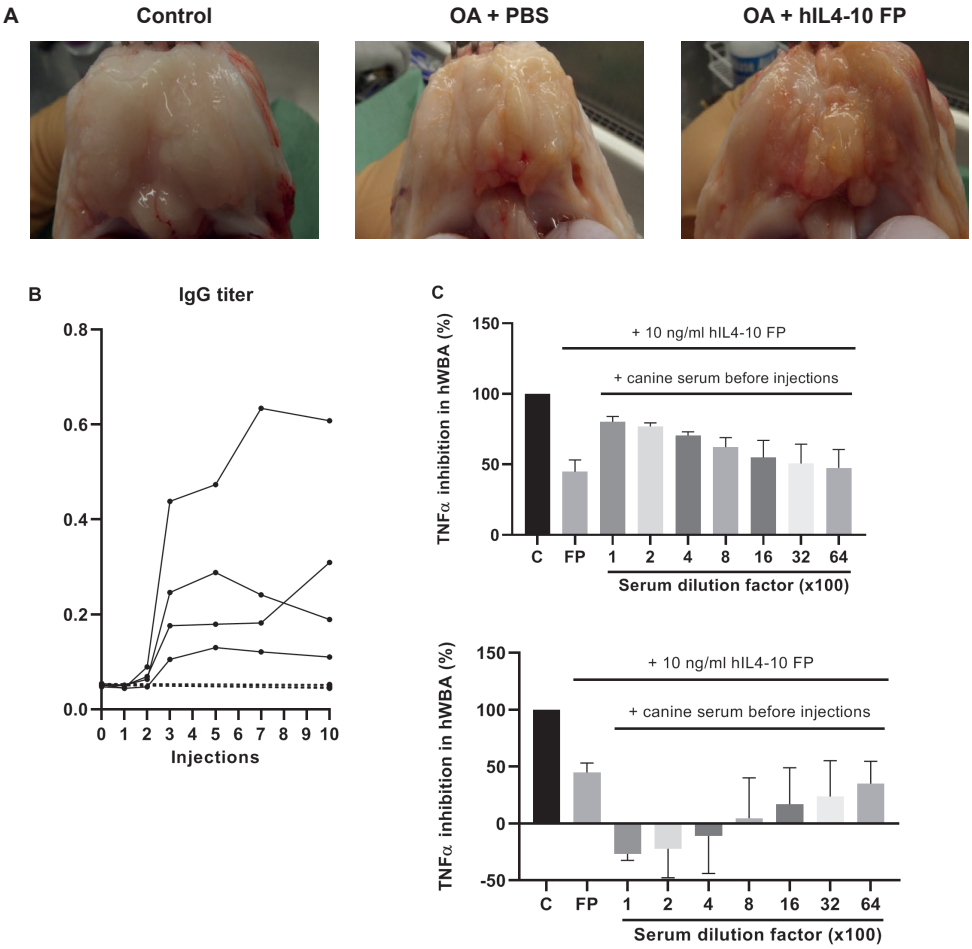
Statistical analyses were done using SPSS Statistics 25. A Wilcoxon-signed rank test was used to analyze the *in vitro* bioactivity. For FPA, a linear mixed model, correcting for time, baseline values, and pre-injection values was used to evaluate the effects on joint loading 24 hours after intra-articular injection. For inflammation and cartilage degeneration parameters mean differences between experimental knees and contralateral knees were determined and considered to represent the effect of OA induction and treatment with either IL4-10 FP or PBS within the experimental knees. The effects within the experimental knees were compared between PBS and IL4-10 FP group using Mann-Whitney U tests. P-values <0.05 were considered as statistically significant for all tests.

Results

Canine IL4-10 FP production and characterization

Human IL4-10 FP was immunogenic in dogs upon repeated intra-articular injections, leading to the formation of antibodies after only three repetitive intra-articular injections (fig. 2b). Whole blood assays evaluating the TNFα inhibition of hIL4-10 FP after adding serum of hIL4-10 FP injected dogs after ten injections showed a complete loss of activity of the hIL4-10 FP, demonstrating a neutralizing effect of the IgGs (fig. 2c). Moreover, the immunogenicity caused severe synovial inflammation (fig. 2a), visible as diffuse discoloration due to neovascularization as well as tissue proliferation with villi formation.

Figure 2. Immune response against hIL4-10 FP.

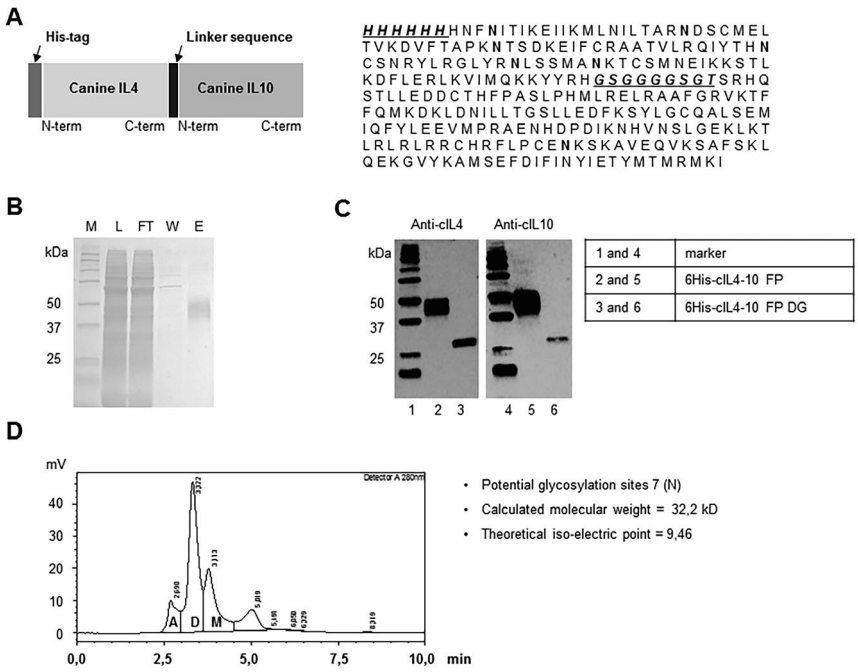


A. Microscopic pictures of synovial fatpad of control, PBS injected and hIL4-10 FP injected knees. B. IgG titers in serum of PBS injected dog (dotted line) and hIL4-10 FP injected dogs (continuous lines). C. TNFα inhibition of hIL4-10 FP after adding serum taken before intra-articular injections (above) and after intra-articular injections with hIL4-10 FP (below). TNFα inhibition of hIL4-10 FP is expressed as percentage of inhibition in serum of non-injected dogs.

Therefore, a canine variant of the IL-4 -10 FP was developed. In figure 3a schematic representation is shown of the cIL4-10 FP and its amino acid sequence, including the linker sequence and seven predicted glycosylation sites. Purified cIL4-10 FP was detected on Coomassie stained SDS gel as a smear composed of multiple protein bands corresponding to a molecular mass of 37 – 50kDa (fig. 3b). A smeared protein band was also detected on a Western Blot by anti-IL-4 and anti-IL-10 antibodies, while after deglycosylation of cIL4-10 FP with peptide:N-glycosidase F only one sharp protein band of 30kDa was detected, as

expected based on amino acid sequence (fig. 3c), indicating the apparent smear represented different glycoforms of cIL4-10 FP. HP-SEC analysis showed that cIL4-10 FP preparations mainly consisted of non-covalently linked dimers with a mass of ~70 kDa, as well as some monomers of ~35 kDa (fig. 3d). Both monomer and dimer have been demonstrated to be biologically active.

Figure 3. Molecular characterization of cIL4-10 FP.



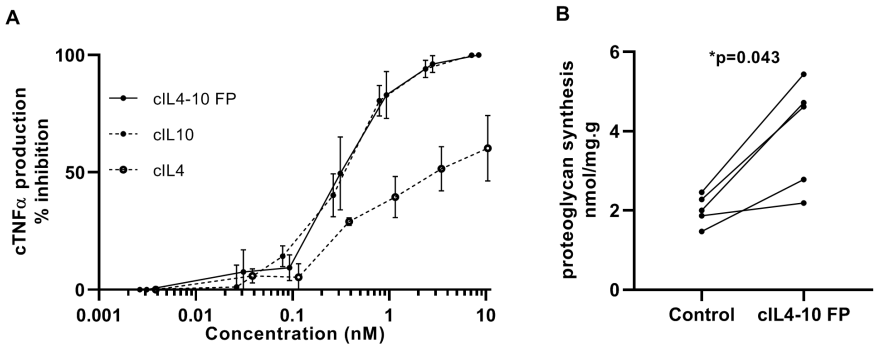
A. Schematic overview of the cIL4-10 FP. B. Coomassie Brilliant Blue stained SDS gel in ni-NTA protein purification steps. C. Western blot analysis of purified cIL4-10 FP (untreated and DG: deglycosylated). D. HP-SEC profile showing A: Aggregate, D: Dimer, and M: Monomer. M: Marker, L: Load, FT: Flow-through, W:Wash, E: Elution.

Bioactivity of canine IL4-10 FP in vitro

The bioactivity of cIL4-10 FP was evaluated *in vitro* by its ability to diminish TNFα production in an LPS-stimulated canine whole blood culture. cIL4-10 FP dose-dependently inhibited TNFα production, with maximal inhibition at 3.0 nM. Bioactivity of the canine IL4-10 FP was comparable to that of canine IL-10, whilst canine IL-4 was less efficient (fig. 4a).

To test the chondroprotective activity of cIL4-10 FP, PG synthesis in TNFα stimulated canine cartilage explants was evaluated *in vitro*. cIL4-10 FP statistically significantly improved PG synthesis in TNFα compromised cartilage explants as compared to untreated TNFα compromised cartilage explants (4.0 (95%CI 2.2; 5.7) *vs* 2.0 (95%CI 1.5; 2.5), p=0.043, fig. 4b).

Figure 4. *In vitro* activity of cIL4-10 FP.



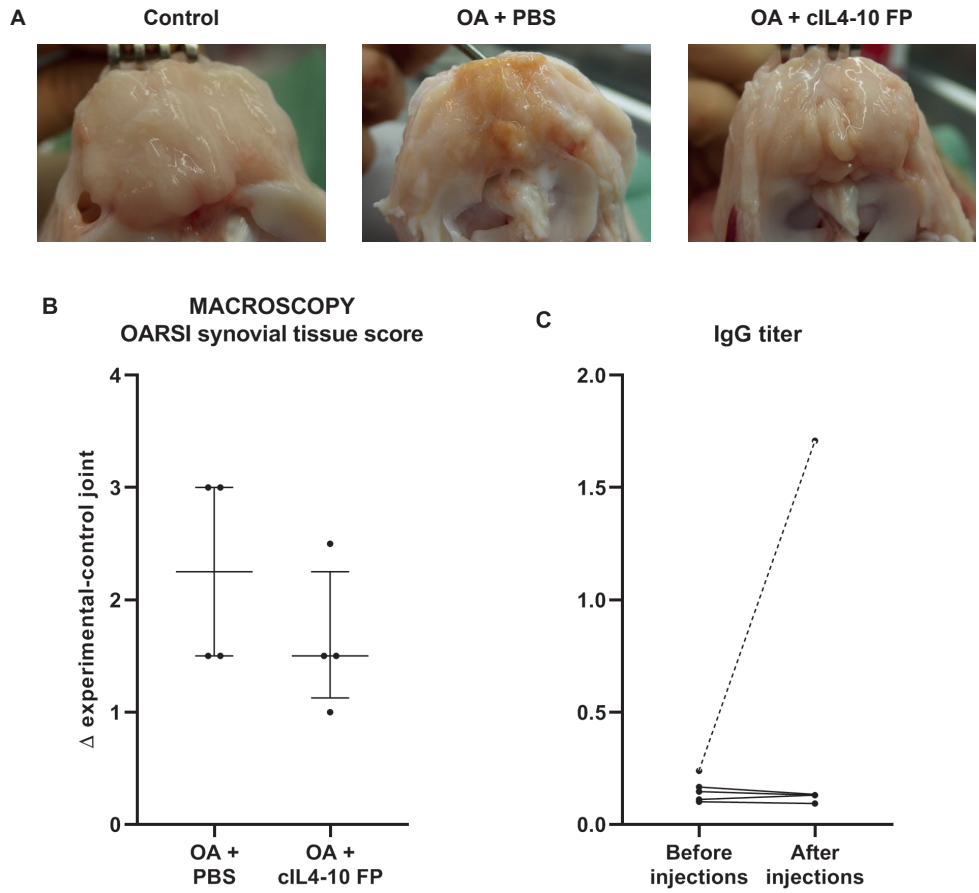
A. Anti-inflammatory activity of cIL4-10 FP. Whole blood was stimulated with LPS with or without cIL4-10 FP, IL-10, or IL-4 for eighteen hours. Canine TNFα was measured using ELISA. B. Chondroprotective activity of cIL4-10 FP. Healthy canine cartilage explants were cultred in TNFα containing medium with or without cIL4-10 FP for four days. Proteoglycan synthesis rate was measured using liquid scintillation of $^{35}SO_4^{2-}$labelled GAGs. Results are expressed per dog and are the mean of eight tissue samples per dog. Mean proteoglycan synthesis rate was 2.0 and 4.0 for controls and cIL4-10 FP treated conditions, respectively.

Canine IL4-10 FP in the canine Groove model of OA

Immunogenicity

After ten injections of cIL4-10 FP, OD450 values of serum in the ELISA for IgG antibodies against cIL4-10 FP were comparable to values obtained with baseline samples, showing that ten repeated intra-articular injections with species specific protein did not lead to antibody formation against cIL4-10 FP (fig. 5c). Intermediate time points and neutralizing effects were not evaluated any further.

Figure 5. Immunogenicity of cIL4-10 FP

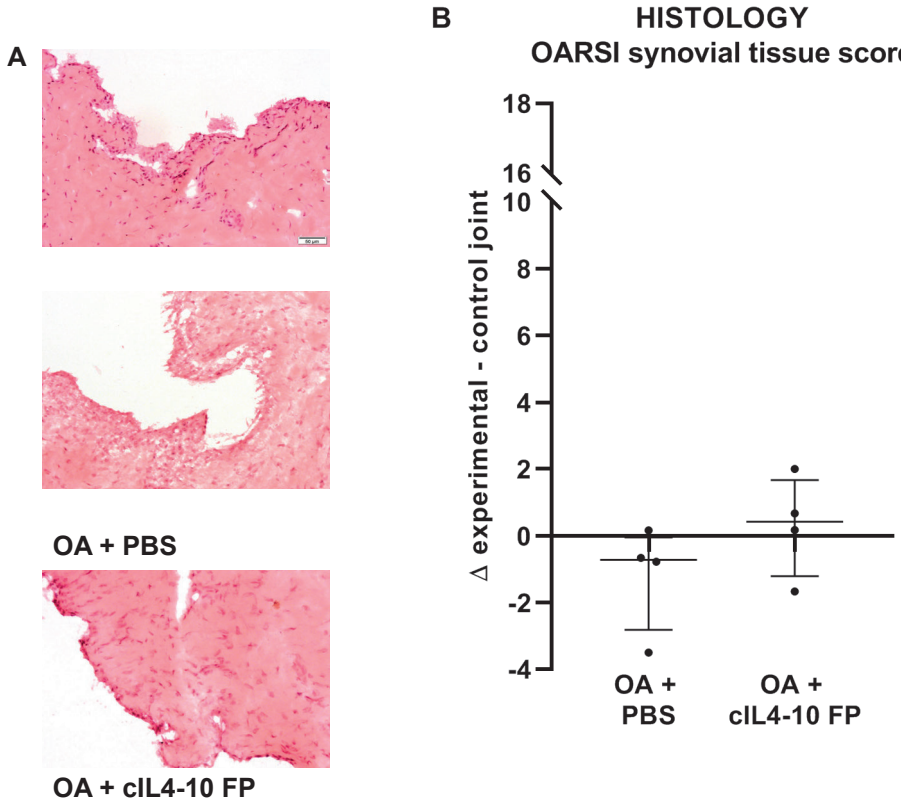


A. Macroscopic pictures of synovial fat pad of control, PBS injected and cIL4-10 FP injected knees. B. Differences in macroscopic synovial inflammation (OARSI score) between experimental and contralateral joint, representing a change from baseline to end of the study for the experimental joint. Values are expressed per dog (dots) and as median±IQR (dash with whiskers). Mean macroscopic OARSI scores in contralateral joints were 0.5 (95%CI-0.2; 1.2) and 0.4 (95%CI -0.4; 1.1) in PBS group and cIL4-10 FP group, respectively. Mean macroscopic OARSI scores in experimental joints were 2.8 (95%CI 0.9; 4.6) and 2.0 (95%CI 1.4; 2.7) in PBS group and cIL4-10 FP group, respectively. C. IgG titers in serum of cIL4-10 FP injected dogs (continuous line) and, as a positive control, a hIL-4-10FP injected dog (dotted line).

Synovial inflammation

The Groove model is characterized by minimal signs of synovial inflammation[30,32]. Macroscopic and histologic OARSI scoring of synovial inflammation showed indeed only minimal inflammation (fig. 5a shows representative macroscopic images and fig. 5b shows scores of the individual animals). As such analyzing anti-inflammatory activity was limited to the canine *in vitro* assays. Mean changes in histologic inflammation grade were -1.2 (95%CI -3.7; 1.3) and 0.3 (95%CI -2.1; 2.7) out of 18 for PBS injected dogs and cIL4-10 FP injected dogs, respectively (fig. 6). None of these changes were statistically significant (table 1).

Figure 6. Anti-inflammatory effects of cIL4-10 F

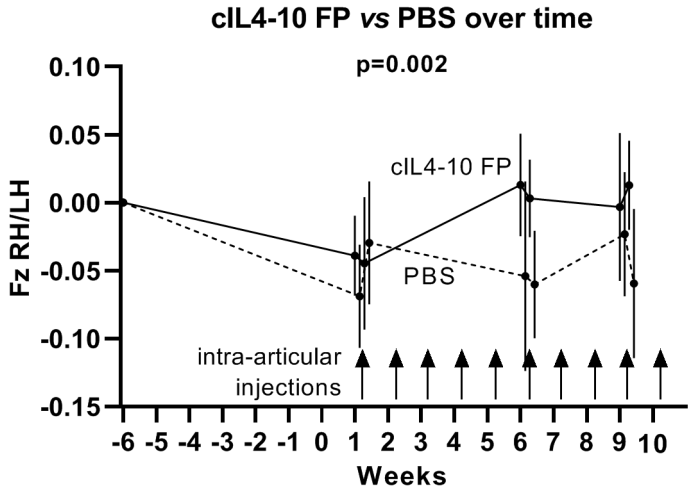


A. Representative images of Haematoxylin-eosin stained synovial tissue sections of control (without Groove surgery), PBS injected and cIL4-10 FP injected knees. Samples were collected at the end of the experiment; sixteen weeks after Groove surgery and after ten weekly intra-articular injections. B. Differences in histologic synovial inflammation (OARSI score) between experimental and contralateral joint, representing a change from baseline to end of the study for the experimental joint. Values are expressed per dog (dots) and as median±IQR (dash with whiskers). Mean histologic OARSI scores in contralateral joints were 3.0 (95%CI 1.7; 4.3) and 1.3 (95%CI -0.1; 2.6) in PBS group and cIL4-10 FP group, respectively. Mean histologic OARSI scores in experimental joints were 1.8 (95%CI -0.8; 4.5) and 1.5 (95%CI-0.8; 3.8) in PBS group and cIL4-10 FP group, respectively.

Force plate analysis

Groove surgery induced a reduction in stance (Fz) and propulsive force (Fy+) in both groups. After ten intra-articular injections with cIL4-10 FP, Fz and Fy+ were restored to pre OA values. Overall, cIL4-10 FP increased Fz and Fy+ 24 hours after intra-articular injections compared to PBS injected dogs (p=0.002 and p=0.01 for Fz and Fy+, respectively, fig. 7). Similarly, the breaking force (Fy-) trended to a decrease after surgery and a recovery after intra-articular cIL4-10 FP injections, although not statically significant.

Figure 7. Analgesic effects of cIL4-10 FP



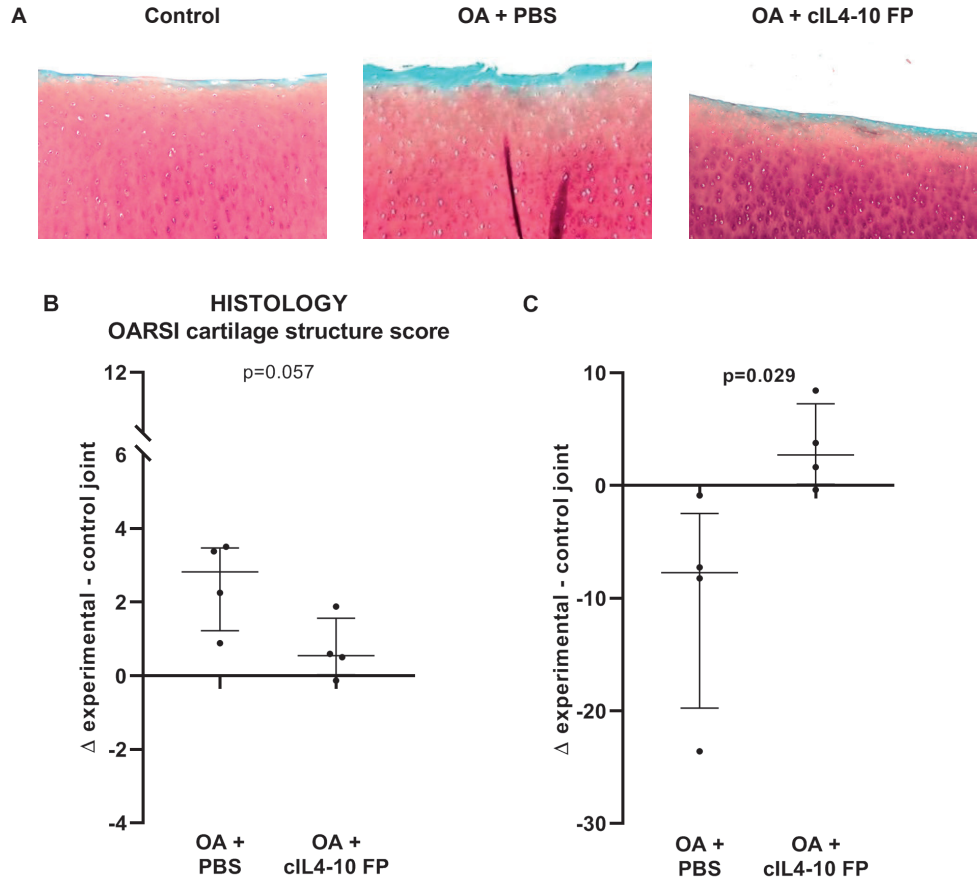
Six weeks after Groove surgery dogs received weekly intra-articular injections with cIL4-10 FP or PBS for ten weeks. Objective force plate analysis was used to determine joint loading as a proxy for pain. After OA induction, Fz RH/LH was clearly reduced. After treatment the Fz RH/LH statistically significant increased in the cIL4-10 FP treated group (continuous line, n=4) compared to the PBS injected group (dotted line, n=4); p=0.002 over the whole time span. Values are expressed as a mean of four dogs ± SEM.

Cartilage damage

Evaluation of Saf-O stained cartilage sections showed a small increase in OARSI cartilage structural damage score in experimental (OA) knees compared to contralateral control knees (2.5 (95%CI 0.6; 4.4) points out of 12 points) in the PBS treated group, which was normalized in the cIL4-10 FP treated group (0.7 (95%CI -0.6; 2.0) points), at borderline significance (p=0.057, fig. 8b). The other subscales of the OARSI score, chondrocyte pathology and proteoglycan staining, as well as total OARSI score, showed similar trends, although not statistically significant (table 1).

The protective effect on cartilage was corroborated by PG content. This PG content was decreased in the OA joints injected with PBS (-10.0 (95%CI -25.3; 5.3) mg/g; p=0.068) whereas in the cIL4-10 FP injected OA joints there was no difference in PG content compared to the contralateral control joint (+3.4 (95%CI -2.6; 9.4) mg/g; p=0.144). cIL4-10 FP significantly normalized the reduced PG content; p=0.029, compared to the PBS treated group (fig. 8c). Changes in PG synthesis, release, and retention did not statistically significantly differ between both treatment groups although differences were mostly in favor of the cIL4-10 FP group (table 1).

Figure 8. Chondroprotective effects of cIL4-10 FP



A. Representative images of Safranin-O stained tibial cartilage section of control (without Groove surgery), PBS-injected and cIL4-10 FP injected knees. Samples were collected at the end of the experiment; sixteen weeks after Groove surgery and after ten weekly intra-articular injections. B. Differences in tibial cartilage structure (part of the OARSI score) between experimental and contralateral joint, representing a change from baseline to end of the study for the experimental joint. Values are expressed per dog (dots) and as median±IQR (dash with whiskers). Mean scores in contralateral joints were 1.3 (95%CI-0.4; 2.5) and 1.4 (95%CI 0.0; 2.8) in PBS group and cIL4-10 FP group, respectively. Mean histologic OARSI scores in experimental joints were 3.8 (95%CI 3.0; 4.5) and 2.1 (95%CI 0.4; 3.8) in PBS group and cIL4-10 FP group, respectively. C. Differences in proteoglycan content in tibia of injected knees. Values are expressed per dog (dots) and as median±IQR (dash with whiskers). Mean proteoglycan content in contralateral joints were 37.8 (95%CI 20.4; 55.2) and 30.7 (95%CI 27.3; 34.1) in PBS group and cIL4-10 FP group, respectively. Mean proteoglycan content in experimental joint were 27.8 (95%CI 24.6; 31.0) and 34.1 (95%CI 28.3; 39.9) in PBS group and cIL4-10 FP group, respectively.

Table 1. Canine IL4-10 FP in the canine Groove model of OA

	PBS			*cIL4-10 FP*			
	Control	***OA***	***ΔContr.-OA***	***Control***	***OA***	***ΔContr.-OA***	***p-value***
FORCE PLATE ANALYSIS*							
Vertical peak force (Fz)							**0.002**
Braking force (Fy+)							**0.010**
Propulsive force (Fy-)							0.067
SYNOVIAL INFLAMMATION							
Macroscopy	0.5 *(-0.2; 1.2)*	2.8 *(0.9; 4.6)*	2.3 *(0.9; 3.6)*	0.4 *(-0.4; 1.1)*	2.0 *(1.4; 2.7)*	1.6 *(0.6; 2.6)*	0.343
Histology	3.0 *(1.7; 4.3)*	1.8 *(-0.8; 4.5)*	-1.2 *(-3.7; 1.3)*	1.3 *(-0.1; 2.6)*	1.5 *(-0.8; 3.8)*	0.3 *(-2.1; 2.7)*	0.886
CARTILAGE DAMAGE							
Macroscopy	0.5 *(0.2; 0.8)*	1.8 *(0.1; 3.4)*	1.3 *(-0.1; 2.6)*	0.4 *(0.1; 0.6)*	1.6 *(1.2; 1.9)*	1.2 *(0.6; 1.8)*	0.886
Histology	4.2 *(1.2; 7.2)*	9.2 *(5.7; 12.7)*	5.1 *(-1.3; 11.4)*	6.0 *(1.7; 10.3)*	7.2 *(2.8; 11.5)*	1.2 *(-4.1; 6.4)*	0.343
Cartilage structure	1.3 *(-0.4; 2.5)*	3.8 *(3.0; 4.5)*	2.5 *(0.6; 4.4)*	1.4 *(0.0; 2.8)*	2.1 *(0.4; 3.8)*	0.7 *(-0.6; 2.0)*	0.057
Chondrocyte pathology	0.5 *(-0.2; 1.1)*	2.0 *(-0.7; 4.7)*	1.5 *(-1.7; 4.8)*	2.3 *(-0.3; 4.8)*	1.9 *(-0.6; 4.4)*	-0.4 *(-4.5; 3.6)*	0.686
Proteogylcan staining	2.5 *(1.2; 3.8)*	3.5 *(2.2; 4.8)*	1.0 *(-1.3; 3.3)*	2.3 *(1.2; 3.7)*	2.6 *(1.9; 3.3)*	0.3 *(-1.2; 1.8)*	0.486
CARTILAGE BIOCHEMISTRY							
Proteoglycan synthesis	3.5 *(-0.2; 7.2)*	1.4 *(0.1; 2.7)*	-2.1 *(-5.4; 1.2)*	6.1 *(-1.7; 13.9)*	3.5 *(-3.4; 10.3)*	-2.7 *(-9.3; 4.0)*	0.686
Proteoglycan retention	79.7 *(61.1; 97.7)*	92.0 *(84.4; 99.6)*	12.4 *(-10.8; 5.6)*	75.7 *(50.2; 101.2)*	89.2 *(74.4; 104.0)*	13.5 *(-6.6; 33.6)*	0.886
Proteoglycan content	37.8 *(20.4; 55.2)*	27.8 *(24.6; 31.0)*	-10.0 *(-25.3; 5.3)*	30.7 *(27.3; 34.1)*	34.1 *(28.3; 39.9)*	3.4 *(-2.6; 9.4)*	**0.029**
Proteoglycan release	10.5 *(9.6; 11.4)*	12.0 *(10.7; 13.5)*	1.6 *(-0.4; 3.5)*	12.6 *(12.0; 13.2)*	13.8 *(10.9; 16.7)*	1.2 *(-1.1; 3.6)*	0.886

* For Force Plate Analysis a linear mixed model was used to analyze multipe timepoints.

Overview of all outcome parameters of the *in vivo* experiment using canine IL4-10 FP. Values are expressed as mean and 95%CI.

PBS: Phosphate buffered saline, cIL4-10 FP: Canine IL-4-10 fusion protein, OA: Osteoarthritis.

Discussion

In a previous explorative *in vivo* study, no antibody formation was observed after two repeated injections of human IL4-10 FP in dogs[28]. This encouraged us to test the efficacy of multiple repeated injections of human IL4-10 FP in this canine model. However, ten intra-articular injections with human IL4-10 FP induced significant IL4-10 FP neutralizing antibody formation that prevented evaluation of the effects of human IL4-10 FP. Although apparently obvious, this demonstrates that testing a human protein for longer periods in an animal model may induce unwanted antibody formation that may prevent therapeutic efficacy. Therefore, in this study we designed a canine variant of the IL4-10 FP and tested this for its DMOAD activity in the *in vivo* canine Groove model.

A clear limitation of this study is the number of dogs treated with the cIL4-10 FP and, as a result, the lack of power. Due to ethical approval and budget reasons it was not possible to extend the number of dogs per group to the originally intended n=8. The study also lacks a healthy control group. The use of the contralateral knee as an internal control is reported to be valid[31,32] but has its limitations as altered loading or systemic alterations by development of the OA as well as the treatment may have been of influence. Nevertheless, the value of using a large animal model with the financial and ethical limitations but significantly higher translational value to the human situation may be appreciated.

To ensure comparable pharmacological activity, the design of the canine IL4-10 FP, was similar to that of human IL4-10 FP and the *in vitro* bioactivity was evaluated in the same type of species specific assays. Either fusion protein consisted of full length species specific IL-4 and IL-10 sequences, connected with the same flexible glycine-serine-rich linker sequence. Indeed, cIL4-10 FP showed anti-inflammatory and chondroprotective activity in canine *in vitro* models, similar to the activity of hIL4-10 FP in human *in vitro* models[27,28,35]. In addition, cIL4-10 FP showed analgesic and chondroprotective effects in an *in vivo* canine model of OA, although the second part of the study was limited to four dogs per group. Importantly, canine IL4-10 FP did not induce antibody formation upon multiple intra-articular injections in this canine model. The human variant of the IL4-10 FP has been demonstrated to be stable under different conditions and not delink. As such the delinking of the canine variant is not anticipated, due to its same construction, although not studied in detail.

According to previous findings, in this OA model only minimal synovial inflammation is observed in PBS injected knees, as well as in cIL4-10 FP injected knees, underlining the non-immunogenic nature of the species specific IL4-10 FP. The low grade synovial inflammation in this model was an advantage in demonstrating potential intra-articular immunogenic responses as well as inflammation independent chondroprotective effects, but more or less ruled out the evaluation of the anti-inflammatory activity of the cIL4-10 FP. Nevertheless, *in vitro* cIL4-10 FP had anti-inflammatory properties, shown by the ability to

reduce LPS-induced TNFα production in canine whole blood. Moreover, hIL4-10 FP lowered the release of inflammatory cytokines from cartilage and synovial tissue, and decreased cartilage destructive properties of inflamed synovial tissue[27,28,35].

In addition to the potential anti-inflammatory effects, we here demonstrated that cIL4-10 FP is also chondroprotective *in vivo*. These findings are in line with the *in vitro* chondroprotective effect of cIL4-10 FP, normalizing the hampered PG synthesis rate of compromised OA canine cartilage. Moreover, hIL4-10 FP normalizes PG release and PG synthesis, as well as PG content in human *in vitro* OA cartilage experiments[28,35].

In the present study, joint loading was used as a proxy for OA pain, induced by Groove surgery. A reduction in FPA parameters represent an objective outcome measure for evaluation of lameness, and to some extent of the magnitude of pain. However these measures do not mean that it tells us exactly how much pain dogs experience or, translating it to the human situation, how it affects quality of life[36]. It might be possible that dogs adapt their joint loading over time despite persisting pain. Irrespectively, we demonstrated a significant improvement in the OA induced reduction in joint loading over the injection period. These data corroborate our previous finding that two intra-articular injections with hIL4-10 FP improved joint loading in the canine Groove model[28] prior to established systemic and local immunogenic response due to a species-mismatch. Overall these findings indicate that IL4-10 FP has analgesic properties. Indeed, the IL4-10 FP also inhibited pain in an *in vivo* rat Groove model[37] and two mouse models, confirming the analgesic capacity of the IL4-10 FP[27,28,38].

Altogether, the above-mentioned anti-inflammatory, chondroprotective, and analgesic properties clearly warrant further research to develop the IL4-10 FP as a DMOAD. In treatment of the larger joints, intra-articular treatment may be preferred above systemic treatment since it needs less (expensive) drug in comparison to systemic application, has a lower risk of systemic side effects, and targets the non-vascularized articular cartilage directly. This would favor patients and healthcare systems. However, application in generalized OA including more and smaller joints will be a challenge. As such, treatment at first focusses on local administration in the larger joints. However, a few issues need to be addressed before it can be brought into a clinical trial. Firstly, injecting patients every week is not feasible in clinical care. To overcome this problem, it is essential to find a way to enhance bioavailability in the joint. Currently, different molecule characteristics and slow release systems are being tested. Secondly, phenotypic differences between OA patients may warrant phenotypic treatment approaches (personalized medicine). IL4-10 FP consists of two anti-inflammatory cytokines, therefore future *in vivo* studies are needed to evaluate whether or not an inflammatory phenotype is the most responsive to this FP.

In conclusion, human IL4-10 FP is immunogenic in dogs upon multiple repeated intra-articular injections. Canine IL4-10 FP can be produced and diminishes canine blood cell inflammatory

activity and improves PG synthesis of canine cartilage. In addition, the canine IL4-10 FP shows promising DMOAD activity *in vivo* in the canine Groove model. Further research to develop the IL4-10 FP as a DMOAD is clearly warranted.

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6 IL4-10 FUSION PROTEIN SHOWS DMOAD ACTIVITY IN A RAT OSTEOARTHRITIS MODEL

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Abstract

Objectives

Ideally, disease modifying osteoarthritis (OA) drugs (DMOAD) should combine chondroprotective, anti-inflammatory, and analgesic effects in a single molecule. A fusion protein of Interleukin-4 (IL-4) and IL-10 (IL4-10 FP) possess these combined effects. In this study, the DMOAD activity of rat IL4-10 FP (rIL4-10 FP) was tested in a rat model of surgically induced OA under metabolic dysregulation.

Methods

rIL4-10 FP was produced with Human Embryonic Kidney 293F cells. Bioactivity of purified rIL4-10 FP was determined in a whole blood assay. Male Wistar rats (n=20) were fed a high-fat diet (HFD) to induce metabolic dysregulation. After twelve weeks, OA was induced according to the Groove model. Two weeks after OA induction, rats were randomly divided into two groups and treated with ten weekly, intra-articular injections of either rIL4-10 FP (n=10) or phosphate buffered saline (PBS; n=10). Possible antibody formation was evaluated using enzyme-linked immunosorbent assay, cartilage degeneration and synovial inflammation were evaluated by histology and mechanical allodynia was evaluated using the von Frey test.

Results

Intra-articular injections with rIL4-10 FP significantly reduced cartilage degeneration ($p=0.042$) and decreased mechanical allodynia ($p<0.001$) compared to PBS. Only mild synovial inflammation was found (ns), limiting detection of putative anti-inflammatory effects. Multiple injections of rIL4-10 FP did not induce antibodies against rIL4-10 FP.

Conclusion

rIL4-10 FP showed chondroprotective and analgesic activity in a rat OA model with moderate cartilage damage, mild synovial inflammation, and pain. Future studies will need to address whether less frequent intra-articular injections, e.g. with formulations with increased residence time, would also lead to DMOAD activity.

Introduction

Osteoarthritis (OA) is the most prevalent chronic degenerative joint disease, predominantly characterised by cartilage damage and pain[1]. Unfortunately, a disease modifying OA drug (DMOAD) is still not available. The Food and Drug Administration and the European Medicines Agency demand a DMOAD that combines chondroprotective and analgesic effects in one molecule[2,3].

Both, interleukin 4 (IL-4) and IL-10, are immune modulatory cytokines that also act on different OA pathways[4]. Besides its anti-inflammatory effects[5], IL-4 reduces cytokine-induced cartilage proteoglycan degradation in bovine cartilage explants[6]. Likewise, IL-10 administered before or after axial compression protected against injury induced apoptosis and extracellular matrix degradation *in vitro*[7,8]. Both cytokines have immune modulatory effects, but IL-4 and IL-10 act differently[9], and possibly synergize in their immunoregulatory activity. IL-4 increases degradation of pro-inflammatory cytokine mRNA, whereas IL-10 inhibits nuclear factor κB and with that the transcription[10]. Moreover, by combining the two cytokines, potential pro-inflammatory effects of IL-10 can be counteracted by IL-4[10,11]. Combining both, suppressed macroscopic signs of inflammation, reduced cellular infiltrates in synovial tissue, and protected against cartilage destruction, better than each of the cytokines alone, in a model of collagen-induced arthritis in mice[12]. In hemophilic arthropathy, a joint disease with clear degenerative and inflammatory characteristics, the combination of IL-4 and IL-10 protected against this blood-induced cartilage damage[13].

To combine the effects of both cytokines in a single molecule and increase bioavailability, a fusion protein of IL-4 and IL-10, IL4-10 FP, has been developed[11]. IL4-10 FP inhibits pain in a mouse model for persistent inflammatory pain through inhibition of spinal neuroinflammation and inhibiting sensory neurons[14,15]. In addition, IL4-10 FP has chondroprotective and anti-inflammatory effects in *in vitro* and *in vivo* models for hemophilic arthropathy[16] as well as in human *in vitro* OA models[17].

In order to achieve a high concentration in the joint and ensure maximal penetration into the joint tissues while minimizing systemic side-effects, the IL4-10 FP is specifically developed for intra-articular application[18]. The chondroprotective and analgesic effects of IL4-10 FP were previously confirmed with intra-articular injections of canine IL4-10 FP in the canine OA Groove model[19].

To test the IL4-10 FP in a rodent model of OA, the rat Groove model was used[20]. This model of surgically induced cartilage damage is mild and allows for tissue repair as there is no permanent trigger for joint damage (as e.g. in instability or chemical induced models). When this damage is induced in rats that are metabolically dysregulated with a high-fat diet (HFD), joint damage increases[21], widening the window to evaluate DMOAD activity.

In the present study rat IL4-10 FP (rIL4-10 FP) was developed and its DMOAD activity was tested upon repeated intra-articular injections in the rat OA Groove model, in rats on HFD, to verify the results found in the canine OA Groove model. Using a rat model makes it possible to increase the study group. Besides, in contrast to previous studies, the model used here reflects a more age/obesity-associated established/late-stage OA, making it more translatable to the human conditions[21,22].

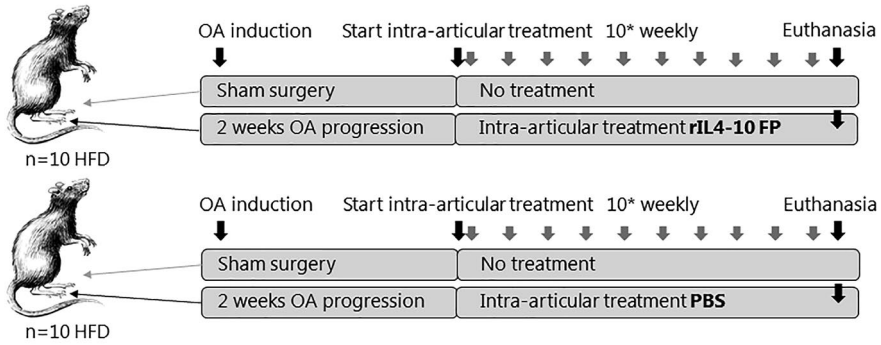
Methods

Study design

Male Wistar rats (n=20), twelve weeks old, were housed two per cage under 12:12 hour light-dark cycle, with access to high-fat food pallets (HFD) and tap water *ad libitum*. After twelve weeks, Groove surgery (OA induction) was performed in the right knee joint of each rat[20]. In short, under general anesthesia, five longitudinal grooves of 150-180µm depth were made in both femoral condyles, without damaging the underlying subchondral bone. Sham surgery was performed in the left knee joint of each rat, to serve as internal control. Analgesia (buprenorphine 10-50µg/kg) was administered to all animals during the first 24h after surgery. Rats were allowed to move freely immediately after surgery.

Subsequently, two weeks after OA development, the rats were randomly divided into two groups of ten rats each to receive ten weekly intra-articular injections of either 0.5µg in 25µL rIL4-10 FP or 25µL phosphate buffered saline (PBS) in the right, OA affected, knee joint, under general anesthesia. The concentration used is comparable to the concentration used in the canine study (10µg in 500µL). The sham operated knee joints were left untouched. After ten weeks with weekly intra-articular injections, rats were euthanized and tissue samples were harvested (fig. 1). Blood samples of all rats in the rIL4-10 FP group were obtained at surgery, to determine baseline values, three weeks after start of intra-articular injections, and at the end of the study, to check for Immunoglobulin G (IgG) antibody formation against the rIL4-10 FP. The study was approved by the Utrecht University Medical Ethical Committee for animal studies (AVD115002016688) and was fully compliant with ARRIVE guidelines.

Figure 1. Experimental set-up



HFD: 24 weeks old, twelve weeks high-fat diet male Wistar rats, OA: Osteoarthritis, PBS: Phosphate buffered saline.

Production and Characterization of rat IL4-10 FP

Production and characterization of rIL4-10 FP are largely done using the same procedures as published previously by this group for cIL4-10 FP[19].

Transfection and cell culture

rIL4-10 FP was produced by transient transfection of Human Embryonic Kidney (HEK) 293F cells with a pcDNA3.1-neo expression vector containing a dual cytomegalovirus promotor. The vector contained two transgenes: cDNA coding for rIL-4-10 and cDNA coding beta-galactosidealpha-2,3-sialyltransferase to optimize glycan capping with sialic acid. To enable purification, a hexa-histidine affinity tag was cloned on the N-terminus of rIL4-10 FP. Cells were cultured in GIBCO® FreeStyle™ 293 Expression Medium. The medium contained no serum or antibiotics. Cells were grown in flasks on a shaker platform in humidified, 5% CO_2 cell culture incubator at 37°C. Cells were split three to four times prior to transfection and they were transfected at the cell viability of 90% and one million cells/ml cell density. The transfection reagent used was 293fectin™. Culture supernatant was harvested 72h after transfection.

Protein purification

rIL4-10 FP was purified from culture medium via His-tag using Nickel-nitrilotriacetic (ni-NTA) agarose according to manufacturer's protocol. In short, protein was purified under native conditions with equilibration buffer (50mM Na_2HPO_4, 0,3 M NaCl, 10mM imidazole) and elution buffer (50mM Na_2HPO_4, 0,3 M NaCl, 250mM imidazole). Purified protein was dialyzed overnight against 2L of PBS (pH=7.4), sterile filtered and stored at -80°C until use. Purity

of rIL4-10 FP batches was evaluated by Coomassie-stained 12% sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and high performance size exclusion chromatography analysis.

Bioactivity assay

The bioactivity of purified rIL4-10 FP was evaluated *in vitro* in a rat whole blood assay. Heparinized rat blood obtained on the day of surgery was diluted 1:10 in Rosswell Park memorial Institue 1640 medium, supplemented with 1% penicillin/streptomycin. Lipopolysaccharide (LPS) was added at 100ng/ml final concentration. rIL4-10 FP as well as controls, recombinant rIL-4, and rIL-10, were simultaneously added and titrated in equal molar ratios, ranging from 0.001-3nM. After 18h incubation at 37°C, 5%CO2, tumor necrosis factor-α (TNFα) was measured in culture supernatants. The inhibition of inflammatory response by cytokines (rIL4-10 FP, rIL-4 or rIL-10) was calculated according to the formula: %inhibition= (1-(A-B)/(C-B)) x 100, where A=TNFα levels in LPS-stimulated cultures treated with cytokines, B=TNFα levels in unstimulated culture, and C =TNFα levels in LPS-stimulated culture.

SDS-PAGE

Samples were diluted 1:1 in 2x Laemmli Sample Buffer containing 100mM DL-dithiothreitol, incubated ten minutes at 95°C, and loaded on a 12% polyacrylamide gel. Electrophoresis was performed at 150V for 1,5h, with reducing conditions (Tris/glycine/SDS buffer). Protein bands in the gel were visualized by InstantBlue protein stain.

Western blotting

After electrophoresis, proteins were transferred to a nitrocellulose membrane. After blotting, membranes were blocked in 5% milk in PBS with 0.1% Tween-20 (PBST) and thereafter incubated overnight with primary antibody (biotinylated goat anti-rat-IL-4; BAF504 0.1µg/mL, or biotinylated goat anti-rat-IL-10; BAF519 0.1µg/mL) in PBST containing 1% milk. Membranes were subsequently incubated with poly-horseradish peroxidase (HRP) for thirty minutes at room temperature. To visualize the bands enhanced chemiluminescence Western Blotting Substrate was added according to the manufacturer's protocol.

Evaluation of immunogenicity of rIL4-10 FP

Immunogenicity of rIL4-10 FP was evaluated by measuring the antibody titer in rat sera using enzyme-linked immunosorbent assay (ELISA). Wells were coated with 1µg/ml of rIL4-10 FP and were allowed to react with appropriately diluted rat sera, followed by incubation with HRP-labeled goat anti-rat IgGs and then 3,3',5,5'-Tetramethylbenzidine substrate solution. Antibody titer was determined using the endpoint dilution. Serial dilutions of sera from rats treated with PBS were used to define the background optical density.

Mechanical allodynia measurement

Mechanical allodynia was assessed in six randomly selected rats of the ten rats in each group on a power calculation with an effect size of 2.667, correction for multiple testing, and a power of 90%. Assessment was performed 24h before, and 24h after each intra-articular injection of either rIL4-10 FP or PBS. The experimenter was blinded to treatment. Rats were acclimatized to the specific set-up three times before testing and placed in enclosures on an elevated wire mesh floor where mechanical allodynia was assessed by applying von Frey hairs to the plantar surface of both hind paws. The hair force was increased or decreased according to the response and the 50% paw withdrawal threshold (PWT) was calculated using the up-and-down method as previously described[23].

Histopathological examination of the knee joint

At the end of the study, the joint degeneration of both knee joints was evaluated using the OsteoArthritis Research Society International (OARSI) histopathology score for rats[24]. In short, knee joints were fixed in formalin and subsequently embedded in paraffin. Coronal plane sections of 5µm thickness were made at 100µm intervals. Safranin-O (Saf-O) staining was used to assess joint histopathology. The total OARSI score is based upon the sum of the following sub sections: cartilage matrix loss width (0-2), cartilage degeneration (0-5), cartilage degeneration width (0-4), osteophytes (0-4), calcified cartilage and subchondral bone damage (0-5) and synovial membrane inflammation (0-4). Sections were scored in random order by two experienced observers, blinded for treatment. The surgically applied grooves were not taken into account during scoring.

Statistical analysis

To evaluate the analgesic effects of rIL4-10 FP a linear mixed model was used to account for the repeated PWT over time within subjects. The difference in PWT between the treated paw and control paw was used as outcome in this analysis and a random intercept at the level of subject was used. Treatment week, injection time (pre- or post-injection) and treatment groups (PBS or rIL4-10 FP) were used as fixed independent variables. To test whether the effect of injections over time (pre- *vs* post-injection) was different between PBS or rIL4-10 FP treated subjects, the interaction between group and injection times was tested. Separate analysis within treatment groups were also performed, and the stability of the treatment effect over time was tested.

Histological data is presented as mean±SD. Changes between OA joints and control joints were calculated. In three rats of each group, the experimental joint as well as the control joint could not be evaluated due to technical reasons (incorrect views) and as such not reliable assessed. The remaining material was insufficient to repeat the procedure. One additional control joint could not be scored in each group. The value for this control joint

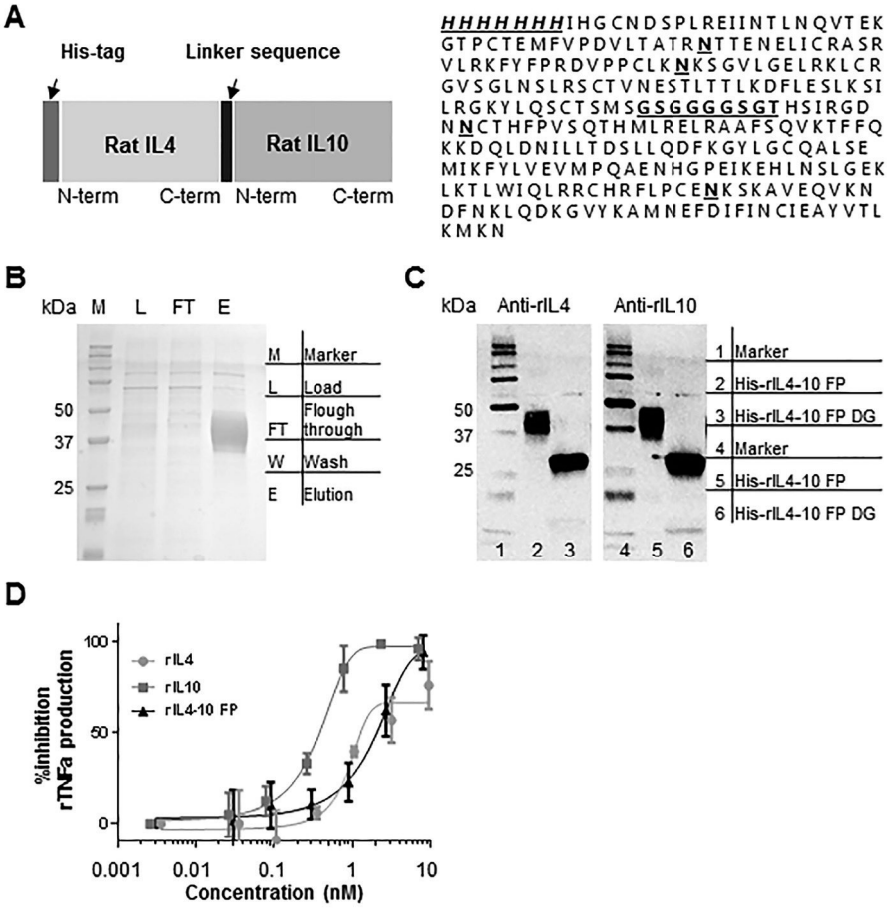
was imputed by taking the mean of the other six control joints. Outliers defined by values higher or lower than mean±2*SD were excluded, (n=1 for each group). Mann-Whitney U tests were used to compare change scores between rIL4-10 FP and PBS group.

Results

rIL4-10 FP characteristics

A schematic representation of the rIL4-10 FP and its amino acid sequence, including the linker sequence and four predicted glycosylation sites are depicted in figure 2a. Protein was produced by transient transfection of HEK293F cells and purified from culture supernatant by Ni-NTA affinity chromatography. Purified rIL4-10 FP was observed on Coomassie stained SDS gel as a smear composed of multiple protein bands with a molecular mass of 35-45kDa (fig. 2b). Smeared protein bands were also detected on a Western blot by anti-IL-4 and anti-IL-10 antibodies, while upon deglycosylation with PNGaseF only one sharp protein band of 30kDa was detected (fig. 2c), indicating that the multiple bands correspond to different glycoforms of rIL4-10 FP. The bioactivity of rIL4-10 FP was evaluated *in vitro* by its ability to inhibit TNFα production in a LPS-stimulated rat whole blood culture (fig. 2d). The full inhibition of TNFα production was achieved with rIL4-10 FP at 8.3nM, whilst IL-10 fully inhibited LPS-induced TNFα at 0.8nM. Thus, *in vitro* rIL4-10 FP activity is approximately 10-fold lower compared to IL-10.

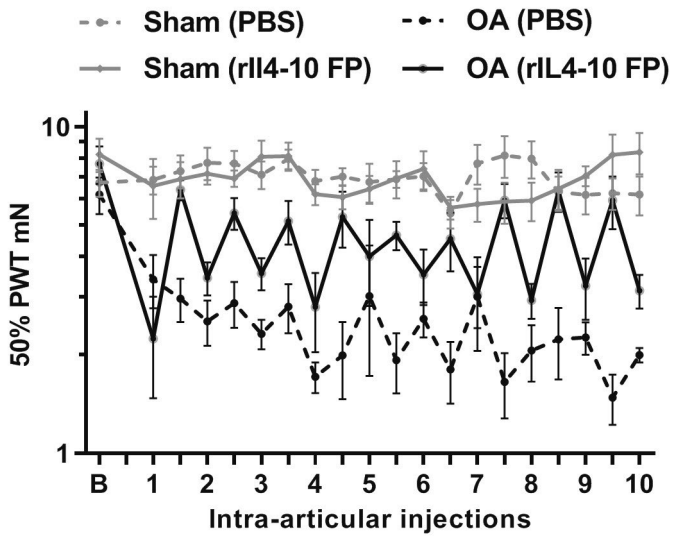
Figure 2. Molecular characterization of the rIL4-10 FP



Production and characterization of rIL4-10 FP are largely done according to the procedures as published previously by this group for cIL4-10 FP[19]. A. Schematic overview of the rIL4-10 FP and its amino acid sequence. N-terminal His-tag and linker sequence are indicated in bold italic. Potential N-linked glycosylation sites are indicated in bold. B. Coomassie stained SDS gel of Ni-NTA protein purification steps. M: Marker, L: Load, FT: Flow-through, W: Wash, E: Elution, C. Western blot analysis of purified rIL4-10 FP (untreated and deglycosylated). D. Bioactivity assay performed in rat whole blood culture. Blood was collected for *in vitro* testing at day of surgery, independent of treatment. Activity of rIL4-10 FP was evaluated *in vitro* according to its ability to inhibit TNFα secretion as a response to LPS-stimulation.

Mechanical allodynia

Figure 3. Effects of rIL4-10 FP on mechanical allodynia



The difference in 50% paw withdrawal thresholds (PWT) between osteoarthritis (OA) paws and control paws reduced 24 hours after intra-articular injection with rIL4-10 FP compared to 24 hours before intra-articular injection.

The PWT in experimental and contralateral paws in both groups over time are presented in figure 3. The intercept of the model indicating average difference in baseline PWT (i.e. before intra-articular treatment) between control and experimental paws was -4.46mN (95%CI -5.56; -3.36, $p<0.001$) indicating that pain hypersensitivity developed over time in the OA Groove model.

In the linear mixed model, a statistically significant interaction was found between treatment group and the effect of intra-articular injections ($p<0.001$) indicating that the effect of rIL4-10 FP injections on PWT is beneficial over PBS injections with an average difference of 2.68mN (95%CI 1.50; 3.83).

Analysing only rats treated with rIL4-10 FP injections showed that, on average, an intra-articular injection with rIL4-10 FP reduced the difference in PWT between OA and control paw after injection (compared to before injection) with 2.45 (95%CI 1.52; 3.37, $p<0.001$, table 1). This effect was not found to be different over time ($p=0.779$). Analysing only PBS injected rats showed no statistically significant effects on PWT.

Table 1. Effects of rIL4-10 FP on mechanical allodynia using a linear mixed model analysis

			95%CI	
Independent variable	***Coefficient***	***p-value***	***Lower bound***	***Upper bound***
Intercept	-3.82	**<0.001**	-7.86	0.22
Injection	2.45	**<0.001**	1.52	3.37
Week	0.02	0.803	-0.15	0.19

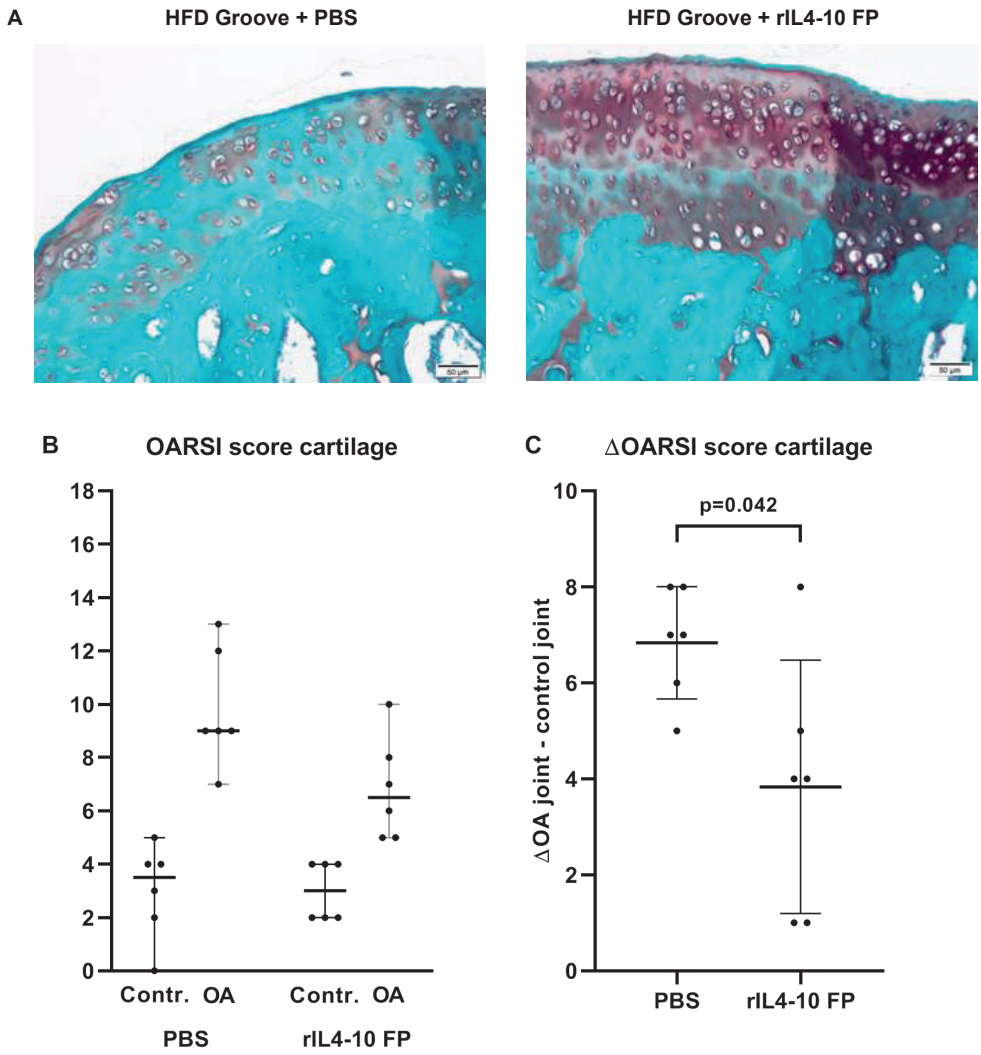
Pain was measured by determining the 50% Paw Withdrawal Thresholds (PWT) using von Frey test. The intercept indicates the average difference in baseline PWT between control and experimental (Grooved) paws before intra-articular treatment.

Joint histopathology

Local cartilage damage induced according to the Groove model resulted in increased joint degeneration twelve weeks post-surgery (two weeks OA development followed by ten weeks intra-articular injections) compared to the sham operated control knee joints in both groups (OARSI histopathology score 9.8±2.2 *vs* 3.0±1.8 and 6.8±1.9 *vs* 3.0±1.1 for PBS and rIL4-10 FP, respectively). These results are in line with previous published data using this model[21].

The increase in cartilage damage after OA induction was less in the rIL4-10 FP group compared to the PBS injected group (ΔOARSI score +6.8±1.2 *vs* +3.8±2.6 in PBS and rIL4-10 FP injected group, respectively, p=0.042, fig. 4).

Figure 4. Cartilage degeneration

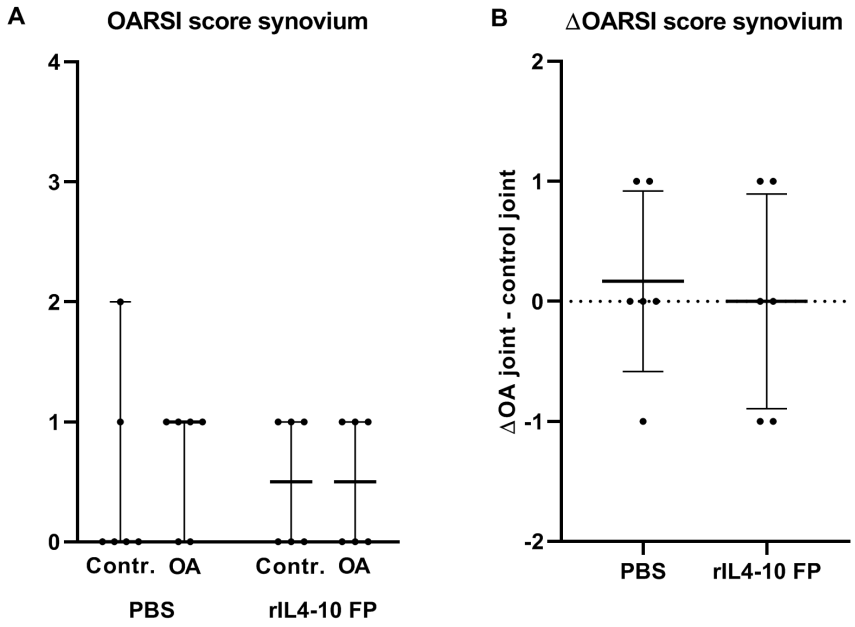


A. Pictures of Saf-O stained joints of PBS injected and rIL4-10 FP injected joints. B. OARSI scores for cartilage and bone damage of sham control and treated OA joints ten weeks after treatment for the PBS and the rIL4-10 FP injected animals. Average scores (median values with 95%CI) are provided for each group. Maximum score is twenty points. C. Differences in cartilage degeneration between sham control joints and treated OA joints for each individual animal. Average scores (median values with 95%CI) are provided for each group.

PBS: Phosphate buffered saline, OARSI: Osteoarthritis Research Society International, OA: Osteoarthritis, HFD: High-fat diet.

Only mild synovial inflammation was found; 0.5±0.8 *vs* 0.7±0.5 for sham operated *vs* PBS injected joints (ns), and 0.5±0.5 *vs* 0.5±0.5 for sham operated *vs* rIL4-10 FP injected joints (ns), preventing evaluation of anti-inflammatory effects (fig. 5). The change in inflammation after OA induction was not different between both groups (0.1±1.0 *vs* 0.5±0.9, for rIL4-10 FP and PBS, respectively).

Figure 5. Synovial inflammation

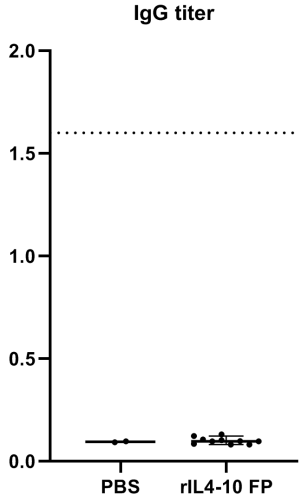


A. OARSI synovial inflammation scores of sham control and treated OA joints ten weeks after treatment for the PBS and the rIL4-10 FP injected groups. Average scores (median values with 95%CI) are provided for each group, maximum score is four points. B. Differences in synovial inflammation between sham control joints and treated OA joints. Average scores (median values with 95%CI) are provided for each group.
PBS: Phosphate buffered saline, OARSI: Osteoarthritis Research Society International, OA: Osteoarthritis.

Immunogenicity of rIL4-10 FP

The IgG antibody titer found in sera of PBS treated rats (n=2) and rIL4-10 FP treated rats (n=10) was 1/1000, corresponding to the background signal, indicating that rIL4-10 FP was not immunogenic in rats after ten weekly intra-articular injections (fig. 6).

Figure 6. Immunogenicity of rIL4-10 FP



IgG antibody titers in sera of PBS injected rats (n=2) and rIL4-10 FP injected rats (n=10). The dotted line represents the titer of 1.6 after an immunogenic response on human IL4-10 FP in the canine model.

PBS: Phosphate buffered saline, IgG: Immunoglobulin G.

Discussion

This study for the first time shows the disease modifying effects of a species specific fusion protein of IL-4 and IL-10 in a rat OA model. The concentration used in this rat study (500ng/25µL) is comparable to the concentration used in the canine model (10µg/500µL)[19]. Intra-articular treatment started already two weeks after OA induction. The OA status after two weeks is unknown but considered mild, therefore the described effects of rIL4-10 FP treatment possibly represent the prevention of (further) development of OA after surgically induced cartilage damage, rather than actual treatment of more established OA. Clinical application of the fusion protein is also anticipated at a relative early stage of the disease, arresting the degenerative process.

In the canine Groove model, human IL4-10 FP (hIL4-10 FP) led to IgG antibody titers of 1.6, whereas a species specific canine IL4-10 FP (cIL4-10 FP) did not lead to antibody formation[19]. In the present study, the absence of IgG antibody formation in case of rIL4-10 FP injections in rats confirmed the non-immunogenic nature of a species specific IL4-10 FP.

Repeated intra-articular injection with rIL4-10 FP restored the by the OA model increased mechanical allodynia to values comparable to the control paws. Additionally, the histologically observed joint damage, developed over a 12-week period in the OA joints, was significantly less in the rIL4-10 FP injected group as compared to the PBS injected group. The histopathology clearly shows severe progression of tissue damage in the PBS treated animals, with the OARSI histopathology scores reaching high values on average, as compared to the rIL4-10 FP treated animals, with lower scores on average and clearly more healthy joint tissue.

Analgesic and chondroprotective effects are two important features of a DMOAD. The potential of IL4-10 FP as a DMOAD is further supported as these rodent data corroborate comparable DMOAD effects of cIL4-10 FP in the canine Groove model of OA[19] and chondroprotective effects of hIL4-10 FP in human OA cartilage explants and pain relief in a dog model[17]. Moreover, similar analgesic and chondroprotective effects of hIL4-10 FP have also been observed in a mouse model of hemophilic arthropathy[16].

Due to an absence of a significant inflammatory component in this rat model, an anti-inflammatory effect of rIL4-10 FP could not be confirmed. However, previous studies demonstrated anti-inflammatory effects of the IL4-10 FP[13,16,17].

The preclinical evaluation of DMOADs is frequently performed by treating prophylactically or early in the OA process, immediately after OA induction (mostly post-traumatic OA), in young and normal-weight animals. This does not match the clinical OA population, which is focused on age-related established/late-stage OA, frequently associated with obesity[22]. Thus, the OA target population and preclinical phenotype are often mismatched. In this study, a combination of HFD and Groove surgery was used as OA model. This combination results in a more clinically relevant model of OA[21]. Therefore it was anticipated that this model is suitable to evaluate in a more translational approach the DMOAD activity of rIL4-10 FP.

Pain is the predominant symptom of OA and the reason why OA patients seek medical assistance[1]. In this study a transient analgesic effect of intra-articular injections with a species specific IL4-10 FP was found. Pain was assessed in a limited number of rats (six per group), nevertheless, the results are in line with previous results, found in an *in vivo* canine model of OA[19].

In vitro, the activity of rIL4-10 FP is 10-fold lower compared to the activity of solely IL-10 (fig. 2d). However, full inhibition is achieved by both, and combining IL-10 with IL-4 increases bioavailability and effectuates possible synergy between both cytokines.

The mechanisms by which intra-articular injected IL4-10 FP reduced mechanical hypersensitivity are not yet clear. However, recently we have shown that IL4-10 FP, when injected intrathecally, inhibits inflammatory pain through direct signaling to sensory

neurons[15]. Indeed various studies showed that cytokines, such as IL-10 and IL-4 may have direct effects on neurons and control their excitability[25-29]. Although IL4-10 FP may have direct analgesic properties through direct effects on sensory neurons, subchondral bone osteoclasts and chondrocytes also contribute to osteoarthritis pain[30,31] and are known to express IL-4 and IL-10 receptors[13,32,33]. Thus it is likely that the observed analgesic effects of IL4-10 FP are also mediated (in part) through indirect actions through osteoclasts and chondrocytes or even other intermediate cells.

In general, rapid clearance from the joint cavity is a major drawback of intra-articular administration of drugs and requires repetitive injections after relatively short time periods. In a murine model for persistent inflammatory pain, intra-articular injection did not influence hyperalgesia at all, whereas intrathecal treatment with IL4-10 FP reduced pain for a time period of 2-4 days[14]. Nevertheless, this is still too short for use in clinical practice. These results clearly show that, despite the analgesic activity, more sustained analgesic effects upon a single intra-articular injection or other delivery routes become key in future studies. Therefore, an important goal in the development of local OA treatment should be extending the duration of effects within the joint to enhance clinical relevance. A slow-release formulation of a hydrogel has previously been proposed with good results in animal studies[34].

In conclusion, repeated (weekly) intra-articular injections of rIL4-10 FP in this rat OA model with OA development in a metabolic dysregulated background result in relief of OA induced pain and prevent/slow down joint damage. As such, DMOAD activity of IL4-10 FP in this OA model but also its activity in an *in vivo* canine OA model and *in vitro* human OA models warrant further research. In particular an improved understanding of the requirements for an increased duration of action will be needed to enable clinical development of this potential DMOAD.

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PART II:
FIRST STEPS TOWARDS A NEW APPROACH

7 Cohort profile: The IMI-APPROACH study: A 2-year, European, cohort study to describe, validate, and predict phenotypes of osteoarthritis using clinical, imaging, and biochemical markers

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Abstract

Objectives

The IMI-APPROACH consortium intends to prospectively describe in detail, pre-selected patients with knee OA, using conventional and novel clinical, imaging, and biochemical markers, to support OA drug development.

Methods

Innovative Medicines Institute Applied Public-Private Research enabling OsteoArthritis Clinical Headway (IMI-APPROACH) is a prospective cohort study including 297 patients with tibiofemoral OA, according to the ACR classification criteria. Patients were (pre-)selected from existing cohorts using machine learning models, developed on data from the Cohort Hip & Cohort Knee (CHECK) cohort, to display a high likelihood of radiographic joint space width (JSW) loss and/or knee pain progression.

Results

Selection appeared logistically feasible and baseline characteristics of the cohort demonstrated an OA population with more severe disease: age 66.5 (SD 7.1) *vs* 68.1 (7.7) years, minJSW 2.5 (1.3) *vs* 2.1 (1.0) mm, and KOOS pain 31.3 (19.7) *vs* 17.7 (14.6), except for age, all: $p<0.001$, for selected *vs* excluded patients, respectively. Based on the selection model, this cohort has a predicted higher chance of progression.

Future plans

Patients will visit the hospital again at 6, 12, and 24 months for physical examination, pain and general health questionnaires, collection of blood and urine, MRI scans, radiographs of knees and hands, CT scan of the knee, low radiation whole-body CT, HandScan, motion analysis, and performance-based tests.

After two years, data will show whether those patients with the highest probabilities for progression experienced disease progression as compared to those with lower probabilities (model validation) and whether phenotypes can be identified and predicted to facilitate targeted drug therapy.

Strengths and limitations of this study

The IMI-APPROACH cohort is part of a larger consortium, bringing together a highly qualified and multidisciplinary group of stakeholders in the form of a public-private partnership of engaged, knowledgeable and complementary industrial, academics, and patient experts.

The IMI-APPROACH cohort is unique in its selection process, recruiting patients from existing cohorts based on machine learning models with encouraging results of which the actual utility needs to be demonstrated at the end of the 2-year follow-up.

The IMI-APPROACH cohort will provide 2-year follow-up data of 297 knee OA patients including conventional and novel, explorative, imaging, biochemical, clinical, and demographic (bio) markers according to strict protocols for acquisition and evaluation with the aim to identify phenotypes and develop predictive models for progression of these phenotypes.

The main limitation of the study is the descriptive phase in which the study is at present and despite the 297 patients still limited in size for the large number of outcome parameters.

Introduction

Osteoarthritis (OA) is characterized by changes in all (peri-)articular tissues[1,2], causing pain, stiffness, and loss of function, usually following a slowly progressive and nonlinear course[2]. OA of the knee is the most common and most disabling, accounting for 83% of total OA burden[3]. In 2010, the global prevalence of knee OA was estimated to be 4.7% in females and 2.6% in males and incidence peaked around the age of 50[4]. Knee OA accounted at that time for 14.218 of total Years Lived with Disability (YLD). This is a 64.8% increase compared to 1990 (8.627), emphasizing the increasing burden of OA[3]. Estimated healthcare costs of knee OA are €4.257 (€383 - €7.675) per patient per year. Non-healthcare-related costs of knee OA, like productivity loss, are estimated to be €1.519 per patient per year[5]. Ageing of the population, an increasing active life style at older age, and the current obesity pandemic all contribute to an even further increase of the incidence and prevalence of OA and its societal burden[6].

Despite this growing OA burden and the still unmet need for effective treatment, pharmaceutical companies seem to have lost their confidence in drug development because clinical trials with potential disease modifying OA drugs (DMOADs) could not demonstrate efficacy. This disappointing result likely has multiple origins.

The typically slow and heterogeneous OA course makes trials easily fall short in terms of size and length for demonstrating treatment efficacy[7]. This issue is further aggravated by the use of relatively insensitive outcome measures (patient reported outcome measures), pain and radiographic joint space changes (x-ray), required by regulatory agencies for a drug to be certified as a DMOAD. Moreover, an incomplete understanding of the OA pathobiology obscures identification of proper treatment targets. This is complicated by the increasing knowledge that the pathobiological mechanisms driving the OA process differs between patients, (type of) joints, and disease stages[2].

This to-date concept of a highly heterogeneous disease contrasts with the one-size-fits-all treatment approach used in most trials and the focus on radiographic joint space narrowing (JSN) and pain as outcome parameters.

New (combinations of) sensitive and robust (bio)markers could importantly contribute to overcome the aforementioned challenges, improving the design of clinical trials in the OA field. Biomarkers with the ability to predict the likely disease course in an untreated individual, so-called prognostic markers, could be employed to identify subjects that will show significant progression of the disease on relevant outcome parameter(s) over the study period. Biomarkers that show a biological response to treatment, response markers, could serve as sensitive outcome parameters, supplementing (or even replacing) radiographic joint space changes and MRI read-outs. These biomarkers could also help to identify vital components of the OA pathobiology and with that distinguish between phenotypes. This

will help to forecast the potential response to treatments targeted to specific mechanisms. Altogether, such biomarkers could importantly improve the quality and effectiveness of trials of potential DMOADs and joint preserving surgical treatments, in terms of selection of study participants, outcome parameters, and study size and length[8].

IMI-APPROACH

Although currently available cohort studies, like the Dutch CHECK[9] and the US OAI with the FNIH[10] have increased our knowledge of the disease, these attempts still have not resulted in clearly distinctive phenotypes with predictive biomarkers. Therefore, the current Applied Public-Private Research enabling OsteoArthritis Clinical Headway (APPROACH) cohort uses a novel strategy and extends on previous studies in several ways. The study is part of a larger consortium being conducted under the Innovative Medicines Initiative (IMI), bringing together a highly qualified and multidisciplinary group of stakeholders in the form of a public-private partnership of engaged, knowledgeable and complementary industrial, academic, and patient experts.

The IMI-APPROACH cohort is unique in its attempt to recruit patients primarily from existing cohorts using machine learning (ML) models (adjusted to the specific cohorts) trained using patient data from the CHECK cohort to increase the likelihood of radiographic joint space width (JSW) loss and/or knee pain progression during a limited, 2-year, follow-up period. The relative short two years period is chosen to facilitate translation of results to pragmatic trial design.

In addition to this unique pre-selection of patients, the IMI-APPROACH cohort combines a very broad spectrum of conventional and novel, explorative, imaging, biochemical, clinical, and demographic markers. Using data science techniques suitable to analyze these 'big data', algorithms of biomarkers will identify and predict phenotypes of OA that share distinct underlying pathobiological mechanisms with their structural and function consequences, relevant for practical and targeted clinical trials.

The objectives of the cohort study are:

- To validate and refine the prediction model for sustained pain and decrease in (minimum) JSW as developed for the selection of patients.
- To develop and validate sensitive markers of/predictive for OA progression by use of conventional and novel clinical, imaging, and biochemical (bio)markers.
- To discover and predict novel OA phenotypes (e.g. post-traumatic, metabolic, ageing, inflammatory, bone driven, and genetic) and (their) disease progression.
- To prospectively describe in detail the discovered phenotypes by use of conventional and novel clinical, imaging, and biochemical (bio)markers.

Methods

Cohort description

The prospective follow-up of the 297 included patients will be two years. A large spectrum of conventional and novel (bio)markers for discovery (baseline, 1-, and 2-year follow-up), and prediction (baseline and change over 6 months) of knee OA phenotypes will be gathered.

Patient selection

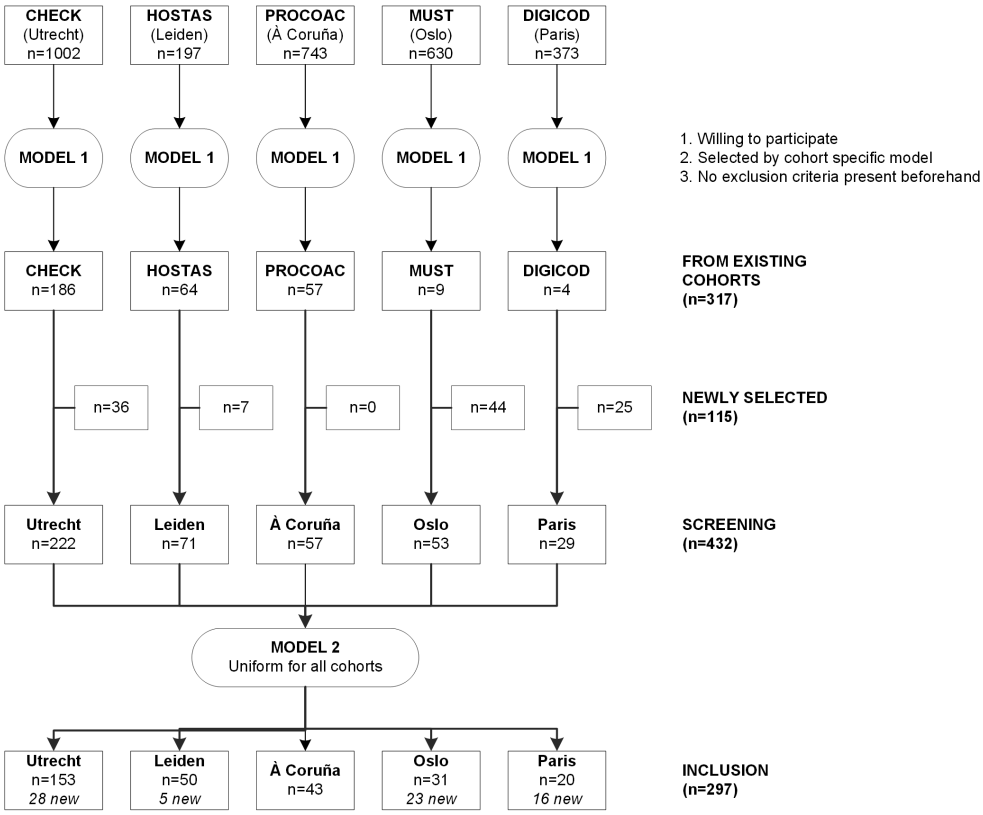
Patients were stepwise selected for a high chance of structural progression (JSN) and/or pain progression/sustained severity over two years, using two ML models for the likelihood of each patient to be a 'progressor'.

The selection process will be described in detail elsewhere[11]. In summary, patients with predominant tibiofemoral OA were selected from five European observational OA cohorts (CHECK[9], HOSTAS[12], MUST[13], PROCOAC[14], and DIGICOD) using a ML approach, trained on longitudinal data from the CHECK cohort, and adjusted for the specific cohorts using the available data from each of the cohorts. Separate models for prediction of structural progression and for sustained significant knee pain or pain progression were used. Structural progression was defined as a reduction in JSW of ≥0.3mm per year over a period of 2-3 years (0.7mm is the minimal detectable difference in radiographic JSW)[15]. Sustained significant pain and pain increase were defined as at least one of the three following: KOOS pain (on a 0 to 100 scale; 0 meaning maximal pain, 100 meaning no pain) decrease ≥5/year and ≤60 at two years, KOOS pain decrease ≥10/year and ≤65 at two years, or ≤60 at both baseline and two years. Three types of progression were defined: pain progression, structural progression, and both pain and structural progression.

All identified patients of the existing cohorts (ranking the highest for predicted progression in the first ML model) willing to participate were invited for a screening visit. During this visit, inclusion and exclusion criteria were checked and an index knee was selected based on American College of Rheumatology (ACR) criteria[16]. If both knees fulfilled the criteria, patients indicated their own index knee based on severity of complaints, in case equal the right knee was selected as the index knee. Key predictors from the first predictive ML model, e.g. Knee injury and Osteoarthritis Outcome Score (KOOS)[17] and Knee Digital Image Analysis (KIDA) parameters[18], were collected and fed into a subsequent predictive ML model that was uniform for all cohorts. The patients that ranked the highest in this second model, were included and invited for a baseline visit.

Because the existing source cohorts could not all provide sufficient patients due to the selection process, patients withdrawing consent, and non-compliance with inclusion criteria, a small number of additional patients were recruited from outpatient departments and invited for a screening visit (fig. 1). These newly recruited patients were also ranked and selected using the second, uniform predictive ML model.

Figure 1. Selection process of the IMI-APPROACH cohort



Inclusion criteria

- Able to walk unassisted
- ≥18 years of age
- Capable of understanding the study
- Capable of communicating in local language
- Predominantly tibiofemoral OA, satisfying the clinical ACR classification criteria:
  - Knee pain, AND:
  - Three or more of the following:
    - >50 years of age
    - <30 minutes of morning stiffness
    - Crepitus on active motion
    - Bony tenderness
    - Bony enlargement
    - No palpable warmth

- High probability of progression based on the algorithm using the following parameters:
  - Reduced version of KOOS questionnaire (pain, stiffness and function)
  - Body Mass Index (BMI)
  - Numeric rating scale (NRS) pain[19] of index knee at moment of screening visit
  - NRS pain of index knee in last week before screening visit
  - Age
  - Gender
  - KIDA parameters of the index knee determined on standard weight bearing radiograph taken at screening[18]

Exclusion criteria

- Inability to comply to the protocol
- Participation in a trial of local therapeutic intervention for index knee OA or potential systemic DMOADs at the time of inclusion, within six months before inclusion, and/or anticipated during two years of follow-up. Participation in non-interventional studies was allowed
- Surgery of the index knee in the six months before inclusion and/or scheduled or expected surgery of the index knee during follow-up
- Current pregnancy or planned pregnancy during follow-up (because of imaging)
- Predominantly patellofemoral knee OA
- Secondary knee OA. e.g. due to severe leg deformity (knee varus or valgus>10°), inflammatory joint disease (either autoimmune, infectious or crystal-induced), severe chondrocalcinosis, Paget's disease of the bone, ochronosis, acromegaly, haemochromatosis, Wilson's disease, osteochondritis dissecans, haemophilia
- Alternative/additional causes of joint pain, e.g. rheumatic symptoms due to malignancies, primary osteochondromatosis, osteonecrosis
- Generalized pain syndrome, e.g. fibromyalgia
- Patients with contraindications for undergoing Magnetic Resonance Imaging (MRI) or Computed Tomography (CT)
- Previous hip replacement or expected hip replacement within six months
- Osteosynthesis material near the knee
- Self-reported severe spine OA
- Current knee prosthesis; in case of surgical replacement of the index or contralateral knee during follow-up, images of that joint will be considered irrelevant and not be obtained. All other acquisitions will be performed as scheduled and patients will remain in the study.

Baseline characteristics of the IMI-APPROACH cohort

The baseline characteristics of the IMI-APPROACH cohort study in total and per center are shown in table 1.

Table 1. Baseline characteristics of the IMI-APPROACH cohort study

	Total n=297	***Utrecht n=153***	***Leiden n=50***	***À Coruña n=43***	***Oslo n=31***	***Paris n=20***	***p-value (ANOVA)***
DEMOGRAPHICS							
Age (years)	66.5 (7.1)	67.5 (6.5)	65.0 (7.0)	66.1 (6.9)	64.6 (8.9)	66.8 (8.8)	0.106
Female (%)	230 (77%)	109 (71%)	39 (78%)	39 (91%)	23 (74%)	20 (100%)	**0.008**
BMI (kg/m^2)	28.1 (5.3)	27.1 (4.4)	27.4 (5.2)	31.3 (5.9)	28.7 (6.4)	29.3 (6.0)	**<0.001**
QUESTIONNAIRES							
KOOS							
Symptoms	69.5 (17.2)	75.2 (15.7)	65.5 (19.9)	61.9 (13.0)	63.7 (16.0)	62.0 (17.7)	**<0.001**
Pain	66.4 (18.8)	73.1 (17.1)	66.8 (19.1)	52.9 (12.7)	56.4 (17.1)	58.9 (19.9)	**<0.001**
ADL	69.1 (19.0)	76.6 (16.5)	69.7 (20.9)	54.0 (10.0)	60.5 (17.3)	56.9 (19.3)	**<0.001**
Sports	42.9 (26.8)	52.1 (27.2)	38.0 (27.4)	28.5 (11.6)	31.8 (23.6)	33.8 (25.7)	**<0.001**
Quality of life	52.9 (20.7)	60.5 (19.0)	52.7 (18.9)	38.7 (12.5)	45.2 (19.1)	39.7 (27.8)	**<0.001**
NRS pain (0-10) Index knee	4.6 (2.7(	3.8 (2.6)	4.3 (2.6)	6.7 (2.0)	5.4 (2.4)	5.7 (2.8)	**<0.001**
RADIOGRAPHY							
KIDA							
Mean JSW index knee (mm)	5.5 (1.0)	5.6 (1.0)	5.4 (1.0)	5.3 (1.1)	5.2 (1.1)	5.3 (0.9)	0.158
Minimum JSW index knee (mm)	2.5 (1.3)	2.7 (1.2)	2.5 (1.3)	2.3 (1.1)	1.8 (1.3)	2.6 (1.3)	**0.008**
KL grade							**0.048**
Grade 0	51 (17%)	36 (24%)	6 (12%)	7 (16%)	0 (0%)	2 (10%)	
Grade 1	90 (30%)	41 (27%)	18 (36%)	14 (33%)	11 (36%)	6 (30%)	
Grade 2	88 (30%)	37 (24%)	14 (28%)	17 (40%)	11 (36%)	9 (55%)	
Grade 3	54 (18%)	30 (20%)	10 (20%)	3 (7%)	9 (29%)	2 (10%)	
Grade 4	10 (3%)	8 (5%)	1 (2%)	1 (2%)	0 (0%)	0 (0%)	

Continuous variables are given as mean (SD). Categorical varibles are given as absolute numbers and percentages. BMI: Body Mass Index, KOOS: Knee injury and Osteoarthritis Outcome Score. ADL: Acitivities of daily living, NRS: Numeric Rating Scale. KIDA: Knee Image Digital Analysis, JSW: Joint Space Width, KL: Kellgren and Lawrence.

Despite ranking of all screened patients from the different cohorts in one uniform ML model, baseline characteristics differed between the patients that were included from the different cohorts, representing the characteristics of the original source cohorts.

Investigation schedule

Conventional and novel clinical, imaging, biochemical, and kinetic markers of the index knee and other joints were obtained at baseline and will be obtained at 6, 12, and 24 months (table 2).

Table 2. Investigation schedule of the IMI-APPROACH cohort study

	Screening	***Baseline***	***M006***	***M012***	***M024***
Medical history	x	x	x	x	x
PHYSICAL EXAMINATION - GENERAL					
Height	x				
Weight	x	x	x	x	x
Waist circumference		x	x	x	x
Blood pressure and pulse rate		x	x	x	x
PHYSICAL EXAMINATION - JOINTS					
ACR criteria for knee OA	x				
Knee		x	x	x	x
Hand		x	x	x	x
Hip		x	x	x	x
IMAGING					
Radiography					
Index knee	x		x	x	x
Contralateral knee		x			x
Hands		x			x
CT-scan					
Index knee		x			x
Whole Body Low Dose CT (WBLDCT)		x			x
MRI-scan of index knee					
Thickness and volume of cartilage, denude bonearea		x	x	x	x
MOAKS assessment		x	x	x	x
T2 mapping		x	x		
HandScan		x			x
FUNCTION TESTS					
Motion analysis (GaitSmart®)		x	x		x
Performance-based tests					
40-meter self-paced walk test		x	x		x
30-second chair-stand test		x	x		x

QUESTIONNAIRES					
KOOS (pain, stiffness, function)	x				
KOOS		x	x	x	x
HOOS		x			x
ICOAP index knee		x	x	x	x
ICOAP hip		x			x
FIHOA		x			x
Pain NRS index knee	x	x	x	x	x
Pain NRS other joints	x	x	x	x	x
PainDETECT		x	x	x	x
SF-36		x	x	x	x
BIOLOGICAL SAMPLES					
Serum		x	x	x	x
Plasma		x			
DNA/RNA		x			x
Urine		x	x	x	x

ACR: American College of Rheumatology, CT: Computed Tomography, MRI: Magnetic Resonance Imaging, MOAKS: MRI Osteoarthritis Knee Score, KOOS: Knee injury and Osteoarthritis Outcome Score, HOOS: Hip disability and Osteoarthritis Outcome Score, ICOAP: Intermittent and Constant OsteoArthritis Pain questionnaire, FIHOA: Functional Index for Hand OsteoArthritis, NRS: Numeric Rating Scale, SF-36: Short Form 36 Health Survey.

Parameters for description of OA progression and phenotypes

OA progression and phenotype of the index knee over two years will be described by changes from baseline to the 1- and/or 2-year visit.

The parameters used to define structural progression will be:

- Radiographic parameters of knee OA severity; JSW and osteophytes using KIDA measurements, Kellgren and Lawrence (KL) grading, and Osteoarthritis Research Society International (OARSI) grading[18,20,21]
- Quantitative MRI parameters for cartilage including thickness, volume, and denuded bone areas in the tibiofemoral joint[22]
- Semi quantitative MRI scoring of cartilaginous and non-cartilaginous components including bone marrow oedema, meniscal alteration, and synovitis, assessed separately and under a global score[23]
- Advanced radiographic parameters; bone shape analyses and subchondral bone architecture on standard radiographs and high-resolution CT representing OA related bone and trabecular deformations/adaptations[24]
- (Bio)markers in blood and urine representing cartilage, bone and synovial matrix turnover and inflammation.

The parameters for pain and function will be:

- KOOS questionnaire[17]
- Knee Intermittent and Constant OsteoArthritis Pain (ICOAP) questionnaire[25]
- General pain and function parameters

Parameters for prediction of index knee OA progression and phenotypes

Prediction of OA progression (phenotype specific), will be evaluated using ML taking into account the parameters mentioned above in addition to explorative markers at baseline and, if available, at six months:

- Qualitative MRI parameters; T2 relaxation MRI representing cartilage collagen distribution[26]
- Advanced radiographic imaging parameters; bone shape analysis on MRI representing bone area and shape[27]
- Motion analysis (GaitSmart®)[28]
- Performance-based tests (40-meter self-paced walk test and 30-second chair-stand test)[29]

Co-variables

Additionally to the above-mentioned parameters, the following co-variables are available for the ML modeling and analyses:

- Contralateral knee OA
  - KIDA measurements[18], KL grading[20] and OARSI grading[21]
- Hand OA
  - Inflammation of hand joints (HandScan)[30]
  - OA features of hand joints on standard radiographs; KL grading[20], OARSI scoring[21] and Verbruggen-Veys grading[31]
  - Functional Index for Hand Osteoarthritis (FIHOA) questionnaire[32]
- Hip OA
  - OA features of the hips; Whole Body Low Dose CT (WBLDCT)[33]
  - Hip disability and Osteoarthritis Outcome Score (HOOS)[34] and hip ICOAP[25]
- Facet joint OA and intervertebral disc (IVD) degeneration
  - WBLDCT
- OA features of glenohumeral and acromioclavicular joints
  - WBLDCT
- General pain and function parameters
  - Short Form 36 Health Survey (SF-36) questionnaire for quality of life[35]
  - Pain with concomitant pain medication registration in a custom made one-month pain diary
  - Pain NRS of contralateral knee, both hips, both hands and spine[19]

  - PainDETECT questionnaire used to identify the likelihood of a neuropathic pain component[36]
  - Motion analysis (GaitSmart®) at 24 months[28]
  - Performance-based tests at 24 months[29]
  - Physical examination of contralateral knee, hips and hands
- Optional systemic biochemical (bio)markers
  - Epigenetic, genomic, transcriptomic, proteomic, lipidomic, and metabolomic markers (to be defined)
- General clinical data
  - History and type of knee traumatism and surgery
  - Smoking habits
  - Menopausal status
  - Concomitant OA treatment
- Advanced parameters
  - Bone shape analysis on radiographs of contralateral knee
  - Subchondral bone analysis on radiographs of contralateral knee
  - Bone shape analysis of the hip on WBLDCT

Statistical analysis

Statistical analyses of baseline data for the current manuscript were performed using IBM SPSS Statistics version 25.0.0.2. For evaluation of differences between included and excluded patients Student's *t*-tests were used, except for gender, for which X^2 test was used. Differences between the five centres were evaluated using ANOVA followed by Tukey's post-hoc tests or X^2 test (for gender). P-values <0.05 were considered as statistically significant.

Future analysis plan

Statistical analyses will be in line with the objectives of the original project. At time of data analysis the best methods to address the aims of IMI-APPROACH will be defined as this systems medicine is a fast evolving field. The final analysis plan will be decided on before database lock. In an overview it will comprise:

Validation of the prediction model used in the inclusion process

Model predictions of pain and structural progression will be compared with actual observed progression over 2-year follow-up.

Development and validation of a predictive model for OA progression: Baseline data and/or change over the first six months follow-up will be used to train and test (ML) models for OA progression. External validation of these models will be needed for implementation in practice.

Discovery and prediction of phenotypes

The dataset will be explored by use of different statistical approaches to define subgroups with common characteristics. Identified phenotypes will be be selected in discussion with clinical experts and described and predicted in enough detail to be of use in practical OA diagnosis and patient selection.

Patient and public involvement statement

A Patient Council (PC) was instituted to ensure that patients are represented in IMI-APPROACH. The PC contributed to the design of the clinical study and with that helped shape the project with particular consideration for the interests of study participants. The PC will maintain close contact with the researchers throughout the project.

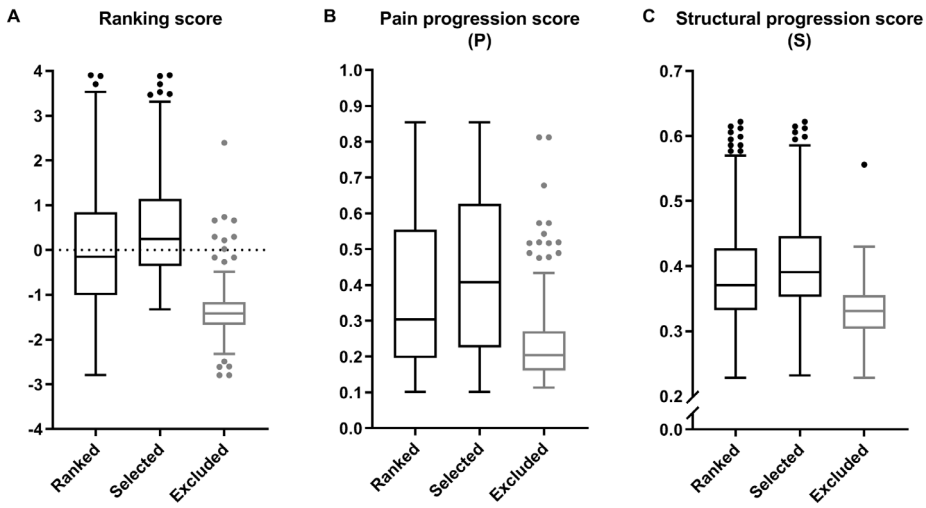
Ethical approval

The study is being conducted in compliance with the protocol, Good Clinical Practice (GCP), the Declaration of Helsinki, and the applicable ethical and legal regulatory requirements (for all countries involved), and is registered under clinicaltrials.gov nr: NCT03883568. All participants have received oral and written information and provided written informed consent.

Results

Figure 2 describes the probability of progression as predicted by the second, uniform ML model using the screening visit data of all patients, those who were finally selected and those that were excluded.

Figure 2. Predicted progression score of the IMI-APPROACH participants.



Combined (A), pain (B), and structural (C) progression scores (confidence estimates) of the ranked (n=409), selected (n=314), and excluded (n=112) patients. Boxplots represent mean±IQR.

Included vs excluded patients

Out of the 314 patients, 297 patients attended their baseline visit and were included in the cohort. The remaining seventeen patients withdrew after initial selection or could not attend the baseline visit before the deadline. All presented baseline parameters were statistically significantly different between included and excluded patients, except for age and mean JSW (table 3).

Table 3. Screening characteristics of total study population

	Included n=297	***Excluded n=109***	***p-value (t-test)***
DEMOGRAPHICS			
Age (years)	66.5 (7.1)	68.1 (7.7)	0.061
Female (%)	230 (77%)	80 (71%)	**0.013**
BMI (kg/m^2)	28.1 (5.3)	26.4 (4.4)	**0.003**
QUESTIONNAIRES			
Adapted KOOS*			
Stiffness	38.5 (21.6)	24.3 (18.5)	**<0.001**
Pain	31.3 (19.7)	17.7 (14.6)	**<0.001**
Function	32.9 (19.1)	19.6 (16.3)	**<0.001**
Total	33.1 (18.8)	19.6 (15.4)	**<0.001**
NRS pain last week (0-10)			
Index knee	4.6 (2.8)	2.6 (2.2)	**<0.001**
Contralateral knee	2.8 (2.5)	1.6 (2.2)	**<0.001**
RADIOGRAPHY			
KIDA			
Mean JSW index knee (mm)	5.5 (1.0)	5.5 (1.0)	0.700
Minimum JSW index knee (mm)	2.5 (1.3)	2.1 (1.0)	**0.001**
KL grade (%)			
Grade 0	51 (17%)	Not determined	Not applicable
Grade 1	90 (30%)		
Grade 2	88 (30%)		
Grade 3	54 (18%)		
Grade 4	10 (3%)		

* a number of KOOS questions was used, weighted to provide a score for stiffness, pain, and function of 0 (no limitations) to 100 (most severe).

Continuous values are given as mean (SD). Categorical values are given as absolute numbers and percentages.

BMI: Body Mass Index, KOOS: Knee injury and Osteoarthritis Outcome Score. NRS: Numeric Rating Scale. KIDA: Knee Image Digital Analysis, JSW: Joint Space Width, KL: Kellgren and Lawrence.

Discussion

The inclusion process of the IMI-APPROACH cohort is considered successful. The dual assessment with the additional screening visit and a second ML has demonstrated the practical value of the chosen recruitment strategy. Although one might have expected higher probabilities of progression from the selection process, opportunities for further optimization were limited due to a narrow time-window and available corresponding data from the source cohorts. Nevertheless, results show a clear differentiation in baseline data of selected and excluded patients, with a predicted increased progression probability of the selected patients. In two years the success of the approach, viz. the true progression of these patients will become clear. The predicted probabilities will not be 100%, so we expect sufficient non-progressive patients, those anticipated with the lowest probabilities for progression, that will serve as controls.

Data from the 2-year longitudinal cohort will provide valuable insights into the relevance of conventional and novel clinical, imaging, and biochemical markers. Changes of these markers over the first 6 months will likely extend the ability to predict the likelihood for OA progression at 12 and 24 months (either pain, structural, or both pain and structural) and distinguish between different OA phenotypes. New markers to identify relevant OA phenotypes based on imaging, locomotion and biochemical/omics methods will be developed and validated. This will enable classification of each knee OA patient on a phenotype-specific progression scale. Ultimately, the IMI-APPROACH cohort intends to provide a basis for phenotype tailored trials of potential DMOADs, decrease the required number of study subjects and trial duration, and therewith form the basis for personalized/stratified medicine in OA.

The IMI-APPROACH cohort is part of a larger consortium, bringing together a highly qualified and multidisciplinary group of stakeholders in the form of a public-private partnership of engaged, knowledgeable and complementary industrial, academics, and patient experts. The IMI-APPROACH cohort is unique in its selection process, recruiting patients from existing cohorts based on ML models with encouraging results of which the actual utility needs to be demonstrated at the end of the 2-year follow-up. The IMI-APPROACH cohort will provide 2-year follow-up data of 297 knee OA patients including conventional and novel, explorative, imaging, biochemical, clinical, and demographic (bio)markers according to strict protocols for acquisition and evaluation with the aim to identify phenotypes and develop predictive models for progression of these phenotypes. The relative limited 2-year follow-up allows translation of results to pragmatic trial design in the future. The main limitation of the study is the descriptive phase in which the study is at present and the still limited number of included patients related to the large number of outcome parameters.

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7

8 Baseline clinical characteristics of predicted structural and pain progressors in the IMI-APPROACH knee osteoarthritis cohort

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Abstract

Objectives

To describe the relations between baseline clinical characteristics of the IMI-APPROACH participants and their predicted probabilities for knee osteoarthritis (OA) structural (S) progression and/or pain (P) progression.

Methods

Baseline clinical characteristics of the IMI-APPROACH participants were used for this study. Radiographs were evaluated according to Kellgren and Lawrence (KL grade) and Knee Image Digital Analysis (KIDA). Knee injury and Osteoarthritis Outcome Score (KOOS) and Numeric Rating Scale (NRS) were used to evaluate pain. Predicted progression scores for each individual were determined using machine learning models. Pearson's correlation coefficients were used to evaluate correlations between scores for predicted progression and baseline characteristics. Student's *t*-tests and X^2 tests were used to evaluate differences between participants with high *vs* low progression scores.

Results

Participants with high S progression scores were found to have statistically significantly less structural damage compared to participants with low S progression scores (minJSW 3.56mm *vs* 1.63mm; p<0.001, KL grade; p=0.028). Participants with high P progression scores had statistically significantly more pain compared to participants with low P progression scores (KOOS pain 51.71 *vs* 82.11; p<0.001, NRS pain 6.7 *vs* 2.4; p<0.001).

Conclusion

The baseline minJSW of the IMI-APPROACH participants contradicts the idea that the (predicted) course of knee OA follows a pattern of inertia, where patients who have progressed previously are more likely to display further progression. In contrast, for pain progressors the pattern of inertia seems valid, since participants with high P score already have more pain at baseline compared to participants with a low P score.

Introduction

One of the major challenges in knee osteoarthritis (OA) clinical trials is the selection of patients. Because actual cure is not anticipated, patients who will sufficiently progress without intervention are needed to provide an opportunity to observe arrest or reduction of disease progression. The Food and Drug Administration and European Medicines Agency still define OA progression by radiographic joint space narrowing (structural progression) and by progressive or sustained significant pain (pain progression)[1,2]. Since progression in OA is on average (very) slow, without pre-selection of fast progressive patients, clinical trials require large group sizes and long follow-up. The course of knee OA might follow a pattern of inertia, meaning that knees that are progressing are more likely to continue to progress, and stable knees are more likely to remain unchanged[3]. Unfortunately, at the moment of inclusion in a clinical trial, it is usually not known how the OA progressed in the years previous to inclusion and with that progression during the trial period is difficult to predict.

Despite efforts over the past decades to develop markers of disease, imaging procedures and biochemical marker analyses need to be improved and possibly extended with more specific and sensitive methods to reliably describe disease processes, to diagnose the disease at an early stage, to classify patients according to their prognosis, and to follow the course of disease and treatment effectiveness. The Applied Public-Private Research enabling OsteoArthritis Clinical Headway (IMI-APPROACH) consortium has set up a broad database of different OA patients and a longitudinal cohort to combine conventional and new disease markers, and identify different OA phenotypes. The IMI-APPROACH cohort study is a 2-year observational study using a unique, multi-step selection of participants, to include people with an increased likelihood of structural progression and/or pain progression[4]. Structural progression was defined as a reduction in Joint Space Width (JSW) of ≥0.3mm per year over a period of 2-3 years (0.7mm has been described as the minimal detectable difference in radiographic JSW)[5]. Pain progression was defined as at least one of the three following: KOOS pain (on a 0 to 100 scale; 0 meaning maximal pain, 100 meaning no pain) decrease ≥5 points/year with a pain level ≤60 at two years, or KOOS pain decrease ≥10 points/year with a pain level ≤65 at two years, or a pain level ≤60 at both baseline and two years. The participants were selected from existing OA cohorts (CHECK[6], HOSTAS[7], MUST[7], PROCOAC[9], and DIGICOD[10]), or from outpatient departments (in case the original cohort did not provide sufficient participants). As a first step, historical data was used to train machine learning (ML) models to provide a S(tructural) and P(ain) progression score for each individual, reflecting the probability to become a 'progressor' on these outcomes[4]. So the S and P progression score represent throughout the manuscript the predicted progression, not the actual progression. These predicted progression scores were combined into one ranking score which orders patients by the likelihood of progression in general, throughout the manuscript described as ranking score. Participants with the highest rank were selected for

a screening visit and ranked again using collected up-to-date information on disease status (demographics, radiographs, and questionnaires). Then 75% of the screened participants with the highest rank (most likely to progress) were included in the study. As a consequence, each included participant was assigned a S progression score, P progression score, and a ranking score.

The predictions of the final ML model are based on cross-sectional data of patients at the moment of or shortly before inclusion in the cohort, viz. baseline data. Participants are subsequently followed for two years, which is at present ongoing, to study actual progression with the use of a large number of conventional and exploratory measures. Irrespective of the actual progression, this analysis provides the clinical characteristics (demographics, radiographic features, and pain severity) of the selected patients in the IMI-APPROACH prospective cohort in relation to their S progression, P progression, and ranking score. The present study provides insight in the actual values of these baseline parameters, and with that the clinical characteristics of predicted S and/or P progressors.

Methods

Patient selection

In this analyses, all included participants (n=297) of the IMI-APPROACH study were used. The study protocol and the general cohort profile have been described previously[11]. Age (mean 66.5 years, SD 7.1), BMI (mean 28.1, SD 5.3), sex (female/male ratio 230/67), and ethnicity (Caucasian/White, Black/African American, Asian, or Other) as well as structural damage and pain were included in the current analyses.

Evaluation of structural damage

Posterior-anterior weight bearing semi-flexed knee radiographs were obtained according to the protocol of Buckland-Wright[12]. These radiographs were graded according to Kellgren and Lawrence (KL grade)[13] and evaluated by Knee Image Digital Analysis (KIDA)[14]. Minimum Joint Space Width (minJSW) in mm, osteophyte area (mm^2), and subchondral bone density (mm Aluminum Equivalents; mm Alu Eq.) were used for analyses.

Pain evaluation

Pain was evaluated using a Numeric Rating Scale (NRS) and the pain subscale of the Knee injury and Osteoarthritis Outcome Score (KOOS)[15]. This subscale is calculated from nine questions, each with a 5-point Likert scale. It is normalized to a 0-100 range, where 0 means maximal limitations (maximal pain) and 100 means no limitations (no pain).

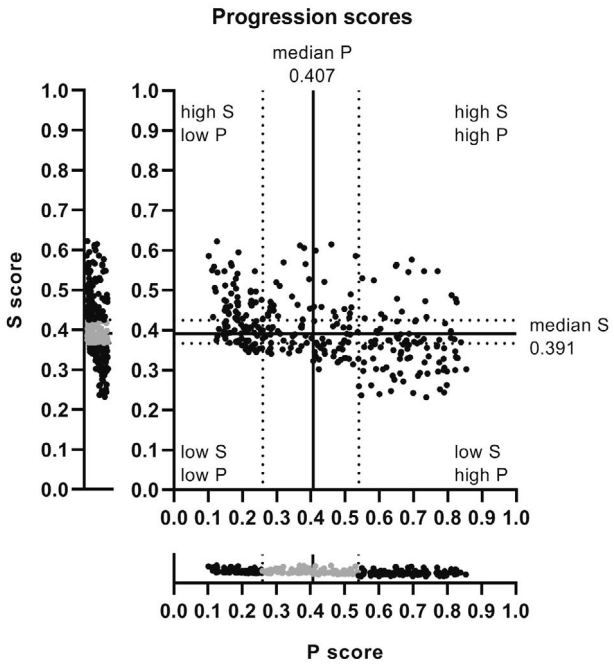
Statistical analysis

IBM SPSS Statistics 25.0.0.2 was used for statistical analysis. Baseline data are presented in relation to the S progression, P progression, and final ranking score. Pearson's correlation coefficient was used to determine correlations between progression/ranking scores and clinical, structural, and demographic variables. Correlations were considered weak when $r<0.3$, moderate when $0.3 \geq r < 0.5$, and strong when $r \geq 0.5$. Additionally, patients were partitioned into three equal size groups (tertiles) with low, medium, and high values of the scores. Baseline clinical, structural, and demographic characteristics were compared between patients with lowest (one-third) or highest (one-third) scores using Student's *t*-test (continuous variables) or chi-squared test (categorical variables). For all tests $p<0.05$ was considered statistically significant.

Results

Figure 1 shows the individual S and P progression scores of all 297 participants.

Figure 1. Combined distribution of the S progression and P progression scores of all participants



Solid lines indicate median values of S progression score (0.391) and P progression score (0.407). Dotted lines indicate tertile borders (0.367 and 0.425 for S progression score, 0.259 and 0.540 for P progression score). Part of the distribution colored grey is the middle tertile.

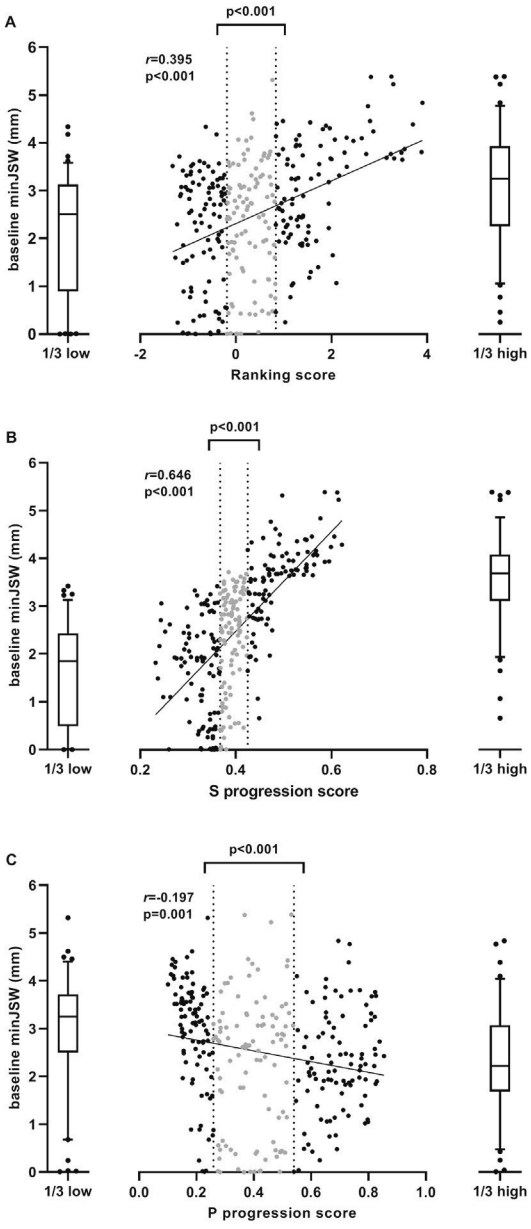
Baseline characteristics of participants with lowest and highest S progression, P progression or ranking scores are presented in table 1. For majority of the characteristics the difference between participants with the highest and lowest S progression, P progression or ranking scores are statistically significant. Age was higher in participants with the lowest S progression scores compared to participants with the highest S progression scores (p=0.029). The same trend was found for P progression scores, although not statistically significant. There was no difference in BMI for the S progression scores. The difference in osteophyte area was statistically significant different only for the P progression scores.

Table 1. Baseline characteristics of the one-third of participants with the lowest and one-third of participants with the highest S progression score, P progression score, and ranking score

	Low S score *n=99*		*High S score* *n=99*		*P-value*	*Low P score* *n=99*		*High P score* *n=99*		*P-value*	*Low rank* *n=99*		*High rank* *n=99*		*P-value*
	Mean	*SD*	*Mean*	*SD*		*Mean*	*SD*	*Mean*	*SD*		*Mean*	*SD*	*Mean*	*SD*	
Age (years)	67.2	7.0	64.9	7.7	**0.029**	67.3	6.1	65.5	7.2	0.051	68.3	6.3	64.2	7.3	**<0.001**
BMI	28.3	5.1	27.2	5.5	0.159	25.5	4.2	29.4	5.5	**<0.001**	26.4	4.6	29.2	5.3	**<0.001**
Caucasian (%)	91.9		98.0		0.052	99.0		92.9		**0.030**	96.0		94.9		0.733
Female (%)	74.7		77.8		0.616	74.7		84.8		0.077	71.7		80.8		0.133
KOOS pain	60.2	19.0	69.2	18.7	**0.001**	82.1	12.5	51.2	14.9	**<0.001**	75.9	16.1	57.9	18.12	**<0.001**
KOOS ADL	61.0	17.7	73.2	19.0	**<0.001**	85.9	11.5	52.6	14.3	**<0.001**	79.4	15.5	59.6	18.53	**<0.001**
NRS pain	5.2	2.6	4.2	2.7	**0.006**	2.4	2.0	6.7	2.0	**<0.001**	3.2	2.5	5.6	2.6	**<0.001**
KL grade (%)					**0.028**					0.347					0.106
Grade 0	14.4		22.2			23.2		13.4			19.4		16.2		
Grade 1	25.8		33.3			37.4		36.1			34.7		37.4		
Grade 2	33.0		32.3			28.3		33.0			22.4		30.3		
Grade 3	20.6		12.1			10.1		16.5			17.3		16.2		
Grade 4	6.2		0.0			1.0		1.0			6.1		0.0		
minJSW (mm)	1.6	1.05	3.6	0.86	**<0.001**	3.00	1.10	2.3	1.07	**<0.001**	2.10	1.23	3.08	1.12	**<0.001**
Osteophytes (mm^2)	20.2	19.6	20.4	18.0	0.947	14.8	10.8	19.8	16.2	**0.012**	18.1	19.3	21.6	19.3	0.203
Subchondral bone density (mm Alu. Eq.)	31.9	4.7	30.4	5.1	**0.037**	29.3	4.2	32.0	5.0	**<0.001**	29.5	4.2	31.5	5.5	**0.004**

Figure 2 shows the correlations between different progression scores (S progression, P progression, and ranking score) and baseline minJSW. A moderate positive correlation of the ranking score with minJSW was observed (r=0.395, p<0.001). The S progression score strongly positively correlated with minJSW (r=0.646, p<0.001), whereas the P progression score only weakly negatively correlated with minJSW (r=-0.197, p=0.001). The participants with the highest ranking scores had a statistically significant higher minJSW compared to participants with the lowest ranking scores (3.08mm *vs* 2.14mm, p<0.001). The same was observed for the S progression scores (3.56mm *vs* 1.63mm, p<0.001). In contrast, participants with the highest P progression scores had a statistically significant lower minJSW compared to participants with the lowest P progression scores (2.29mm *vs* 2.99mm, p<0.001).

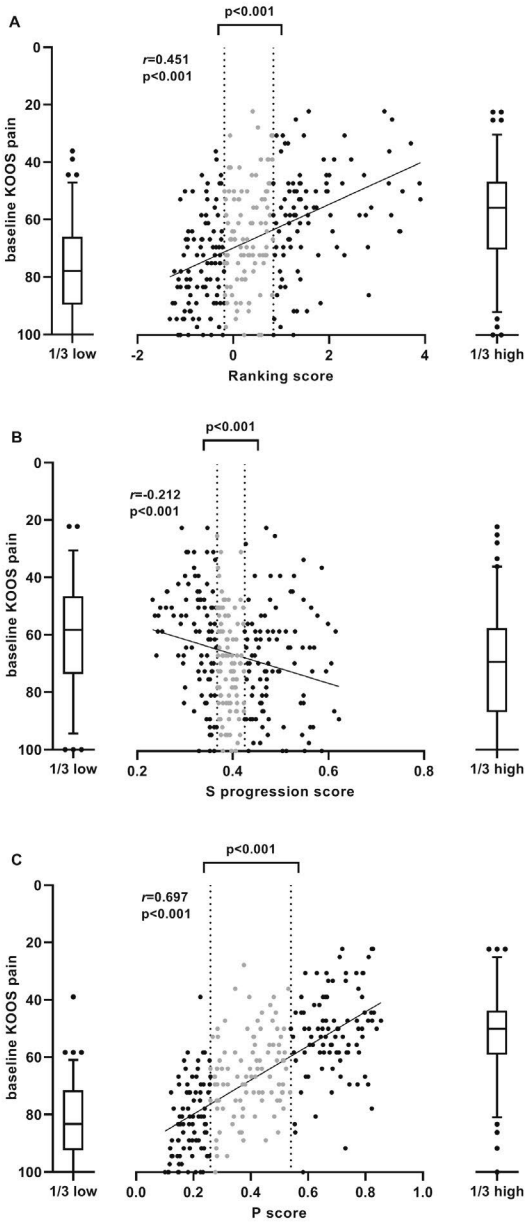
Figure 2. Relationship between minJSW and progression/ranking scores



The middle panels of each plot show correlation between minJSW and ranking score (A), S progression score (B), and P progression score (C). Each dot represents a single participant; dots in grey show the middle tertile of the score. Left and right boxplots show the score distribution (median value with 95%CI) of the participants in the lowest (left) and highest (right) tertile of ranking score (A), S progression score (B), an P progression score (C).

Figure 3 shows the correlations between the different progression scores (S progression, P progression, and ranking score) and baseline KOOS pain. A moderate positive correlation between ranking score and KOOS pain was observed (r=0.451, p<0.001). The P progression score correlated strongly positively with KOOS pain (r=0.697, p<0.001), whereas S progression score correlated weakly and negatively with KOOS pain (r=-0.212, p<0.001). Participants with the highest ranking scores had statistically significant more pain compared to participants with the lowest ranking scores (57.87 *vs* 75.87, p<0.001). The same was observed for P progression scores (51.17 *vs* 82.11, p<0.001). In contrast, participants with the highest S progression scores had statistically significant less pain compared to participants with the lowest S progression scores (69.21 *vs* 60.23, p<0.001).

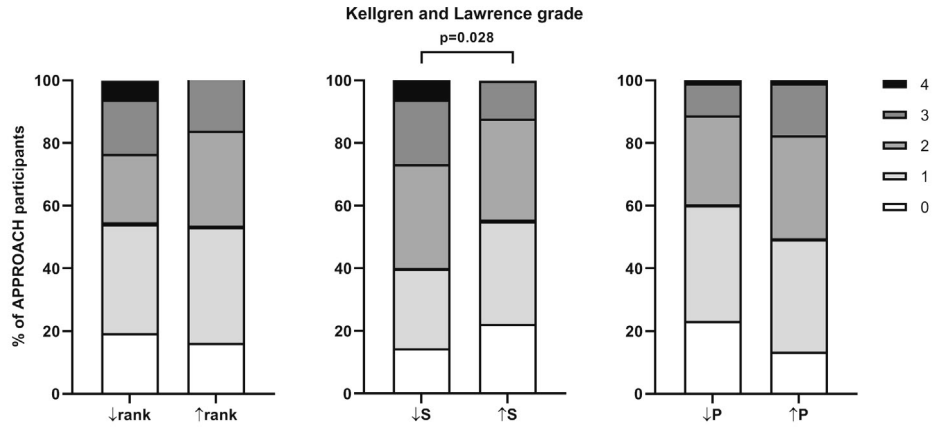
Figure 3. Relationship between KOOS pain and progression/ranking scores



The middle panels of each plot show correlation between KOOS pain and ranking score (A), S progression score (B), and P progression score (C). Each dot represents a single participant; dots in grey show the middle tertile of the score. Left and right boxplots show the score distribution (median value with 95%CI) of the participants in the lowest (left) and highest (right) tertile of ranking score (A), S progression score (B), an P progression score (C).

Distribution of KL grades is presented in figure 4. In accordance with the correlation between the S progression score and minJSW, participants with the highest S progression scores had statistically significant lower KL grades, compared to participants with the lowest S progression scores. For ranking score and P progression score KL grade was not statistically significantly different between groups (p=0.347 and p=0.106, respectively).

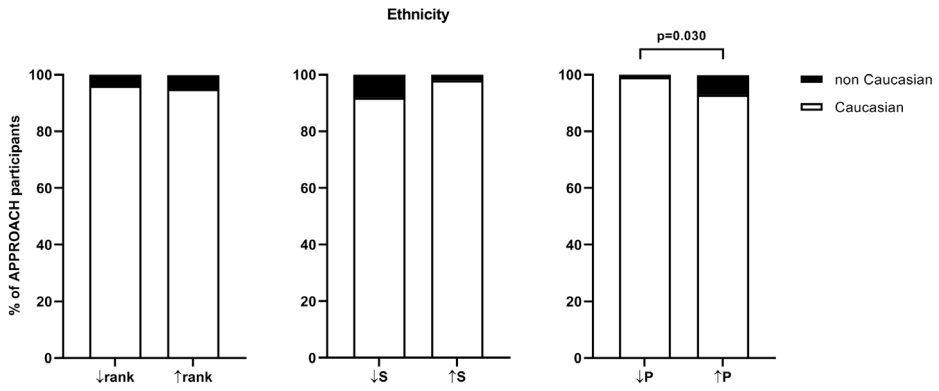
Figure 4. Relationship between Kellgren and Lawrence grade and progression/ranking scores



P-value was calculated using X^2 test. Thick black line in the middle separates KL grade 0-1 (no or doubtful radiographic damage) and KL grade 2-4 (certain rediographic damage).

The analysis of ethnicity is presented in figure 5. The difference was statistically significant only for P progression scores (7.1% *vs* 1.0%). For ranking score and S progression score ethnicity was not statistically significantly different between groups (p=0.733 and p=0.052, respectively).

Figure 5. Relationship between ethnicity and progression/ranking scores



P-value was calculated using X^2 test.

Discussion

Within the IMI-APPROACH cohort study, a higher S progression score implicates a higher likelihood for structural progression. According to the general perception that the course of knee OA follows a pattern of inertia, participants with a higher S score were expected to have lower minJSW (since progression already happened before inclusion). However, in our analysis, the opposite was observed and participants with a high S progression score had a statistically significantly higher minJSW and lower KL grade compared to participants with a low S progression score. In contrast, Halilaj et al. found no differences in baseline characteristics, including JSW, between fast-progressors and non-progressors within the Osteoarthritis Initiative (OAI) incidence cohort[16,17]. The higher minJSW and lower KL grade implicate presence of more cartilage that still can deteriorate, and therefore provide an opportunity for structural progression. The ranking from the ML model reflects this.

For KOOS and NRS pain the contrary was found. Participants with a high P progression score had more pain at baseline compared to participants with a low P progression score. Again, in the OAI incidence cohort the contrary was found. Patients with improving WOMAC scores had more pain at baseline, compared to patients with stable pain or patients with pain progression[17]. The OAI incidence cohort, included patients who were at high risk of developing OA during the study, while patients in the APPROACH cohort already had OA and were selected based on a high change for progression.

The likely explanation for our result lies in the definition of pain progression. While structural progression is solely based on JSW narrowing, the definition for pain progression was more complex, not only including progression but also high sustained pain over the past years. Although still speculative, most participants with a high P progression score may have been provided by patients with a high sustained score. Indeed, low pain scores reduced the probability of being a P progressor[4], supporting the idea of a pattern of inertia, but contrasting the window for change as observed for structure progression.

The ranking score, on which the patient selection was based, combines both progression scores using normalization of the individual progression scores[4]. Participants with a high ranking score had statistically significantly higher minJSW, lower KL grade (contrasting inertia but providing a window for change), and more pain (according to the inertia concept but without the window for change regarding progression) compared to participants with a low ranking score. Although these baseline characteristics seem to be counter-intuitive, this combination provides potentially the best patient selection for treatment modalities that decrease pain (initially low KOOS score) and prevent, stop, or slow-down structural progression (initially large JSW). With all present knowledge on treatment modalities thus far, this supports testing treatment modalities that are able to halt tissue structure damage and decrease pain. As cartilage structure repair is still challenging, this may turn out to be the best approach for patient selection for clinical trials.

The limited number of participants included in the study made the IMI-APPROACH cohort difficult to compare different ethnicities within the non-Caucasian group. However, differences in pain severity were previously described between Non-Hispanic Blacks and Non-Hispanic Whites[18, 19], and Asians and Caucasians[20].

The main limitation of this analysis is the availability of only baseline data. The predicted S progression score, P progression score, and ranking score used in this manuscript only represent the likelihood of becoming a progressor. The actual progression can only be evaluated when all 2-year follow-up data is available, and may enable fine-tuning or, when needed, correction of the baseline predictive parameters. Nevertheless, the relationships between baseline characteristics and the predicted progression scores, provide insights on clinical characteristics of this unique cohort.

In conclusion, the selected IMI-APPROACH cohort consists of patients with high pain levels and low structural damage, suitable for evaluation of treatment modalities that decrease pain and arrest or slow-down tissue structural damage.

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9 Neuropathic pain in the IMI-APPROACH cohort: prevalence and phenotyping

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Abstract

Objectives

Osteoarthritis (OA) patients with a neuropathic pain (NP) component may represent a specific phenotype. This study compares joint damage, pain, and functional disability between knee OA patients with a likely NP component, and those without a likely NP component.

Methods

Baseline data from the IMI-APPROACH knee OA cohort study were used. Patients with a painDETECT score ≥19 (with likely NP component, n=24) were matched on a 1:2 ratio to patients with a painDETECT score ≤12 (without likely NP component), and similar knee and general pain (KOOS and SF-36 pain). Pain, physical function and radiographic joint damage of multiple joints were determined and compared between OA patients with and without a likely NP component.

Results

OA patients with painDETECT scores ≥19 had statistically significant less radiographic joint damage ($p \leq 0.04$ for KIDA parameters and KL grade), but an impaired physical function ($p < 0.003$ for all tests) compared to patients with a painDETECT score ≤12. In addition, more severe pain was found in joints other than the index knee ($p \leq 0.001$ for hips and hands), while joint damage throughout the body was not different.

Conclusion

OA patients with a likely NP component, as determined with the painDETECT questionnaire, may represent a specific OA phenotype, where local and overall joint damage is not the main cause of pain and disability. Patients with this NP component will likely not benefit from general pain medication and/or disease modifying (DMOAD) therapy. Reserved inclusion of these patients in DMOAD trials is advised in the quest for successful OA treatments.

Introduction

Osteoarthritis (OA) is a degenerative joint disease leading to pain, stiffness, and loss of function. Despite the increasing prevalence and great burden, there is still no cure. Treatment is focused on relieving symptoms and controlling inflammation if present. Multiple international guidelines recommend the use of topical/oral non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of OA pain, if non-pharmacological interventions fail[1-3]. However, in a meta-analysis evaluating the analgesic efficacy of NSAIDs and selective cyclooxygenase-2 inhibitors (coxibs) in knee OA, an effect size of 0.32 was found[4], suggesting the effect is limited and/or that at least part of the OA patients does not benefit from this approach sufficiently.

A possible explanation for the limited efficacy of current analgesic drugs is the variety in pathophysiologic mechanisms between different OA patients. OA is considered a heterogeneous disease, existing of multiple phenotypes, with different causes for similar clinical symptoms including pain[5]. Pain is currently categorized into nociceptive pain resulting from tissue damage; neuropathic pain (NP), involving nerve damage; and nociplastic pain, which is caused by altered nociception without clear evidence of actual tissue damage[6]. OA pain is classically considered a nociceptive pain that arises from joint tissue damage and inflammation. However, an evident relation between the (radiographic) tissue damage with (reported) pain has not been identified[7]. Not all patients respond to general pain medication, such as acetaminophen, NSAIDs, coxibs, and tramadol[8-11]. Moreover, in a substantial proportion of knee OA patients, pain persists after removing the damaged tissue during total knee arthroplasty (TKA)[12]. These observations indicate that the classification of OA pain as purely nociceptive is erroneous.

Patients with OA who experience pain that is not a pure nociceptive pain but also has neuropathic features could be recognized as a distinct subgroup. Identification of this subgroup may allow clinicians to improve the management of symptoms of this specific OA phenotype, using distinct treatments focused on this NP component[6]. Moreover, excluding these patients from disease modifying OA drug (DMOAD) trials may benefit the quest for discovery of tissue structure modifying medication accompanied by analgesic activity as requested by EMA and FDA[13,14].

Although definite NP requires an objective diagnostic test to confirm a lesion or disease of the somatosensory system, questionnaires like the painDETECT, assessing pain characteristics suggestive for NP, show high sensitivity and specificity to distinguish NP from non NP[15]. The painDETECT questionnaire predicts the likelihood of a NP component in patients with chronic low back pain[16], and has widely been used in OA studies as well. A low score on the painDETECT questionnaire (≤12) means that the presence of a NP component is unlikely (<15%), while a high score (≥19) indicates that the presence of a NP component is likely (>90%)[16].

In general, OA patients with a high painDETECT score (more likely to have a NP component) also have higher scores on other pain questionnaires like Western Ontario McMaster Universities Osteoarthritis Index and Numeric Rating Scales (NRS)[17-23], hampering in this respect selection of patients.

In the present study, for the first time, knee OA patients with a likely NP component (as defined by painDETECT score ≥19) and knee OA patients without a likely NP component (as defined by painDETECT score ≤12) were matched for knee pain levels, to compare differences in clinical characteristics. We hypothesize that knee OA patients with a likely NP component form a specific OA phenotype, without a relation between joint damage and clinical symptoms.

Methods

Overall study population

Patients were selected from the Innovative Medicines Initiative Applied Public-Private Research enabling OsteoArthritis Clinical Headway (IMI-APPROACH) clinical cohort[24], a 2-year follow-up study including 297 knee OA patients. The IMI-APPROACH consortium provides a broad database of OA patients and a longitudinal cohort study to combine conventional and new disease markers to identify different OA phenotypes. The study is being conducted in compliance with the protocol, Good Clinical Practice (GCP), the Declaration of Helsinki, and the applicable ethical and legal regulatory requirements (for all countries involved). The study is registered under clinicaltrials.gov nr: NCT03883568. All patients have received oral and written information and provided written informed consent. The present analysis used baseline data.

Identification of patients with and without a likely NP component

The painDETECT questionnaire[16] was used to identify patients with a high likelihood of a NP component. This questionnaire contains nine questions: seven questions to characterize pain, one for the pain course pattern and one for the presence or absence of radiating pain, leading to a final score ranging from -1 to 38. -1 is scored when all seven questions about pain characteristics are answered with 'never', the pain course pattern is 'persistent pain with pain attacks' and no radiation is present. In case of a score ≤12 a NP component is unlikely (<15%), whereas in case of a score ≥19 a NP component is likely (>90%)[16]. In-between scores provide doubtful classification and patients with these intermediate scores are therefore left out of the current analyses.

Evaluation of joint pain

Multiple questionnaires were used to evaluate joint pain. The pain subscales of the Knee injury and Osteoarthritis Outcome Score (KOOS)[25] and its equivalent for the hip (HOOS)[26] consist of nine questions for pain, each scored on a 5-point scale. A normalized score is calculated where 0 means maximal pain and 100 means no pain. The Short Form 36 Health Survey (SF-36) contains 36 questions and measures eight domains of health status, including bodily pain ranging from 0-100, where 0 means no pain and 100 mean maximal pain[27]. A numeric rating scale (NRS) for pain[28] was used for both knees, both hips, both hands, and the lower back. It consists of an 11-point scale on which patients score pain from 0 (no pain) to 10 (worst imaginable pain). The Intermittent and Constant OA Pain (ICOAP) questionnaire[29] for knee and hip contains eleven questions, five for constant pain and six for intermittent pain, each question scored on a 5-point scale. A higher total score reflects more pain.

Evaluation of physical function

The Functional Index for Hand OA (FIHOA)[30] comprises ten questions, scored on a 4-grade scale. Score ranges from 0 (no difficulties) to 30 (maximal difficulties). In addition, two performance-based tests, recommended by Osteoarthritis Research Society International, were used in APPROACH. For the 30s chair-stand test (chair) patients had to stand up completely from a sitting position in the middle of a seat with feet shoulder width apart, flat on the floor, arms crossed at chest, and then sit completely. The result is the number of repetitions completed in thirty seconds. The 40m self-paced walk test (walk) records time in seconds needed to walk as quickly but as safe as possible (regular walking, no running) to a mark 10m away, return, and repeat for a total distance of 40m.

Evaluation of structural joint damage in the index knee

For each patient an index knee was selected based on American College of Rheumatology (ACR) clinical criteria[31]. If both knees fulfilled the criteria, the most painful knee was selected as the index knee. If equal, the right one was selected as the index knee. Standardized semi-flexed posterior-anterior weight bearing knee radiographs were taken according to Buckland-Wright[32]. KL grading was performed by one blinded observer. The intra- and inter-observer correlation were both previously found to be good (>0.83)[33], and in IMI-APPROACH an intraclass correlation coefficient (ICC) of 0.88 was found (using 10% of the radiographs). Additionally, Knee Images Digital Analysis (KIDA)[34] was performed by one single experienced observer. Minimum Joint Space Width (minJSW) of the index knee (mm), osteophyte area (mm^2), and subchondral bone density (mm Alu Eq.) were used as radiographic parameters. Previous studies demonstrated an ICC of 0.73-0.99 for the different features[35].

Evaluation of OA grades of other joints

Whole-body Low-dose Computed Tomography (WBLDCT) was performed to assess concomitant OA or degenerative disc disease (DDD) in case of intervertebral discs. Scans were evaluated using the OsteoArthritis Computed Tomography (OACT) score, grading all joints on a 0-3 scale. For intervertebral discs, DDD of the two most degenerated levels of each region (cervical, thoracic, lumbal) were scored. Next to grades per joint, the OACT provides a score for total body OA, ranging from 0-72 (24 joints with a maximum score of 3 per joint). Kappa values for the intra-observer reliability for individual joints ranged from 0.79 to 0.95, and for inter-observer reliability from 0.48 to 0.95. ICC for the total OA body score ranged from 0.94-0.97 for different observers[36].

Statistical analysis

First, all patients with a painDETECT ≥19 (NP) were matched in a 1:2 ratio to patients with a painDETECT score ≤12 (non-NP), using the MatchIt package from the R statistical package. Subjects were matched using KOOS pain (as a knee specific pain measure) and SF-36 pain (as a general pain score) based on the 'nearest neighbor' principle as well as using a caliper for KOOS pain (as the primary matching criterion) of ten points. These matching variables were chosen because they are known to have a significant relation with painDETECT scores[17,20-23]. Further analyses were performed using IBM SPSS Statistics version 26.0.0.1. Differences between patients with a likely NP component and matched patients without a likely NP component were evaluated using Student's *t*-tests (for continuous variables), and X^2 or Fisher's Exact test (for categorical variables) when assumptions for X^2 were not met. P-values <0.05 were considered statistically significant.

Results

Patient characteristics

In the whole cohort of 297 knee OA patients, 24 patients (8.2%) scored ≥19 on the painDETECT questionnaire at time of inclusion, whereas 220 patients (74.8%) scored ≤12. Fifty (17.0%) patients had an in-between score (three patients did not have a painDETECT score at baseline). The characteristics of patients with a likely NP component (NP, n=24), and the matched patients without a likely NP component (non-NP, n=48) are shown in table 1. No statistically significant differences were found between groups for demographics or the matching variables (as expected).

Table 1. Participant characteristics

	NP n=24	*non-NP n=48*	*p-value (t-test)*
DEMOGRAPHICS			
Age (years)	64.5 (6.9)	65.7 (7.3)	0.491
BMI (kg/m^2)	30.7 (5.9)	29.1 (6.2)	0.295
Female, n (%)	20 (83%)	38 (79%)	0.761*
MATCHING VARIABLES			
KOOS pain	50.8 (15.1)	49.8 (13.1)	0.777
SF-36 pain	34.1 (22.8)	39.8 (16.8)	0.285

Values are given as mean (SD).
NP: Patients with neuropathic pain, non-NP: Matched controls, BMI: Body Mass Index, KOOS: Knee injury and Osteoarthritis Outcome Score, SF-36: Short form 36 Health Survey.
*Fisher's Exact Test

Differences in radiographic joint damage in patients with and without a likely NP component
Differences in radiographic parameters of the index knee between patients with and without a likely NP component (matched for KOOS and SF-36 pain) are shown in table 2. Patients with a likely NP component have statistically significantly less radiographic damage in their index knee (KL grade p=0.003; minJSW 3.0 *vs* 2.1, p=0.002; osteophyte area 12.7 *vs* 25.5, p=0.001; subchondral bone density 30.1 *vs* 32.4, p=0.037).

Table 2. Radiographic damage in NP and non-NP patients

	NP n=24	*non-NP n=48*	*p-value (t-test)*
RADIOGRAPHY			
KL grade, n (%)			
Grade 0	8 (33.3)	4 (8.3)	**0.003***
Grade 1	8 (33.3)	11 (22.9)	
Grade 2	8 (33.3)	19 (39.6)	
Grade 3	0 (0)	13 (27.1)	
Grade 4	0 (0)	1 (2.1)	
minJSW (mm)	3.0 (1.0)	2.1 (1.4)	**0.002**
Osteophyte area (mm^2)	12.7 (11.1)	25.5 (19.1)	**0.001**
Mean subchondral bone density (mm Alu. Eq.)	30.1 (4.3)	32.4 (4.7)	**0.037**

Values are given as mean (SD).
NP: Patients with neuropathic pain, non-NP: Matched controls, KL grade: Kellgren and Lawrence grade, minJSW: minimum Joint Space Width, mm Alu Eq.; mm aluminium equivalent.
*Fisher's Exact Test

Differences in physical function in patients with and without a likely NP component
Differences in physical function between patients with and without a likely NP component are shown in table 3. Patients with a likely NP component have statistically significant worse hand function (FIHOA 12.3 *vs* 5.3, p<0.001), and perform worse on both performance-based tests (chair test 7.3 *vs* 9.7, p<0.001; walk test 38.1 *vs* 29.5, p=0.003).

Table 3. Physical function in NP and non-NP patients

	NP n=24	***non-NP n=48***	***p-value (t-test)***
PHYSICAL FUNCTION			
FIHOA	12.3 (6.9)	5.3 (6.3)	**<0.001**
Chair (# standing up)	7.3 (2.2)	9.7 (3.2)	**<0.001**
Walk (s)	38.1 (12.1)	29.5 (8.2)	**0.003**

Values are given as mean (SD).
NP: Patients with neuropathic pain, non-NP: Matched controls, FIHOA: Functional Index for Hand OsteoArthritis.

Differences in generalized joint pain between patients with and without a likely NP component
Differences in pain in joints other than the index knee between patients with and without a likely NP component are shown in table 4. As anticipated based on the matching process, ICOAP knee and NRS of the index knee did not differ between groups. In contrast, OA patients with a likely NP component had statistically significantly more pain in the contralateral knee (NRS 5.8 *vs* 4.0, p=0.012), hips (HOOS 61.0 *vs* 82.8, p=0.001; ICOAP hip 16.8 *vs* 6.7, p<0.001; NRS left hip 4.5 *vs* 1.9, p=0.001; NRS right hip 5.0 *vs* 1.5, p<0.001) and hands (NRS left hand 6.4 *vs* 3.9, p=0.001; NRS right hand 6.5 *vs* 3.7, p<0.001). Also, NRS lower back was higher, although not statistically significant (p=0.343).

Table 4. Pain scores in NP and non-NP patients

	NP n=24	***non-NP n=48***	***p-value (t-test)***
INDEX KNEE			
ICOAP knee	13.0 (8.7)	10.5 (9.1)	0.252
NRS index knee	6.3 (2.4)	6.6 (2.2)	0.680
CONTRALATERAL KNEE			
NRS contralateral knee	5.8 (2.5)	4.0 (2.8)	0.012
HIPS			
HOOS pain	61.0 (24.2)	82.8 (20.9)	**0.001**
ICOAP hip	16.8 (10.1)	6.7 (9.0)	**<0.001**
NRS left hip	4.5 (3.1)	1.9 (2.7)	**0.001**
NRS right hip	5.0 (3.1	1.5 (2.6)	**<0.001**
HANDS			
NRS left hand	6.4 (2.4)	3.9 (3.2)	**0.001**
NRS right hand	6.5 (2.4)	3.7 (3.1)	**<0.001**
LOWER BACK			
NRS lower back	5.6 (3.4)	4.4 (3.2)	0.343

NP: Patients with neuropathic pain, non-NP: Matched controls, ICOAP: Intermittent and Constant Osteoarthritis Pain, NRS: Numeric Rating Scale, HOOS: Hip disability and Osteoarthritis Outcome Score.

Differences in OACT grades of other joints between patients with and without a likely NP component

Differences in OACT grades between patients with and without a likely NP component are shown in table 5. The OACT grade of the index knee in patients with a likely NP component was lower compared to patients without a likely NP component, indicating less joint damage and supporting the data of the standard measurements in table 2. OACT grading of other joints did not differ between both groups, except for the right acromioclavicular joint (p=0.023; less damage in NP group), and the worst degenerated intervertebral disc of the lumbal spine (p=0.042; more damage in NP group).

Table 5. OsteoArthritis Computed Tomography grades in NP and non-NP patients

	NP n=24	***non-NP n=48***	***p-value (t-test)***
OACT GRADES			
Index knee			**0.010***
0	7 (29.2)	4 (8.3)	
1	9 (37.5)	10 (20.8)	
2	7 (29.2)	22 (45.8)	
3	1 (4.2)	12 (25.0)	
Contralateral knee			0.199*
0	4 (16.7)	4 (8.3)	
1	14 (58.3)	20 (41.7)	
2	5 (20.8)	16 (33.3)	
3	1 (4.2)	8 (16.7)	
Index patellofemoral			0.409
0	6 (25.0)	6 (12.5)	
1	10 (41.7)	20 (41.7)	
2	3 (12.5)	12 (25.0)	
3	5 (20.8)	12 (25.0)	
Contralateral patellofemoral			0.329
0	7 (29.2)	7 (14.6)	
1	9 (37.5)	15 (31.3)	
2	4 (16.7)	14 (29.2)	
3	4 (16.7)	12 (25.0)	
Left hip			0.638*
0	16 (66.7)	31 (64.6)	
1	7 (29.2)	16 (33.3)	
2	1 (4.2)	0 (0.0)	
3	0 (0.0)	1 (2.1)	
Right hip			0.868*
0	14 (58.3)	28 (58.3)	
1	7 (29.2)	16 (33.3)	
2	3 (12.5)	4 (8.3)	
3	0 (0.0)	0 (0.0)	

Left ankle			0.268*
0	21 (87.5)	33 (68.8)	
1	3 (12.5)	13 (27.1)	
2	0 (0.0)	0 (0.0)	
3	0 (0.0)	0 (0.0)	
Right ankle			0.420*
0	20 (83.3)	31 (64.6)	
1	4 (16.7)	13 (27.1)	
2	0 (0.0)	2 (4.2)	
3	0 (0.00	0 (0.0)	
Left acromioclavicular			0.705*
0	11 (45.8)	16 (33.3)	
1	5 (20.8)	14 (29.2)	
2	2 (8.3)	3 (6.3)	
3	6 (25.0)	15 (31.3)	
Right acromioclavicular			**0.023**
0	11 (45.8)	7 (14.6)	
1	6 (25.0)	13 (27.1)	
2	1 (4.2)	8 (16.7)	
3	6 (25.0)	20 (41.7)	
Left glenohumeral			0.480*
0	21 (87.5)	36 (75.0)	
1	2 (8.3)	10 (20.8)	
2	1 (4.2)	1 (2.1)	
3	0 (0.0)	0 (0.0)	
Right glenohumeral			0.340*
0	19 (79.2)	38 (79.2)	
1	2 (8.3)	8 (16.7)	
2	3 (12.5)	2 (4.2)	
3	0 (0.0)	0 (0.0)	
C1			0.786*
0	1 (4.2)	2 (4.2)	
1	5 (20.8)	7 (14.6)	
2	7 (29.2)	11 (22.9)	
3	11 (45.8)	28 (58.3)	

C1 facet			0.943
0	3 (12.5)	6 (12.5)	
1	5 (20.8)	11 (22.9)	
2	6 (25.0)	9 (18.8)	
3	10 (41.7)	22 (45.8)	
C2			0.083
0	6 (25.0)	8 (16.7)	
1	3 (12.5)	12 (25.0)	
2	10 (41.7)	9 (18.8)	
3	5 (20.8)	19 (39.6)	
C2 facet			0.676
0	8 (33.3)	15 (31.3)	
1	7 (29.2)	9 (18.8)	
2	3 (12.5)	10 (20.8)	
3	6 (25.0)	14 (29.2)	
T1			0.422
0	0 (0.0)	0 (0.0)	
1	4 (16.7)	15 (31.3)	
2	10 (41.7)	19 (39.6)	
3	9 (37.5)	14 (29.2)	
T1 facet			0.705*
0	10 (41.7)	25 (52.1)	
1	8 (33.3)	10 (20.8)	
2	4 (16.7)	8 (16.7)	
3	2 (8.3)	5 (10.4)	
T2			0.334*
0	1 (4.2)	1 (2.1)	
1	7 (29.2)	24 (50.0)	
2	11 (45.8)	15 (31.3)	
3	4 (16.7)	8 (16.7)	
T2 facet			0.916*
0	16 (66.7)	28 (58.3)	
1	5 (20.8)	13 (27.1)	
2	3 (12.5)	6 (12.5)	
3	0 (0.0)	1 (2.1)	

L1			**0.042***
0	2 (8.3)	5 (10.4)	
1	2 (8.3)	13 (27.1)	
2	13 (54.2)	11 (22.9)	
3	6 (25.0)	18 (37.5)	
L1 facet			0.277*
0	14 (58.3)	16 (33.3)	
1	3 (12.5)	12 (25.0)	
2	1 (4.2)	5 (10.4)	
3	5 (20.8)	14 (29.2)	
L2			0.772
0	5 (20.8)	15 (31.3)	
1	7 (29.2)	15 (31.3)	
2	8 (33.3)	12 (25.0)	
3	3 (12.5)	5 (10.4)	
L2 facet			0.357*
0	18 (75.0)	31 (64.6)	
1	5 (20.8)	8 (16.7)	
2	0 (0.0)	3 (6.3)	
3	0 (0.0)	5 (10.4)	
Total body score, mean (SD)	25.4 (8.4)	29.9 (9.0)	**0.044**

Values are given as n (%).

NP: Patients with neuropathic pain, non-NP: Matched controls, OACT: OsteoArthritis Computed Tomography, C/T/L-1: Worst degenerated level, C/T/L-2: Second worst degenerated level

*Fisher's Exact Test

Discussion

In the IMI-APPROACH knee OA cohort 24 patients out of 297 (8%) had a likely NP component. Interestingly, despite similar general knee pain levels (due to matching), patients with a likely NP component had less radiographic damage in their index knee, but a significant more impaired physical function. This might be explained by higher pain scores in joints other than the index knee, although OACT grades of these joints did not statistically significantly differ between patients with and without a NP component. The total body OACT score was even lower in patients with a likely NP component. These data indicate that patients with a likely NP component, determined with the painDETECT questionnaire, represent a specific phenotype, where local and overall joint damage is not the main cause of pain and disability.

Although questionnaires like the painDETECT show high sensitivity and specificity to distinguish NP from non NP[15], an objective measurement to show presence of a lesion of

the somatosensory system is required, which has not been done within IMI-APPROACH. As a consequence, other pain mechanisms than a pure neuropathic pain component, such as nociplastic pain or occurrence of central sensitization may play a role in these patients as well. Although fibromyalgia patients were excluded from inclusion in the IMI-APPROACH cohort, the presence of comorbidities/generalized pain syndromes was only assessed by asking the patients directly at time of inclusion. Objective scoring to determine specific pain syndromes was not used. Therefore, the presence of other pain phenotypes cannot be excluded.

The majority of the patients with a likely NP component (67%) had KL grade of 0 or 1. This raises the question whether these subjects are actually knee OA patients. The patients of the IMI-APPROACH knee OA cohort were included based on the clinical ACR criteria for knee OA. The agreement between these criteria and symptomatic radiographic knee OA is known to be low[37]. The IMI-APPROACH also did use a knee radiograph, taken at a screening visit, for final selection of patients, which was afterwards scored for KL grading performed by one observer blinded for the source of material. In the whole cohort, 47% of patients had a KL grade of 0 or 1 at baseline. The follow-up data of the study is currently being analyzed and will demonstrate whether these patients are actual knee OA patients.

In our study, 8% of patients reported a painDETECT score ≥19. The presence of painDETECT scores ≥19 in knee OA patients in other studies ranges from 5-67%[17-22,38,39]. In a recently published systematic review, a prevalence of 19% (CI 15-24%) in patients with hip and knee OA was found, although the prevalence depended on population type: community based 17% (CI 11-24%), hospital based 25% (CI 15-36%), randomized controlled trial patients 15% (CI 5-26%), end-stage knee OA 16% (CI 8-26%)[40]. In the general population these numbers ranged from 1%-14%[41-43].

In our study, patients with a likely NP component showed less radiographic damage compared to patients without a likely NP component that were matched for KOOS and SF-36 pain. Others compared patients with clinical knee OA with and without a NP component and found no differences in duration of OA symptoms[44], refuting the argument of NP being simply a symptom of OA progression. Duration of the OA was not assessed in the IMI-APPROACH study, irrespectively, it supports the concept of OA patients with a likely NP component as a specific phenotype, not related to radiographic severity (our data) or duration[44] of disease.

Age, BMI, and gender did not differ between patients with and without a likely NP component, confirming results from previous studies[19,22]. However, in other studies OA patients with higher painDETECT scores were younger[44] and had a higher BMI[18,44]. In contrast to our study, these studies included patients with painDETECT scores from 13-18 (uncertain result) in their analysis, giving a possible explanation for the different results.

In our study, patients with a likely NP component had a worse self-reported hand function (FIHOA) and worse outcomes on the two performance-based tests compared to patients

without a likely NP component. Others also found OA patients with NP-like symptoms had impaired walk and stair climb activity[21], but no difference in sit-to-stand activity[17,21]. Self-reported joint related function is also diminished in knee OA patients with a likely NP component compared to patients without a likely NP component[17,18]. Possibly, the impaired function is caused by OA pain in other joints. Indeed, more pain was found in the other joints, something that was also found previously[20]. However, joint damage of joints other than the index knee, assessed by OACT grades, did not differ between both groups. The total body score was even lower in patients with a likely NP component compared to patients without a likely NP component. Nevertheless, increased lumbar spine damage in patients with a likely NP component could contribute to the worse results on the two performance-based tests. Although, this does not explain the observed limited hand function. Besides, NRS pain of the lower back did not statistically differ between both groups. In addition to more severe overall pain, other causes for the physical impairment should be considered as well. Psychosocial factors, less physical activity, and other comorbidities are factors known to interfere with pain and physical impairment[45].

In agreement with the abovementioned finding that in patients with a likely NP component local joint damage is not the main cause of pain (similar pain level but less radiographic joint damage), these findings show that this is the case for other joints as well (more pain but comparable joint damage).

Based on our results it is possible that the generally found discordance between radiographic damage and pain in OA studies, is explained by the inclusion of patients with a NP component. Table 6 shows that the relationships between knee pain and radiographic damage are very small in the whole IMI-APPROACH. Excluding patients with a likely NP component from this cohort, increases the relationships. The relationships increased even more when patients with an in-between painDETECT score (13-18) were also excluded. These data indicate that OA patients with a likely NP component indeed weaken the relationship between joint damage and pain.

Table 6. Relationships between radiographic parameters and knee pain

		*Whole cohort n=297**		*Without likely NP n=270*		*non-NP n=220*	
KOOS pain	KL grade	***r***	**-0.186**	***r***	**-0.237**	***r***	**-0.292**
		p	**0.001**	**p**	**<0.001**	**p**	**<0.001**
	minJSW (mm)	***r***	**0.140**	***r***	**0.185**	***r***	**0.232**
		p	**0.017**	**p**	**0.002**	**p**	**0.001**
	Osteophyte area (mm^2)	***r***	**-0.147**	***r***	**-0.178**	***r***	**-0.251**
		p	**0.012**	**p**	**0.004**	**p**	**<0.001**
	Subchondral bone density (mm Alu. Eq.)	***r***	**-0.164**	***r***	**-0.183**	***r***	**-0.217**
		p	**0.005**	**p**	**0.003**	**p**	**0.001**
NRS index knee	KL grade	***r***	**0.147**	***r***	**0.171**	***r***	**0.204**
		p	**0.013**	**p**	**0.006**	**p**	**0.003**
	minJSW (mm)	***r***	**-0.142**	***r***	**-0.179**	***r***	**-0.198**
		p	**0.016**	**p**	**0.004**	**p**	**0.004**
	Osteophyte area (mm^2)	*r*	0.075	*r*	0.094	***r***	**0.145**
		p	0.206	p	0.130	**p**	**0.035**
	Subchondral bone density (mm Alu. Eq.)	***r***	**0.127**	***r***	**0.154**	***r***	**0.145**
		p	**0.032**	**p**	**0.013**	**p**	**0.035**

* Three patients did not have a painDETECT score at baseline and could not be assigned to one of the subgroups.
NP: Participants with neuropathic pain, non-NP: Matched controls, KOOS: Knee injury and Osteoarthritis Outcome Score, KL grade: Kellgren and Lawrence grade, minJSW: minimum Joint Space Width, NRS: Numeric Rating Scale.

Clearly, in OA patients with a likely NP component, joint damage itself is not the driving factor of pain and loss of function. This implies that pain and physical function are less likely to improve after e.g. TKA in this specific group. In general, about 20% of TKA is not successful[46]. Indeed, multiple studies found that OA patients with a likely NP component were twice as likely to experience pain after TKA than those without a likely NP component[47-49]. This effect may also interfere in case of DMOAD trials where the combination of tissue structure modification and pain control is needed (as requested by EMA and FDA[13,14]).

Obviously, the small number of patients with a NP component (n=24) is a clear limitation of this study. The main objectives of the IMI-APPROACH cohort study did not include evaluation of a NP component, therefore the current analysis should be considered as post-hoc analysis with limited power. Nevertheless, the results are important to consider in patient selection of future OA clinical trials and further research to characterize this specific group is warranted.

In conclusion, this study shows that OA patients with a likely NP component possibly reflect an OA phenotype where local tissue damage is not the leading cause for pain and physical impairment. As a consequence, the general one-size fits all approach, focused on treatment of nociceptive pain (resulting from tissue damage), might be inappropriate in this specific patient group. Therefore, it is advised to use the painDETECT questionnaire or an alternative measure to assess NP in OA patients. Identifying these OA patients and offering them a more personalized treatment[6,15] as well as excluding them from DMOAD trials could increase successes.

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10 Relationship between motion, using the GaitSmart® system, and radiographic knee osteoarthritis: an explorative analysis in the IMI-APPROACH cohort

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Abstract

Objectives

The currently used methods are insufficient to describe all aspects of osteoarthritis (OA). There is still an unmet need for non or minimal invasive techniques that add to the evaluation of this disease. Gait analysis might be of additional value as objective non-invasive measurement for assessing OA.

Methods

GaitSmart® analysis was performed during baseline visits of participants of the IMI-APPROACH cohort (n=297). Principal component analyses (PCA) were performed to explore structure in relationships between GaitSmart® parameters alone and in addition to radiographic parameters and patient reported outcome measures (PROMs). Logistic and linear regression analyses were performed to analyze the relationship of GaitSmart® with the presence (KL≥2 in at least one knee) and severity of radiographic OA (ROA).

Results

284 successful GaitSmart® analyses were performed. The PCA identified five underlying GaitSmart® domains. Radiographic parameters and PROMs formed additional domains indicating that GaitSmart® largely measures separate concepts. Several GaitSmart® domains were related to the presence of ROA as well as the severity of joint damage in addition to demographics and PROMs with an AUC-ROC of 0.724 and explained variances (adjusted R^2) of 0.107, 0.132 and 0.147 for minimum joint space width, osteophyte area and mean subchondral bone density, respectively.

Conclusion

GaitSmart® analysis provides additional information over established OA outcomes. GaitSmart® parameters are also associated with the presence of ROA and extent of radiographic severity over demographics and PROMS. These results indicate that Gaitsmart® may be an additional outcome measure for the evaluation of OA.

Introduction

Conventional radiography, despite its limitations, is the gold standard imaging technique to assess progression of tissue damage in osetoarthritis (OA). It enables detection of OA-associated bony features but lacks the ability to directly detect changes in other articular tissues (e.g. synovial tissue, meniscus and cartilage)[1]. Besides, clinical signs and symptoms of OA might be present even 2-3 years before radiographic changes on conventional images appear[2]. Magnetic resonance imaging (MRI) techniques do have the ability to visualize pathologies that are not detectable on radiographs. However, the high costs make it less suitable for standard use in clinical practice[1].

OA patients learn to avoid pain, but this avoidance leads to functional limitations and may change movement patterns. Structural changes may lead to functional limitations with a corresponding change in gait. Questionnaires assessing pain and functional limitations have the drawback of reflecting the subjective opinion of a patient rather than an objective measurement of the functional severity of OA. As such, there is still an unmet need for non- or minimal invasive techniques that add to the evaluation of OA.

Gait analysis might be such an additional measurement. Significant correlations were found between gait and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)[3] and The Short Form (36) Health Survey (SF-36)[4] subscales in patients fulfilling the American College of Rheumatology (ACR) clinical criteria for knee OA[5], radiographically confirmed according to Kellgren and Lawrence (KL)[6, 7].

A commonly used gait parameter from tests performed in an optical gait lab, the peak knee adduction moment (KAM), was found to have a negative correlation with cartilage thickness in OA knees, defined by KL grade ≥2[8]. KAM is increased in patients with OA, compared to controls, and this increase is higher for patients with severe OA than for patients with mild OA[9, 10]. General gait parameters also differed between patients with knee OA and matched (for sex, age, height, and weight) control subjects, walking at a similar speed. Knee flexion at heel strike (beginning of stance phase) was less in OA patients compared to controls[10]. Disadvantages of the optical gait lab are the time and costs required to complete one analysis.

The GaitSmart® system might be a user-friendly and objective method to assess gait. It takes about 10-15 minutes and can be carried out virtually everywhere. Knee flexion range of motion (ROM) in stance and swing phase, measured using an earlier version of the GaitSmart® system as used in this study, is significantly lower in OA patients fulfilling the ACR clinical criteria for OA, compared to healthy volunteers. A cut-off value of 13.6° of knee ROM in stance phase could discriminate between knee OA patients and healthy controls with a specificity of 0.952 and sensitivity of 0.783. Knee ROM in swing phase was less discriminative[11].

As such, gait analysis, as an additional measurement, may improve the assessment of presence and severity of OA, in addition to standard outcome measures based on radiographic measurements and patient reported outcome measures (PROMs). The objectives of this study are to (I) assess underlying domains measured by GaitSmart® parameters and whether these are additional to established OA markers including PROMs and radiographic parameters, (II) to evaluate if gait analysis using GaitSmart® is related to the presence of radiographic knee OA (ROA), and (III) to the severity of ROA both above demographics and PROMS. If GaitSmart® provides a potential useful additional measurement to asses OA we hypothesize that GaitSmart® parameters measure domains different from PROMS and radiographic outcomes, and that these GaitSmart® domains add to the relationship of demographics and PROMS with the presence and severity of ROA.

Methods

Participants

297 people with knee OA were included in the Applied Public-Private Research enabling OsteoArthritis Clinical Headway (IMI-APPROACH) study from January 2018 until April 2019 (age; 66.5±7.1, female; 230 (77%), BMI; 28.1±5.3)[12]. IMI-APPROACH is an exploratory, European, 5-centre, 2-year prospective follow-up cohort study. It obtains extensive clinical, imaging, biomechanical, and biochemical parameters of participants recruited using machine learning models based on retrospective and, to a limited extent, prospectively collected patient data, to display a high likelihood of radiographic joint space width loss and/or knee pain over the 2-year course of the study. For each participant the index knee was selected based on ACR clinical criteria for knee OA, using history and physical examination. If both knees fulfilled these criteria, the index knee was the most painful knee according to the participant. If both knees were equally painful, the right knee was chosen. A radiograph of the index knee was taken afterwards. Hence, the index knee wasn't necessarily the knee with the highest KL grade, since KL grade was determined after selecting an index knee and index knees can have KL grade 0 or 1.

The study is being conducted in compliance with the protocol, Good Clinical Practice (GCP), the Declaration of Helsinki, and the applicable ethical and legal regulatory requirements (for all countries involved), and is registered under clinicaltrials.gov nr: NCT03883568. All participants have received oral and written information and provided written informed consent. The present analysis focused on the baseline data.

GaitSmart® measurement

The GaitSmart® system uses six inertial measurement units (IMU) to evaluate gait mechanics. These IMUs comprise three tri-axial accelerometers and three tri-axial gyroscopes, making it

possible to measure movements in the sagittal and frontal plane[13]. After synchronizing the IMUs using Poseidon Software they were attached to the body.

Figure 1. Position of IMUs

Two IMUs were placed on the pelvis, under the iliac crest, following the alignment of the pelvis. Then, two other IMUs were placed on the widest part of the thighs, aligned in a straight vertical line. The last two IMUs were placed on the calves, on the belly of the gastrocnemius muscles[11, 13] (fig. 1).

Subsequently, participants were asked to stand still for five seconds to calibrate the IMUs. Then, the participants were asked to walk 15-20m at their own self-selected speed and return. After performing the test, the IMUs were removed and attached to the laptop for analysis. The IMUs are accurate to 0.11°, although the measurement error depends on positioning on the body. A previous study showed a reproducibility of ±2.8° and ±3.4° for knee ROM in swing and stance phase, respectively[11, 14].

Poseidon Software was used to extract and analyze data from the IMU sensors. The result is a report containing ROM of pelvis, hips, thighs, knees in swing and stance phase, and calves in the sagittal plane, stride duration, medial-lateral movement of thighs and calves, and symmetry scores between left and right. All parameters are presented in graphs and tables.

Fifteen GaitSmart® parameters were selected for statistical analysis based on previous research[11, 14] and clinical expertise; ROM for both knees in swing and stance, both hips and both calves were determined. Gait is considered as a measurement at patient level as opposed to a measurement at joint level. Therefore, the differences between both legs were also determined and included in the analysis as separate parameters. In addition, average stride duration, calculated speed and stride length were used.

Radiographic assessments

Standardized semi-flexed posterior-anterior weight bearing knee radiographs of both knees were taken according to Buckland-Wright[15]. KL grading was performed by one blinded observer. The intra- and inter-observer correlation were both previously found to be good (>0.83)[6], and in the current study an intraclass correlation coefficient (ICC) of 0.88 was found (using 10% of the radiographs). Additionally, Knee Images Digital Analysis (KIDA)[16] was performed by one single experienced observer. Minimum Joint Space Width of the tibiofemoral joint (minJSW in mm), osteophyte area (mm^2), and subchondral bone

density (mm Alu Eq.) were used as radiographic parameters. Previous studies demonstrated an ICC of 0.73-0.99 for the different features[17].

Assessment of pain and function

Pain and function were evaluated at patient level using the corresponding subscales of the Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaire[18], assessing pain in the most affected knee (MAK), the Numeric Rating Scale (NRS) pain for both knees and the Intermittent and Constant OsteoArthritis Pain (ICOAP) questionnaire[19], again assessing pain in the MAK. The KOOS questionnaire comprises nine items for pain and seventeen items for activities of daily living (ADL), each question scored on a 5-point scale. A normalized score is calculated where 0 means maximal limitations and 100 means no limitations. The NRS pain consists of an 11-point scale on which participants score pain from 0 (no pain) to 10 (worst imaginable pain). The ICOAP questionnaire contains 11 questions, five for constant pain and six for intermittent pain, each question scored on a 5-point scale. A higher total score reflects more pain.

Statistical analysis

Statistical analysis was performed using IBM SPSS statistics version 25.0.0.2. P-values <0.05 were considered statistically significant.

Relationship between individual GaitSmart® parameters and conventional parameters

A Principal component analysis (PCA) was performed to explore structure in relationships between individual GaitSmart® parameters and to reduce the total set of parameters to a limited set of underlying domains. This analysis was performed with GaitSmart® parameters alone as well as with radiographic parameters or PROMs as additional parameters to see if/how these parameters would underlie the same domains or measure something different.

PCA was also performed in different severity subgroups to investigate the stability of the identified underlying domains, as associations in different OA severity subgroups could differ. Subgroups were based on radiographic parameters (KL grade and minJSW) and PROMs (KOOS pain and ADL) using mean values as cut-offs to dichotomize.

Relationship with presence of radiographic knee OA

Logistic regression was used to evaluate the relationship of identified GaitSmart® domains with the presence of ROA in a patient, defined as KL≥2 in *at least* one knee, in addition to currently used parameters. Independent variables were entered stepwise starting with demographic variables (age, sex, and BMI), then KOOS pain and KOOS ADL, and finally the GaitSmart® domains.

It was also evaluated whether the association of the relevant GaitSmart® domains with the presence of ROA depended on pain severity, by testing interaction terms in the model. Statistically significant interactions were retained in the model. The area under the receiver operating characteristics curves (AUC-ROC) was calculated for all models as a measure of (increase in) model fit.

Relation with severity of radiographic knee OA

To explore the relationship between identified GaitSmart® domains and the severity of ROA, in addition to currently used parameters, linear regression was performed. The value of the MAK regarding minJSW (mm), osteophyte area (mm^2), and mean subchondral bone density (mm Alu Eq.), respectively, was used as outcome within these analyses. The independent variables were again entered stepwise in the same blocks as in the analysis used for the presence of ROA and interactions between KOOS pain and relevant GaitSmart® domains were tested, and, if statistically significant, retained in the model.

Results

Participant characteristics

A successful GaitSmart® analysis was performed for 284 participants. The thirteen missing analyses were due to user errors (n=9) or technical issues (n=4). Patient characteristics of the total population and separately for those with/without ROA are described in table 1. In three participants the radiograph of the index knee was made incorrectly. The thirteen excluded patients did not (statistically) significantly differ from the patients included in the study (data not shown).

Table 1. Baseline characteristics of the included patients

	Total ***n=281***	***ROA present*** ***n=159***	***ROA absent*** ***n=122***
DEMOGRAPHICS			
Age (years)	66.4 (7.0)	66.9 (7.2)	65.8 (6.9)
Female (%)	217 (77%)	126 (79%)	91 (74%)
BMI (kg/m^2)	28.0 (5.4)	28.5 (5.3)	27.4 (5.4)
QUESTIONNAIRES			
KOOS			
Pain	66.4 (18.8)	63.7 (17.8)	69.9 (19.4)
ADL	69.3 (19.0)	67.4 (18.1)	71.6 (19.8)
Symptoms	69.7 (17.0)	66.5 (16.8)	73.7 (16.6)
Sports	43.0 (26.9)	36.6 (23.7)	51.0 (28.6)
Quality of life	53.4 (20.3)	49.5 (18.4)	58.5 (21.6)
NRS (0-10)			
Index knee	4.5 (2.7)	4.7 (2.6)	4.3 (2.8)
Contralateral knee	2.9 (2.6)	3.0 (2.5)	2.9 (2.6)
RADIOGRAPHY			
KL grade (%)			
Grade 0	47 (17%)	3 (2%)	44 (36%)
Grade 1	88 (31%)	10 (6%)	78 (64%)
Grade 2	85 (30%)	85 (54%)	-
Grade 3	51 (18%)	51 (32%)	-
Grade 4	10 (4%)	10 (6%)	-
KIDA			
minJSW (mm)	2.5 (1.2)	2.1 (1.4)	3.1 (0.8)
Osteophyte area (mm^2)	21.2 (19.8)	30.6 (21.5)	8.8 (5.5)
Subchondral bone density (mm Alu Eq.)	31.0 (5.1)	31.5 (5.1)	30.5 (5.0)
GAITSMART®			
Range of motion (°)			
Index knee in stance phase	15.8 (4.9)	15.0 (5.0)	17.0 (4.5)
Index knee in swing phase	58.0 (7.3)	56.4 (6.7)	60.0 (7.5)
Contralateral knee in stance phase	16.8 (5.1)	16.5 (5.0)	17.1 (5.3)
Contralateral knee in swing phase	59.0 (7.1)	58.0 (7.1)	60.3 (6.9)
Index calf	71.9 (6.6)	70.6 (6.9)	73.6 (5.9)
Contralateral calf	72.3 (6.3)	71.7 (6.6)	73.1 (5.9)
Index hip	33.4 (7.5)	33.1 (7.6)	33.7 (7.3)
Contralateral hip	34.0 (7.1)	34.1 (6.9)	33.9 (7.4)
Stride length (m)	1.1 (0.2)	1.0 (0.2)	1.1 (0.2)
Average duration per stride (s)	1.1 (0.1)	1.1 (0.1)	1.1 (0.1)
Speed (m/s)	1.0 (0.2)	1.0 (0.2)	1.0 (0.2)

Values are represented as mean (SD) unless other specified.

BMI: Body Mass Index, KOOS: Knee injury and Osteoarthritis Outcome Score, NRS: Numeric Rating Scale, KL: Kellgren and Lawrence, KIDA: Knee Image Digital Analysis, minJSW: minimum Joint Space Width.

Principal component analysis

The PCA of GaitSmart® parameters (GS) identified five underlying domains (table 2): one mainly related to ROM in hips (GS Hip, component #1), one mainly related to ROM of knees and calves (GS Knee, component #2), and three mainly related to differences in either ROM of knees and calves in swing phase (GS Difference knee, component #3), ROM in hips (GS Difference hip, component #4), and ROM in knees during stance phase (GS Difference stance, component #5). PROMs and radiographic parameters each formed an additional component when added to the PCA, suggesting that the parameters measure different domains of a patient's disease status (table 2). The PCA in different subgroups showed that the domains identified were relatively stable (data not shown). Therefore the Gaitsmart® domains were used in further analyses.

Table 2. Principal component analysis GaitSmart® parameters

		Component				
Domain	**Parameter**	**1**	**2**	**3**	**4**	**5**
GS Knee	ROM index knee in swing	0.134	**0.891**	-0.111	-0.034	-0.058
	ROM contralateral knee in swing	0.164	**0.876**	0.062	-0.046	-0.026
	ROM index calf	0.491	**0.764**	-0.163	-0.011	0.047
	ROM contralateral calf	0.526	**0.745**	-0.019	0.021	0.068
	ROM index knee in stance	0.534	**0.478**	-0.007	-0.132	-0.368
	ROM contralateral knee in stance	0.613	**0.346**	-0.057	-0.121	-0.088
GS Hip	ROM index hip	0.849	0.208	-0.065	0.080	0.059
	ROM contralateral hip	**0.841**	0.184	0.025	0.164	0.065
	Speed (m/s)	**0.919**	0.207	-0.048	-0.175	-0.028
	Average duration per stride (s)	**-0.565**	0.059	0.014	0.513	0.186
	Stride length (m)	**0.761**	0.281	-0.046	0.087	0.101
GS Difference knee	Difference ROM knees in swing	-0.054	-0.105	**0.803**	0.189	-0.067
	Difference ROM calves	-0.043	-0.005	**0.847**	0.189	-0.067
GS Difference stance	Difference ROM knees in stance	0.069	-0.012	0.126	-0.033	**0.912**
GS difference hip	Difference ROM hip	0.099	-0.091	0.060	**0.836**	-0.071

GS: GaitSmart®, ROM: Range of motion.

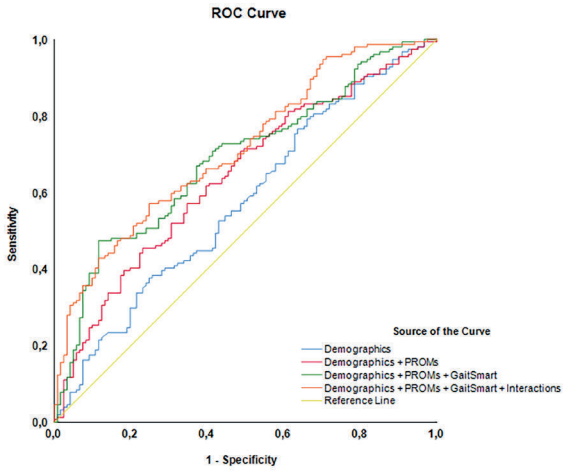
Relation with the presence of radiographic knee OA

159 participants (56%) had ROA in at least one knee (KL grade ≥2). Logistic regression showed that addition of GaitSmart® data to the model with demographics and PROMs improved the association with the presence of ROA (table 3, fig. 2); Nagelkerke R^2 increased from 0.075 to 0.150 when adding GaitSmart® parameters after demographics and PROMs, but the discriminatory value of this model was still only moderate (AUC=0.698, 95%CI 0.637; 0.760).

Sensitivity and specificity were 71.0% and 52.0%, respectively, using a probability of 0.50 as cut-off). KOOS pain (OR=0.964, 95%CI 0.935; 0.994), GS Knee (OR=0.624, 95%CI 0.457; 0.850), and GS Difference knee (OR=1.319, 95%CI 1.004; 1.733) were statistically significant contributing factors.

The association of GS knee and GS Difference knee with ROA statistically significantly depended on the level of pain. With less pain the effect of GaitSmart® domains on the likeliness of having ROA decreased. Including both interaction terms, the models Nagelkerke R^2 increased to 0.212 (table 3). The AUC-ROC increased to 0.724 (95%CI 0.665; 0.783, table 3, fig. 2).

Figure 2. ROC curve



Diagonal segments are produced by ties.
PROM: Patient reported outcome measures. ROC: Receiver operating characteristics.

Table 3. Results of logistic regression analysis on presence of radiographic OA

	OR (95%CI)			
Independent variable	**Model 1**	**Model 2**	**Model 3**	**Model 4**
Constant	**0.058**	0.068	0.104	0.069
Age	1.025 *(0.990; 1.061)*	**1.037** ***(1.000; 1.075)***	1.032 *(0.993; 1.073)*	1.035 *(0.994; 1.077)*
Sex	1.418 *(0.802; 2.509)*	1.165 *(0.643; 2.112)*	0.923 *(0.480; 1.773)*	0.959 *(0.486; 1.894)*
BMI	1.043 *(0.996; 1.092)*	1.045 *(0.994; 1.099)*	1.034 *(0.978; 1.093)*	1.048 *(0.989; 1.109)*
KOOS pain		**0.959** ***(0.931; 0.988)***	**0.964** ***(0.935; 0.994)***	**0.960** ***(0.930; 0.991)***
KOOS ADL		1.029 *(0.999; 1.059)*	1.029 *(0.998; 1.062)*	1.032 *(0.999; 1.066)*
GS Hip			1.079 *(0.819; 1.421)*	1.071 *(0.808; 1.421)*
GS Knee			**0.624** ***(0.457; 0.850)***	2.794 *(0.976; 8.000)*
GS Difference knee			**1.319** ***(1.004; 1.733)***	**4.548** ***(1.453; 14.230)***
GS Difference hip			1.104 *(0.848; 1.438)*	1.162 *(0.882; 1.531)*
GS Difference stance			1.202 *(0.913; 1.582)*	1.213 *(0.916; 1.604)*
KOOS pain*GS Knee				**0.978** ***(0.962; 0.993)***
KOOS pain*GS Difference knee				**0.981** ***(0.964; 0.998)***
Nagelkerke R^2	0.029	0.075	0.150	0.212
Δ R^2 *vs* previous model		0.46	0.075	0.062
AUC (95%CI)	0.578 *(0.510; 0.645)*	0.641 *(0.576; 0.706)*	0.698 *(0.637; 0.760)*	0.724 *(0.665; 0.783)*
Sensitivity	81.9%	74.8%	71.0%	74.2%
Specificity	25.3%	43.9%	52.0%	51.2%

P-values <0.05 are indicated in bold.

OR: Odds Ratio, AUC: Area under the curve, BMI: Body Mass Index, KOOS: Knee injury and Osteoarthrtitis Outcome Score, ADL: Activities of daily living, GS: GaitSmart®.

Relation with severity of radiographic knee OA: minimum JSW

In the model with minJSW as outcome parameter age (B=-0.024, 95%CI -0.044; -0.005), GS Hip (B=-0.647, 95%CI -1.148; -0.146), GS Knee (B=-0.696, 95%CI -1.174; -0.218), GS Difference knee (B=-0.153, 95%CI -0.281;-0.025), and GS Difference stance (B=-0.134, 95%CI -0.262; -0.005) were statistically significant contributing factors (table 4). In this model statistically significant interactions between KOOS pain and GS Hip (B=0.009, 95%CI 0.002; -0.017) and between KOOS pain and GS Knee (B=0.012, 95%CI 0.005; 0.019) were found. The adjusted R^2 of the final model, including both statistically significant interaction terms, was 0.107.

Table 4. Linear regression models for minJSW

	Unstandardized B (95%CI)			
Independent variable	**Model 1**	**Model 2**	**Model 3**	**Model 4**
Constant	**4.074*** ***(2.555; 5.593)***	**3.358*** ***(1.671; 5.045)***	**3.435*** ***(1.717; 5.515)***	**3.398*** ***(1.727; 5.069)***
Age	**-0.021** ***(-0.040; -0.002)***	**-0.240** ***(-0.043; -0.005)***	**-0.024** ***(-0.044; -0.005)***	**-0.024** ***(-0.043; -0.005)***
Sex	0.021 *(-0.296; -0.002)*	0.094 *(-0.227; 0.416)*	0.108 *(-0.230; 0.446)*	0.072 *(-0.257; 0.401)*
BMI	-0.018 *(-0.043; 0.007)*	-0.010 *(-0.037; 0.016)*	-0.007 *(-0.034; 0.021)*	-0.010 *(-0.037; 0.017)*
KOOS pain		0.011 ***(-0.004; 0.026)***	0.007 *(-0.008; 0.023)*	0.005 *(-0.010; 0.020)*
KOOS ADL		-0.001 *(-0.016; 0.015)*	0.000 *(-0.016; 0.016)*	0.003 *(-0.013; 0.019)*
GS Hip			-0.006 *(-0.149; 0.136)*	**-0.647** ***(-1.148; -0.146)***
GS Knee			0.105 *(-0.045; 0.254)*	**-0.696** ***(-1.174; -0.218)***
GS Difference knee			**-0.139** ***(-0.270; -0.008)***	**-0.153** ***(-0.281; -0.025)***
GS Difference hip			-0.049 *(-0.179; 0.081)*	-0.070 *(-0.197; 0.057)*
GS Difference stance			**-0.147** ***(-0.279; -0.015)***	**-0.134** ***(-0.262; -0.005)***
KOOS pain*GS Hip				**0.009** ***(0.002; 0.017)***
KOOS pain*GS Knee				**0.012** ***(0.005; 0.019)***
Adjusted R^2	0.011	0.031	0.054	0.107
Δ R^2 *vs* previous model		0.020	0.023	0.053

P-values <0.05 are indicated in bold, * indicates p-value <0.001. BMI: Body Mass Index, KOOS: Knee injury and Osteoarthritis Outcome Score, ADL: Activities of daily living, GS: GaitSmart®.

Relation with severity of radiographic knee OA: osteophyte area

In the model for osteophyte area sex (B=-10.117, 95%CI -16.409; -3.825), and GS Difference knee (B=2.568, 95%CI 0.120; 5.017) were statistically significant contributors. Only one statistically significant interaction term was found, between KOOS pain and GS Knee (B=-0.228, 95%CI -0.361; -0.095). The final adjusted R^2 was 0.132 (table 5).

Table 5. Linear regression models for osteophyte area

	Unstandardized B (95%CI)			
Independent variable	**Model 1**	**Model 2**	**Model 3**	**Model 4**
Constant	5.257 *(-24.271; 34.785)*	10.876 *(-22.215; 43.968)*	23.592 *(-9.017; 56.202)*	22.923 *(-9.066; 54.930)*
Age	0.174 *(-0.194; 0.542)*	0.224 *(-0.150; 0.598)*	0.085 *(-0.282; 0.451)*	0.074 *(-0.285; 0.433)*
Sex	-5.262 *(-11.424; 0.900)*	-6.258 *(-12.563; 0.046)*	**-10.401** ***(-16.811; -3.991)***	**-10.117** ***(-16.811; -3.991)***
BMI	0.385 *(-0.096; 0.865)*	0.332 *(-0.188; 0.852)*	-0.012 *(-0.534; 0.509)*	0.073 *(-0.442; 0.587)*
KOOS pain		-0.176 *(-0.478; 0.127)*	-0.130 *(-0.426; 0.166)*	-0.119 *(-0.410; 0.171)*
KOOS ADL		0.072 *(-0.235; 0.378)*	0.164 *(-0.142; 0.469)*	0.153 *(-0.147; 0.453)*
GS Hip			-2.031 *(-4.762; 0.641)*	-2.060 *(-4.710; 0.591)*
GS Knee			**-7.118*** ***(-9.956; -4.280)***	7.839 *(-1.305; 16.983)*
GS Difference knee			2.230 *(-0.257; 4.718)*	**2.568** ***(0.120; 5.017)***
GS Difference hip			1.014 *(-1.456; 3.484)*	1.336 ***(-1.095; 3.767)***
GS Difference stance			1.745 *(-0.760; 4.249)*	1.620 *(-0.838; 4.079)*
KOOS pain*GS Knee				**-0.228** ***(-0.361; -0.095)***
Adjusted R^2	0.011	0.013	0.098	0.132
Δ R^2 *vs* previous model		0.002	0.085	0.034

P-values <0.05 are indicated in bold, * indicates p-value <0.001.

BMI: Body Mass Index, KOOS: Knee injury and Osteoarthritis Outcome Score, ADL: Activities of daily living, GS: GaitSmart®.

Relation with severity of radiographic knee OA: subchondral bone density

In the model for mean subchondral bone density age (B=-0.094, 95%CI -0.183; -0.004), sex (B=-2.007, 95%CI -3.573; -0.442), BMI (B=0.250, 95%CI 0.122; 0.377), and GS Knee (B=-0.880, 95%CI -1.573; -0.187) were statistically significant contributing factors (table 6). No statistically significant interaction terms were found. The adjusted R^2 of the final model was 0.147.

Table 6. Linear regression models for subchondral bone density

	Unstandardized B (95%CI)		
Independent variable	**Model 1**	**Model 2**	**Model 3**
Constant	**30.628*** ***(23.665; 37.590)***	**33.067*** ***(25.260; 40.874)***	**34.272*** ***(26.309; 42.234)***
Age	-0.083 *(-0.170; 0.004)*	-0.078 *(-0.166; 0.010)*	**-0.094** ***(-0.180; -0.004)***
Sex	**-1.507** ***(-2.960; -0.054)***	**-1.618** ***(-3.106; -0.131)***	**-2.007** ***(-3.573; -0.442)***
BMI	**0.323*** ***(0.210; 0.436)***	**0.293*** ***(0.171; 0.416)***	**0.250*** ***(0.122; 0.377)***
KOOS pain		-0.008 *(-0.080; 0.063)*	0.002 *(-0.070; 0.074)*
KOOS ADL		-0.019 *(-0.091; 0.054)*	-0.009 *(-0.083; 0.066)*
GS Hip			-0.351 *(-1.011; 0.309)*
GS Knee			**-0.880** ***(-1.573; -0.187)***
GS Difference knee			0.223 *(-0.385; 0.830)*
GS Difference hip			0.094 *(-0.509; 0.697)*
GS Difference stance			0.569 *(-0.043; 1.180)*
Adjusted R^2	0.128	0.129	0.147
Δ R^2 *vs* previous model		0.001	0.018

P-values <0.05 are indicated in bold, * indicates p-value <0.001.

BMI: Body Mass Index, KOOS: Knee injury and Osteoarthritis Outcome Score, ADL: Activities of daily living, GS: GaitSmart®.

Discussion

This study showed that GaitSmart® parameters, as measured at baseline in the IMI-APPROACH cohort, can be grouped in five main underlying domains; one mainly related to ROM in hips (GS Hip, component #1), one mainly related to ROM of knees and calves (GS Knee, component #2), and three mainly related to differences in either ROM of knees and calves in swing phase (GS Difference knee, component #3), ROM in hips (GS Difference hip, component #4), and ROM in knees during stance phase (GS Difference stance, component #5). The GaitSmart® analysis relates to the whole individual, including (possible) OA in multiple joints. To account for this, differences in gait parameters (component 3-5, see above) are also used as input variables. These five domains contain additional information above radiographic parameters and PROMs and appear stable in different subgroups. The adjusted R^2 of the linear regression models shows moderate correlations with the severity of ROA. However, the increase in adjusted R^2 compared to the models using only demographics and PROMs is considerably.

Therefore, combining GaitSmart® parameters in five 'domains' as proposed, provides a concise set of relevant parameters that may have value as additional outcome measurements to assess OA and can be further validated in future analyses.

The main limitation of this study is the translation to the general OA population. IMI-APPROACH participants were selected based on a high probability of structural and/or pain progression. This may restrict the generalizability of the results. However, the domains identified were stable over subgroups of severity and selection bias regarding the associations found, taking into account other demographics and PROMs, is likely limited. However, the specific size of the association may be different in e.g. very early disease. Another limitation is the lack of follow-up data. Any prognostic value of the GaitSmart® parameters or any time relationship (e.g. does progression lead to a difference in GaitSmart® or the other way around), which is highly relevant, could not be evaluated. Furthermore, the development of gait characteristics over time might be of additional value above a single gait analysis.

The association of GaitSmart® (specifically the GS Difference knee domain) additionally to other parameters, was highest for osteophyte area. One can imagine that a certain relationship exists between the size of osteophytes and limitation in knee movement. The concept of mechanical hindering has also been linked to the presence of a relationship between osteophytes and synovial inflammation[20, 21].

In this study, the severity of ROA was evaluated by parameters related to cartilage (minJSW) and bone (mean subchondral bone density). The fact that the association is limited indicates that other joint structures (e.g. ligaments and/or muscles) also play a substantial role in someone's gait. Although the exclusion criteria of IMI-APPROACH rule out secondary osteoarthritis and generalized pain syndromes, other comorbidities influencing

gait (e.g. neuromuscular disorders) might also be present. Therefore, contribution of other joint structures and/or comorbidities related to gait might have influenced the relations found between gait and ROA. Some people are also able to manage pain better when walking than others, and neuropathic OA pain might be involved. These may influence the possibility to obtain strong relations, but given the finding that GaitSmart® measures another underlying domain of OA, these associations probably should not be too strong.

The association with the presence of ROA (KL grade ≥2) was quite strong for GaitSmart®. This is in line with Naili *et al*., who found that peak KAM and a positive KAM impulse were able to discriminate between mild OA (KL grade 1-2) and severe OA (KL grade 3-4)[9]. Using the GaitSmart® system has the benefit of assessing the full motion of walking, in contrast to peak value measurements which only represent a single moment during walking[22].

The gait analysis used by Naili *et al*. was conducted at motion analysis laboratories. At present, 3D optical gait analysis is considered to be the Gold standard for testing a person's movement[23]. The strong advantage of the GaitSmart® system is the possibility to use it in a natural environment, since no cameras and force plates are required. Moreover, significantly less time is needed to perform a GaitSmart® measurement, approximately fifteen minutes, where measurements in gait laboratories require up to half a day[23]. When comparing the use of IMUs to 3D analysis using an optical tracking system (OTS) no differences were found in determining pelvic tilt and knee ROM. The intraclass correlation coefficients were 0.83 (0.72-0.90) for right knee ROM, 0.86 (0.77-0.92) for left knee ROM, 0.75 (0.34-0.89) for right hip ROM and 0.73 (0.22-0.89) for left hip ROM[13]. This indicates that GaitSmart® produces valid data for pelvic tilt and, more importantly in our case, knee ROM.

Factors which alter proper joint biomechanics trigger the onset or acceleration of the degenerative process of OA, facilitating the beginning of structural changes and clinical symptoms[24]. The reverse sequence of events will likely occur as well, degenerative and inflammatory changes in the joint will alter biomechanics. Gait characteristics related to medial compartment knee OA depend on the OA severity[10]. Patients with less severe knee OA may adopt a strategy of gait compensation, lowering the load at the medial compartment, reducing their progression risk, where patients with more severe knee OA are unable to lower the load on the medial compartment, increasing the risk for disease progression[10]. By adapting the gait pattern in an early OA stage, assisting the natural compensation strategy, it might be possible to slow down disease progression and postpone surgery. Therefore, first a prognostic value of GaitSmart® parameters for disease progression should be established. This data becomes available within the IMI-APPROACH project when follow-up data is collected. When gait characteristics prove to be possibly modifiable prognostic factors, early detection of an unfavorable gait in combination with adequate adaptation strategies to this might become a feasible preventive strategy.

Patients scheduled for Total Knee Arthroplasty (TKA) had a typical OA gait pattern (reduced knee ROM in stance and swing phase) before surgery. 52 weeks post-operative two third of the patients still had OA gait characteristics, even though pain was reduced[25]. This study also suggested a potential value of gait analysis, in this case in the rehabilitation after joint replacement. GaitSmart® could monitor ROM progression of patients after TKA and identify patients that do not improve and might benefit from additional rehabilitation.

In conclusion, our study shows that GaitSmart® provides additional information above parameters currently used to asses OA, and is associated with the presence of ROA and, to a limited extent, the severity of OA above demographics and PROMs. This may indicate that GaitSmart® could be an additional parameter to asses OA, but longitudinal studies are required to evaluate how GaitSmart® could optimally serve as an additional non-invasive and easily applicable parameter to assess knee OA.

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11 GaitSmart® motion analysis compared to commonly used function outcome measures in the IMI-APPROACH knee osteoarthritis cohort

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Abstract

Objectives

There are multiple measures for assessment of physical function in knee osteoarthritis (OA), but each has its strengths and limitations. The GaitSmart® system, which uses inertial measurement units (IMUs), might be a user-friendly and objective method to assess function. This study evaluates the validity and responsiveness of GaitSmart® motion analysis as a function measurement in knee OA and compares this to Knee injury and Osteoarthritis Outcome Score (KOOS), Short Form 36 Health Survey (SF-36), 30s chair-stand test (chair), and 40m self-paced walk test (walk).

Methods

Available baseline and sic months follow-up data of the IMI-APPROACH knee OA cohort (n=262) was used. Principal component analysis was used to investigate whether above mentioned function instruments could represent one or more function domains. Subsequently, linear regression was used to explore the association between GaitSmart® parameters and those function domains. In addition, standardized response means (SRM), effect sizes and Student's *t*-tests were calculated to evaluate the ability of GaitSmart® to differentiate between good and poor general health (based on SF-36). Lastly, the responsiveness of GaitSmart® to detect changes in function was determined.

Results

KOOS, SF-36, chair and walk test were first combined into one function domain (total function). Thereafter, two function domains were substracted related to either performance-based (objective function) or self-reported (subjective function) function. Linear regression resulted in the highest R^2 for the total function domain: 0.314 (R^2 for objective and subjective function were 0.252 and 0.142, respectively.). Furthermore, GaitSmart® was able to detect a difference in general health status, and is responsive to changes in the different aspects of objective function (SRMs up to 0.74).

Conclusion

GaitSmart® analysis can reflect performance-based and self-reported function and may be of value in the evaluation of function in knee OA.

Introduction

There are multiple measures for assessment of physical function in knee osteoarthritis (OA)[1], and each has its strengths and limitations. Gait analysis in an optical gait lab is often used as gold standard[2,3] but has disadvantages. It is not always available, very costly, and time consuming. For performance-based tests (PBT) the opposite is true, they are easily performed in an everyday environment and take a few minutes. Limitations of PBT are the poor construct validity and responsiveness to change[4,5]. Besides, PBT do not give any information on quality of movement, in contrast to gait analysis[6]. PBT and gait analysis are said to be objective measures[7,8], containing information about the ability to complete a task. Self-reported measures, like the Knee injury and Osteoarthritis Outcome Score (KOOS) and Short Form 36 Health Survey (SF-36) give information concerning the experience associated with doing the task. Patients are not simply reporting their ability to move around, but their response also includes what they are experiencing during a task[9]. Construct validity and responsiveness to change are better for KOOS than PBT, including subscales activities of daily living (KOOS ADL) and sport and recreational function (KOOS sports)[10]. However, self-reported function is more influenced by pain than performance-based function[11,12]. Pain while performing a task will influence the experience of doing the task, but might not always influence the ability to perform a task. As such, subjective function might be more influenced by pain than objective function.

Self-reported and performance-based measures assess different aspects of function (experience *vs* ability) and are poorly correlated[9,13]. The first month after total knee arthroplasty, PBT and self-reported measures show inverse trajectories of improvement. The poor concurrent validity between both measures implicates that using solely self-reported measures or PBT is not sufficient to fully characterize function[8,12]. Variable correlations between self-reported function and gait parameters have been found as well[14-16]. As such, PBT (objective) and self-reported function (subjective) offer complementary information, essential to clinical research and practice[1,8,9].

The GaitSmart® system, which uses inertial measurement units (IMUs), might be a user-friendly and more objective method to assess function. Because no pressure plates or cameras are needed, it can be carried out virtually everywhere, taking approximately 10-15 minutes. When comparing the use of IMUs to 3D analysis in an optical gait lab, no differences were found in e.g. determining pelvic tilt and knee range of motion (ROM)[3]. It has been shown previously that GaitSmart® analysis gives additional information over patient reported outcome measures and radiographic outcomes for OA[17].

The objective of this study was to investigate construct validity and responsiveness of GaitSmart® as measurement of function in knee OA. For this purpose, multiple questions were answered.

1. Is GaitSmart® related to commonly used outcome measures for function?
2. Can GaitSmart® differentiate between groups with different general health status?
3. Is GaitSmart® able to measure change in function over a six month period?

Methods

Participants

297 people with knee OA were included in the Applied Public-Private Research enabling OsteoArthritis Clinical Headway (IMI-APPROACH) cohort study from January 2018 until April 2019 (age; 66.5±7.1, female; 230 (77%), BMI; 28.1±5.3)[18]. At screening, for each participant an index knee was determined based on American College of Rheumatology clinical criteria[19] or (if equal between two knees) the most painful knee was chosen as index knee. Screenings data was used in machine learning models to determine a predicted progression probability for pain (P) and one for structural progression (S)[20]. Participants with the highest predicted progression scores were included in the IMI-APPROACH cohort[18]. For this study, baseline and six months follow-up (M006) data were used.

GaitSmart® measurement

The GaitSmart® system uses six IMUs to evaluate gait mechanics. These IMUs comprise three tri-axial accelerometers and three tri-axial gyroscopes, making it possible to measure movements in the sagittal and frontal plane[3]. After attaching the IMUs to the body, participants are asked to walk 15-20m at their own self-selected speed and return. Subsequently, data is extracted from the IMUs and analyzed. The resulting report contains ROM of pelvis, hips, thighs, knees in swing and stance phase, and calves in the sagittal plane, stride duration, medial-lateral movement of thighs and calves, and symmetry scores between left and right (extensive description reported previously[17]).

Performance-based tests

Two PBT, recommended by OsteoArthritis Research Society International (OARSI)[21], were used in IMI-APPROACH. For the 30s chair-stand test (chair) participants had to stand up completely from a sitting position in the middle of a seat with feet shoulder width apart, flat on the floor, arms crossed at chest, and then sit completely. The result is the number of repetitions completed in thirty seconds. The 40m self-paced walk test (walk) records time in seconds needed to walk as quickly but as safe as possible (regular walking, no running) to a mark 10m away, return, and repeat for a total distance of 40m.

Function questionnaires

Self-reported function was evaluated using corresponding subscales of the KOOS questionnaire: ADL and sports[22], and the 'physical functioning' (SF-36 physical function) and 'role limitations due to physical health' scales (SF-36 role physical) of the SF-36[23].

Statistical analysis

All individual GaitSmart® parameters were used for analyses. Additionally to these individual GaitSmart® parameters, five GaitSmart® domains: GS Knee, GS Hip, GS Difference knee, GS Difference stance, and GS Difference hip, were also used for analyses. These domains have been identified previously by use of principal component analysis (PCA)[17].

As there is no 'gold standard' instrument to assess function, and several instruments are used, we evaluated whether six commonly used instruments (two PBTs, two KOOS subscales, and two SF-36 subscales) could represent one or multiple function domains (e.g. objective and/or subjective function) using PCA on cross-sectional IMI-APPROACH baseline data. Resulting domain(s) (combining all six common function outcome measures) were used as outcome measures for linear regression analyses.

Relation between GaitSmart® and common outcome measures for function

Linear regression analysis was used to explore whether individual GaitSmart® parameters were associated with derived function domains. Additionally, these analyses were performed using GaitSmart® domains (instead of individual GaitSmart® parameters) as independent variables.

Modelling started with a 'full model' including all GaitSmart® parameters. Then GaitSmart® parameters with a p-value >0.2 were removed, starting with the least statistically significant variable. In case the adjusted R^2 diminished relevantly, the variable was retained. Resulting regression formula(s) were then used to construct GaitSmart® based function scores related to total, objective, and subjective function domains (GS total function, GS objective function, and GS subjective function).

Differentiation between two groups with different general health status

Participants were divided into two subgroups based on the first question of the SF-36:

"In general, would you say your health is": 1. Excellent, 2. Very good, 3. Good, 4. Fair, 5. Poor.

The two groups were defined as either 'poor' general health (4 and 5) or 'good' general health (1 and 2). Cross-sectional data of participants with a succesfull GaitSmart® analysis at baseline were used. Student's *t*-tests and effect sizes (Hedges' g) were calculated to evaluate whether GaitSmart® is able to differentiate between these groups.

Six months change in function

Changes from baseline to M006 were calculated for each separate function outcome measure and GaitSmart® based function scores. Pearson's correlation coefficients were calculated to compare changes between commonly used function outcome measures and GaitSmart® based function scores.

Subsequently, patients were divided based on an increase or decrease of at least the minimal detectable change (MDC) in each of the commonly used function outcome measures[10,24,25] (those without at least a MDC were left out of analyses). For each of these subgroups SRM (mean change (i.e. M006-BL) in outcome variable divided by the standard deviation of this change) within the subgroup was calculated for the other function outcome measures and GaitSmart® based function scores. The difference in change scores between subgroups was compared using Student's *t*-tests and effect sizes (Hedges' g) to evaluate responsiveness of GaitSmart® to clinical change, compared to commonly used function outcome measures. An effect size of 0.5-0.8 is considered moderate, an effect size of 0.8 or higher is considered high.

Statistical analysis was performed using IBM SPSS statistics version 25.0.0.2. P-values <0.05 were considered statistically significant.

Results

Of the IMI-APPROACH cohort (n=297), 284 participants had a successful GaitSmart® measurement at baseline, of which 262 also had a successful GaitSmart® measurement at M006. Missing analyses were due to user errors, technical issues, or drop-outs. Two participants could not perform the chair test at M006, while all 262 successfully performed the walk test. Both KOOS subscales were available for all 262 participants, SF-36 physical function was missing for three participants, and SF-36 role physical was missing for one participant. For each of the analyses the maximum available full data set was used.

Principal component analysis on baseline function outcome measures

Using the default setting of an eigenvalue>1 in the PCA, all six function outcome measures loaded on one domain: total function. We also performed a PCA defining extraction of two components, where we found a division into a more objective function domain (PBT as main loading factors) and a more subjective function domain (KOOS as main loading factors). SF-36 was found to load both components, strongest on subjective function (table 1).

Table 1. Principal component analysis function outcome parameters

	Total function*	Objective function**	Subjective function**
Chair (# standing up)	0.611	**0.808**	0.110
Walk (s)	0.688	**0.792**	0.225
KOOS ADL	0.847	0.297	**0.863**
KOOS sports	0.730	0.081	**0.895**
SF-36 physical function	0.885	0.517	**0.722**
SF-36 role physical	0.697	0.563	0.435

Loadings are given as absolute values. Bold values indicate strong loadings, indicating a strong correlation between the parameter and that component.

KOOS: Knee injury and Osteoarthritis Outcome Score, ADL: Activities of daily living, SF-36: Short Form 36 Health Survey.

*result of principal component analysis when numbers of extracted domains was not set.

**result of principal component analysis defining extraction of two domains

Relation between GaitSmart® and derived function domains

In the model for subjective function, only index side variables (ROM and stance flexion index knee and ROM index hip) were statistically significant (table 2a). The final model had an adjusted R^2 of 0.141.

In the model for objective function, contralateral side (ROM contralateral knee), difference between both sides (difference range calf), and speed were statistically significant (table 2b). The final model had a higher adjusted R^2 compared to the model for subjective function; 0.252.

Finally, in the model for total function, parameters for index side (stance flexion index knee and ROM index hip) as well as contralateral side (ROM contralateral knee), and general parameters (average duration and stride length) were statistically significant (table 2c). The final model had a higher adjusted R^2: 0.314.

Table 2. Linear regression analysis for commonly used function domains

A. Subjective function domain					**Adjusted R^2 0.141**
Independent variable	B. (Unstand.)	95%CI		Beta (stand.)	p-value
Constant	-2.653	-3.587	-1.720		<0.001
ROM index knee	0.025	0.007	0.043	0.184	0.006
Stance flexion index knee	0.029	0.001	0.058	0.143	0.004
ROM index hip	0.022	0.006	0.039	0.167	0.009
B. Objective function domain					**Adjusted R^2 0.252**
Independent variable	B. (Unstand.)	95%CI		Beta (stand.)	p-value
Constant	-3.562	-4.432	-2.691		<0.001
ROM contralateral knee	0.026	0.011	0.040	0.188	0.001
Difference range calf	0.047	0.000	0.094	0.104	0.050
Speed	2.018	1.475	2.560	0.413	<0.001
C. Total function domain					**Adjusted R^2 0.314**
Independent variable	B. (Unstand.)	95%CI		Beta (stand.)	p-value
Constant	-2.744	-4.138	-1.349		<0.001
ROM contralateral knee	0.030	0.014	0.045	0.213	<0.001
Stance flexion index knee	0.029	0.004	0.054	0.141	0.023
ROM index hip	0.026	0.007	0.044	0.194	0.007
Average duration	-0.977	-1.852	-0.102	-0.119	0.029
Stride length	0.760	0.025	1.494	0.144	0.043

ROM: Range of motion.

Differentiation between groups with different general health status

Table 3 shows the results of Student's *t*-tests and effect sizes in the poor and good general health group. All individual commonly used function outcome measures and all three GaitSmart® based function scores are able to discriminate between participants with poor and good general health. Effect sizes are highest for SF-36 subscales. Nevertheless, all effect sizes were found to be high (>0.8).

Table 3. Effect sizes of different functional outcome measures in subgroups based on general health

	Poor general health n=79 Mean (SD)	Good general health n=58 Mean (SD)	Effect size Hedges' g	T-test p-value
SF-36 physical function	44.43 (18.24)	75.09 (17.31)	1.72	**<0.001**
SF-36 role physical	44.38 (23.35)	81.36 (17.85)	1.64	**<0.001**
KOOS ADL	57.52 (17.48)	80.87 (15.94)	1.39	**<0.001**
KOOS sports	29.75 (22.63)	53.88 (27.47)	0.97	**<0.001**
Walk (s)	32.99 (8.40)	24.05 (3.96)	1.30	**<0.001**
Chair (# standing up)	8.95 (3.08)	11.31 (2.47)	0.83	**<0.001**
GS subjective function	-0.19 (0.36)	0.13 (0.37)	0.88	**<0.001**
GS objective function	-0.23 (0.48)	0.29 (0.43)	1.14	**<0.001**
GS total function	-0.26 (0.56)	0.28 (0.53)	0.98	**<0.001**

Bold p-values indicate statistically significant p-value (<0.05).

KOOS: Knee injury and Osteoarthritis Outcome Score, ADL: Activities of daily living, SF-36: Short Form 36 Health Survey.

Cases were excluded listwise.

Six months change in function

Table 4 shows Pearson's correlation coefficients between changes from baseline to M006 (M006-BL) for commonly used function outcome measures and GaitSmart® based function scores. Clearly all GaitSmart® based function scores correlated best with PBTs.

Table 4. Pearson's correlation coefficients between changes from baseline to M006 for all used functional outcome measures

	ΔGS subjective function		ΔGS objective function		ΔGS total function	
	r	p-value	*r*	p-value	*r*	p-value
ΔChair (# standing up)	**0.128**	**0.041**	**0.163**	**0.009**	0.092	0.143
ΔWalk (s)	**-0.263**	**<0.001**	**-0.262**	**<0.001**	**-0.311**	**<0.001**
ΔKOOS ADL	0.084	0.176	0.049	0.431	0.095	0.129
ΔKOOS sports	0.045	0.475	0.039	0.533	0.042	0.508
ΔSF-36 physical function	0.096	0.124	0.105	0.093	0.089	0.156
ΔSF-36 role physical	0.084	0.177	0.098	0.116	0.069	0.273

Statistically significant values are given in bold.

GS: GaitSmart®, KOOS: Knee injury and Osteoarthritis Outcome Score, ADL: Activities of daily living, SF-36: Short Form 36 Health Survey.

GaitSmart® appears more related to PBT than to questionnaires as deduced thus far. Therefore, the study population was divided in two groups based on an increase or decrease of at least the MDC on the chair test (table 5) or the walk test (table 6). SRMs, effect sizes, and results of Student's *t*-tests between those with an in- or decrease are shown for all function tests.

If there is a decrease in sit-to-stand activity (table 5), this decrease is most prominently detected by GS subjective (and total) function score. If there is an improvement in sit-to-stand activity, this is most prominently found in the GS objective function score. Effect sizes for worsening compared to improving are highest for all three GaitSmart® based function scores, meaning these are more responsive to detect an actual change in sit-to-stand activity, as compared to commonly used function paramaters, including the walk test.

Table 5. Standardized response means and effect sizes of different functional outcome measures in subgroups based on MDC (=2) of the 30s chair-stand test

	Worsened chair test n=41		Improved chair test n=79		Effect size	T-test
	M006-BL		M006-BL		Hedges 'g	p-value
	Mean (SD)	SRM	Mean (SD)	SRM		
Walk (s)	0.02 (5.69)	0.00	-1.53 (6.66)	-0.23	0.24	0.205
KOOS ADL	-0.61 (16.07)	-0.04	3.11 (13.27)	0.23	0.26	0.179
KOOS sports	-0.37 (21.13)	-0.02	-0.82 (19.49)	-0.04	0.02	0.906
SF-36 physical function	-2.32 (15.05)	-0.15	-0.95 (14.63)	-0.06	0.09	0.631
SF-36 role physical	-5.04 (23.85)	-0.21	-0.63 (19.80)	-0.03	0.21	0.285
GS subjective function	-0.12 (0.49)	-0.25	0.03 (0.57)	0.05	0.28	0.148
GS objective function	0.00 (0.44)	0.01	0.18 (0.42)	**0.43**	0.41	**0.033**
GS total function	-0.09 (0.32)	**-0.26**	0.06 (0.36)	0.16	0.41	**0.035**

Bold p-values indicate statistically significant p-value (<0.05). Highest SRM for each are given in bold.
SRM: Standardized response mean, M006: Six months follow-up visit, BL: Baseline, KOOS: Knee injury and Osteoarthritis Outcome Score, ADL: Activities of daily living, SF-36: Short Form 36 Health Survey, GS: GaitSmart®

A decrease in walking activity (table 6) is most prominently detected by GS total function score, and an improvement in walking activity is most prominently found by GS objective function score. Effect sizes for worsening compared to improving are highest for all three GaitSmart® based function scores, meaning these are more responsive to detect an actual change in walk activity, as compared to commonly used function parameters. Also the chair test shows a statistically significant difference between the worsened and improved walk test group, however with a lower effect size.

Table 6. Standardized response means and effect sizes of different functional outcome measures in subgroups based on MDC (=0.19m/s) of the 40m self-paced walk test

	Worsened walk test n=43		Improved walk test n=48		Effect size	T-test
	M006-BL		M006-BL		Hedges 'g	p-value
	Mean (SD)	SRM	Mean (SD)	SRM		
Chair (# standing up)	-0.02 (2.47)	-0.00	1.06 (2.32)	0.46	0.45	**0.033**
KOOS ADL	-0.79 (14.98)	-0.05	2.08 (14.62)	0.14	0.19	0.359
KOOS sports	-2.67 (21.39)	-0.13	-6.56 (21.57)	-0.30	0.18	0.391
SF-36 physical function	-3.95 (15.87)	-0.25	-0.83 (16.38)	-0.05	0.19	0.360
SF-36 role physical	-7.27 (21.73)	-0.33	1.69 (22.40)	0.08	0.41	0.057
GS subjective function	-0.15 (0.35)	-0.44	0.11 (0.24)	0.49	0.92	**<0.001**
GS objective function	-0.18 (0.36)	-0.50	0.24 (0.32)	**0.74**	1.23	**<0.001**
GS total function	-0.33 (0.52)	**-0.64**	0.17 (0.33)	0.52	0.92	**<0.001**

Bold p-values indicate statistically significant p-value (<0.05). Highest SRM for each are given in bold.
SRM: Standardized response mean, M006: Six months follow-up visit, BL: Baseline, KOOS: Knee injury and Osteoarthritis Outcome Score, ADL: Activities of daily living, SF-36: Short Form 36 Health Survey, GS: GaitSmart®

For self-reported function outcome measures (KOOS and SF-36 subscales) the same analyses have been performed based on an increase or decrease of at least the MDC (table 7a-b and 8a-b). Worsening and improvement in one of the self-reported function outcome measures are most prominently detected by the other self-reported function outcome measures. Effect sizes for worsening compared to improving were low for GaitSmart® based function scores, meaning these GaitSmart® based function scores are minimally responsive to detect an actual change in self-reported function.

Table 7a. Standardized response means and effect sizes of different functional outcome measures in subgroups based on MDC (=15.7) of the KOOS ADL subscale

	Worsened KOOS ADL n=28		Improved KOOS ADL n=30		Effect size	T-test
	M006-BL		M006-BL		Hedges 'g	p-value
	Mean (SD)	SRM	Mean (SD)	SRM		
Chair (# standing up)	-0.11 (1.99)	-0.06	0.00 (3.27)	0.00	0.04	0.882
Walk (s)	-0.18 (4.30)	-0.04	-2.10 (6.75)	-0.31	0.34	0.205
KOOS sports	-18.75 (25.73)	-0.73	15.33 (18.00)	**0.85**	1.54	**<0.001**
SF-36 physical function	-16.25 (16.59)	**-0.98**	7.67 (19.86)	0.39	1.30	**<0.001**
SF-36 role physical	-13.62 (27.33)	-0.50	6.04 (33.42)	0.18	0.64	**0.018**
GS subjective function	-0.06 (0.28)	-0.21	0.04 (0.33)	0.13	0.33	0.216
GS objective function	0.01 (0.36)	0.03	0.07 (0.36)	0.18	0.15	0.569
GS total function	-0.13 (0.47)	-0.27	0.02 (0.42)	0.04	0.32	0.228

Bold p-values indicate statistically significant p-value (<0.05). Highest SRM for each are given in bold.
SRM: Standardized response mean, M006: Six months follow-up visit, BL: Baseline, KOOS: Knee injury and Osteoarthritis Outcome Score, ADL: Activities of daily living, SF-36: Short Form 36 Health Survey, GS: GaitSmart®.

Table 7b. Standardized response means and effect sizes of different functional outcome measures in subgroups based on MDC (=25.1) of the KOOS sports subscale

	Worsened KOOS sport n=19		Improved KOOS sport n=14		Effect size	T-test
	M006-BL		M006-BL		Hedges 'g	p-value
	Mean (SD)	SRM	Mean (SD)	SRM		
Chair (# standing up)	0.53 (2.07)	0.26	-0.21 (3.66)	-0.06	0.26	0.466
Walk (s)	-1.42 (5.56)	-0.26	1.86 (5.93)	0.31	0.57	0.114
KOOS ADL	-11.54 (11.92)	**-0.96**	15.33 (18.25)	**0.84**	1.80	**<0.001**
SF-36 physical function	-6.58 (12.70)	-0.52	10.00 (23.62)	0.42	0.92	**0.014**
SF-36 role physical	6.25 (19.43)	0.32	5.80 (25.41)	0.22	0.02	0.955
GS subjective function	-0.02 (0.33)	-0.06	0.05 (0.47)	0.11	0.17	0.627
GS objective function	0.04 (0.27)	0.15	0.09 (0.63)	0.14	0.11	0.765
GS total function	-0.12 (0.46)	-0.25	0.00 (0.76)	0.01	0.20	0.570

Bold p-values indicate statistically significant p-value (<0.05). Highest SRM for each are given in bold.
SRM: Standardized response mean, M006: Six months follow-up visit, BL: Baseline, KOOS: Knee injury and Osteoarthritis Outcome Score, ADL: Activities of daily living, SF-36: Short Form 36 Health Survey, GS: GaitSmart®.

Table 8a. Standardized response means and effect sizes of different functional outcome measures in subgroups based on MDC (=-2.46 for worsening, and 2.26 for improvement) of the SF-36 physical function subscale

	Worsened SF-36 n=103		Improved SF-36 n=99		Effect size	T-test
	M006-BL		M006-BL		Hedges 'g	p-value
	Mean (SD)	SRM	Mean (SD)	SRM		
Chair (# standing up)	0.58 (2.16)	0.27	0.44 (2.84)	0.16	0.06	0.697
Walk (s)	-0.27 (5.77)	-0.05	-0.87 (4.45)	-0.16	0.11	0.597
KOOS ADL	-4.17 (14.60)	-0.29	5.55 (13.49)	**0.41**	0.69	**<0.001**
KOOS sports	-8.01 (22.19)	**-0.36**	2.78 (20.01)	0.14	0.51	**<0.001**
SF-36 role physical	-8.43 (23.30)	**-0.36**	7.20 (20.93)	0.34	1.06	**<0.001**
GS subjective function	-0.04 (0.32)	-0.11	0.03 (0.31)	0.10	0.21	0.129
GS objective function	0.04 (0.37)	0.10	0.10 (0.43)	0.23	0.15	0.274
GS total function	-0.09 (0.47)	-0.18	-0.01 (0.50)	-0.02	0.15	0.277

Bold p-values indicate statistically significant p-value (<0.05). Highest SRM for each are given in bold.
SRM: Standardized response mean, M006: Six months follow-up visit, BL: Baseline, KOOS: Knee injury and Osteoarthritis Outcome Score, ADL: Activities of daily living, SF-36: Short Form 36 Health Survey, GS: GaitSmart®.

Table 8b. Standardized response means and effect sizes of different functional outcome measures in subgroups based on MDC (=-9.61 for worsening, and 10.85 for improvement) of the SF-36 role physical subscale

	Worsened SF-36 n=79		Improved SF-36 n=76		Effect size	T-test
	M006-BL		M006-BL		Hedges 'g	p-value
	Mean (SD)	SRM	Mean (SD)	SRM		
Chair (# standing up)	0.23 (2.20)	0.10	0.55 (3.09)	0.18	0.12	0.451
Walk (s)	0.15 (5.92)	0.03	-1.17 (4.39)	-0.27	0.25	0.117
KOOS ADL	-3.33 (15.49)	-0.22	5.47 (15.16)	0.36	0.57	**<0.001**
KOOS sports	-6.27 (16.99)	-0.37	0.86 (24.13)	0.04	0.34	**0.036**
SF-36 physical function	-7.97 (16.44)	**-0.49**	7.04 (15.75)	**0.45**	0.93	**<0.001**
GS subjective function	-0.04 (0.32)	-0.12	0.06 (0.31)	0.18	0.30	0.068
GS objective function	0.04 (0.41)	0.09	0.12 (0.41)	0.31	0.21	0.192
GS total function	-0.10 (0.54)	-0.19	0.02 (0.47)	0.05	0.25	0.127

Bold p-values indicate statistically significant p-value (<0.05). Highest SRM for each are given in bold.
SRM: Standardized response mean, M006: Six months follow-up visit, BL: Baseline, KOOS: Knee injury and Osteoarthritis Outcome Score, ADL: Activities of daily living, SF-36: Short Form 36 Health Survey, GS: GaitSmart®.

Since selection of IMI-APPROACH participants is based on predicted progression scores (S and P, see above) we also evaluated change in commonly used and GaitSmart® based function scores in participants with low predicted progression (S ánd P score below median value) and high predicted progression (S ánd P score above median value). These data demonstrated that GS total function and GS subjective function were also discriminative between these predicted progression subgroups (table 9).

Table 9. Standardized response means and effect sizes of different functional outcome measures in subgroups based on predicted progression probabilities

	Low predicted progression n=33		High predicted progression n=38		Effect size	T-test
	M006-BL		M006-BL		Hedges 'g	p-value
	Mean (SD)	SRM	Mean (SD)	SRM		
Chair (# standing up)	0.45 (1.62)	0.28	0.39 (2.41)	0.16	0.03	0.904
Walk (s)	0.97 (3.30)	0.29	-2.11 (6.30)	**-0.33**	0.60	**0.014**
KOOS ADL	-1.29 (11.38)	-0.11	4.22 (16.88)	0.25	0.38	0.109
KOOS sports	-1.82 (21.17)	-0.09	1.84 (25.21)	0.07	0.16	0.514
SF-36 physical function	-1.06 (10.29)	-0.10	1.97 (20.97)	0.09	0.18	0.433
SF-36 role physical	-4.73 (17.54)	-0.27	1.15 (27.49)	0.04	0.25	0.280
GS subjective function	-0.07 (0.29)	-0.25	0.05 (0.27)	0.18	0.43	0.077
GS objective function	0.05 (0.30)	0.16	0.13 (0.43)	0.29	0.20	0.398
GS total function	-0.13 (0.44)	**-0.30**	0.10 (0.43)	0.22	0.52	**0.033**

Bold p-values indicate statistically significant p-value (<0.05). Highest SRM for each are given in bold.
SRM: Standardized response mean, M006: Six months follow-up visit, BL: Baseline, KOOS: Knee injury and Osteoarthritis Outcome Score, ADL: Activities of daily living, SF-36: Short Form 36 Health Survey, GS: GaitSmart®.

Discussion

GaitSmart® analysis is related to commonly used function outcome measures, specifically more objective outcomes, with good sensitivity to observe short-term changes over time. GaitSmart® is considered of additive value because it is easy to use (contrary to analysis in an optical gait lab), gives information on subjective and objective function (contrary to questionnaires), is sensitive for short-term change (contrary to PBT), and gives information on quality of gait.

As expected, the adjusted R^2 is higher for the objective function domain compared to the subjective function domain. Not surprisingly, GaitSmart®, as objective measurement, did not represent a high proportion of the variability in the subjective function domain (R^2 for subjective function domain is low). This finding is confirmed by longitudinal analyses.

Changes in GaitSmart® based function scores are specifically related to changes in PBT and less to changes in function questionnaires. Nevertheless, the adjusted R^2 is highest in the model for the total function domain. This indicates that both objective function and subjective function contribute to the total function domain. As such, GaitSmart® is of use as function outcome measure, combining evaluation of both constructs of function.

There is a notable difference between the models for objective and subjective function. In the model for subjective function, GaitSmart® parameters that are related to the index leg are statistically significant, this in contrast to objective function where differences between both legs were found to be more dominant. The index leg is the leg which was most painful for participants at screening, indicating pain is more important for subjective function compared to objective function. This is confirmed in a previous study, where better correlations between pain and self-reported function were found compared to those between pain and performance-based measures[11]. Moreover, change in pain was found to be the principal determinant of change in self-reported function[9].

Prediction models using GaitSmart® domains as independent variables show the same trend. In the model for subjective function, 'GS Difference stance' is included. Most likely, stance phase of a stride is more painful than swing phase, because in that phase loading is applied. Therefore, stance phase, and with that 'GS Difference stance', might be more important for a person's view of their function (subjective), compared to their actual function (objective). These findings support the result of the PCA: subjective function is mainly determined by questionnaires, a reflection of people's opinion about their function.

In the model for objective function hip related GS domains are included, suggesting a contribution of the hip joint in someone's objective function, which apparently is less pain related but more related to actual performance.

Not surprisingly, in the analysis where subgroups were based on the first question of the SF-36, highest effect sizes were found for both SF-36 subscales. This question is not part of the SF-36 subscales, but is included in the general health subscale. General health is related to both physical and mental health[26], and it remains uncertain if and how function and general health are influenced by each other. Nevertheless, effect sizes were high (>0.8) for all parameters, including GaitSmart® based function scores, indicating that all function outcome measures are able to differentiate between participants with different general health status.

In case of dichotomisation based on PBT, GaitSmart® based function scores show the highest effect sizes for a six month change. Interestingly, GS subjective function score also shows higher effect sizes for a six month change in the objective PBT. In case of dichotomization based on function questionnaires effect sizes were significantly smaller. This implies that although GaitSmart® includes both subjective and objective function, it best describes objective function.

With respect to the IMI-APPROACH cohort it appeared that GaitSmart® showed the highest SRM for a six month change in the low progression group. Assuming these participants will indeed slowly progress, this shows that GaitSmart® is able to detect small changes in function. Of course final follow-up data is needed to further evaluate usability of GaitSmart® to detect disease progression in different knee OA subgroups.

In conclusion, this study shows that GaitSmart® is related to commonly used function outcome measures and includes evaluation of subjective and objective function with a dominance on objective function. GaitSmart® is responsive to changes in different aspects of objective function. Future studies using GaitSmart® are warranted to validate whether GaitSmart® can be used as clinical outcome measure in research and clinical practice.

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12 Two-year actual and predicted radiographic and pain progression in the IMI-APPROACH knee osteoarthritis cohort

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Abstract

Objectives

The IMI-APPROACH knee osteoarthritis cohort study used machine learning (ML) models to select patients from existing cohorts with an increased likelihood for radiographic and/or pain progression, reflected by a structural (S) and pain (P) progression score. This study evaluates the actual 2-year radiographic progression and pain progression, in relation to the originally assigned progression scores.

Methods

Structural progression was measured using minimum Joint Space Width (minJSW). Pain (progression) was evaluated using the KOOS questionnaire. 2-year progression was presented as actual change (Δ) after two years, and as progression over two years based on a per patient fitted regression line using baseline, 6, 12, and 24 month values. Differences in progression scores between progressors and non-progressors were evaluated. ROC curves were constructed and corresponding AUCs reported. Using Youden's indices optimal cut-offs were chosen to enable evaluation of both progression scores to identify progressors.

Results

Radiographic progressors were initially assigned higher S progression scores compared to radiographic non-progressors. Likewise, pain progressors were assigned higher P progression scores compared to pain non-progressors. The AUC-ROC for the S progression score to identify radiographic progressors was poor (0.612 and 0.599 for ΔminJSW and regression minJSW, respectively). The AUC-ROC for the P progression score to identify pain progressors was good (0.817 and 0.830 for ΔKOOS and regression KOOS pain, respectively).

Conclusion

The S and P progression scores as provided by the ML models developed and used for the selection of IMI-APPROACH patients were to some degree able to distinguish between progressors and non-progressors.

Introduction

One of the major challenges in knee osteoarthritis (OA) clinical trials is the selection of patients. Because actual cure is not anticipated, patients who will sufficiently progress without intervention are needed to provide an opportunity to observe arrest or reduction of disease progression. Since progression in OA is on average (very) slow, without pre-selection of fast progressive patients clinical trials require large group sizes and long follow-up[1]. The Innovative Medicines Initiative Applied Public-Private Research enabling OsteoArthritis Clinical Headway (IMI-APPROACH) consortium brings together European clinical centers, basic research institutes, business, pharmaceutical companies, and a patient council representing the patients voice[2]. The consortium provided a longitudinal cohort study of pre-selected knee OA patients combining conventional and new disease markers, to evaluate innovative selection procedures and to identify different OA phenotypes. This 2-year multicenter observational prospective cohort used a unique, multi-step preselection of participants, to include patients with an increased likelihood of radiographic and/or pain progression from five European observational OA cohorts (CHECK[3], HOSTAS[4], MUST[5], PROCOAC[6], and DIGICOD[7]). Details of the selection procedure and a cohort profile were described previously[8].

To select the most eligible patients after screening, collecting a minimal data set including Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain and radiographic features, all patients were assigned a structure (S) progression score and a pain (P) progression score, using machine learning (ML) models. The S progression score (range 0-1) reflected the probability of showing radiographic progression (yes or no, based on pre-defined criterion) during the follow-up period. Likewise, the P progression score (range 0-1) reflected the probability of showing pain progression (yes or no, based on pre-defined criteria) during the follow-up period. Both scores were normalized and combined into one score for ranking purposes, where the patient with the highest ranking score has the highest chance of showing radiographic and/or pain progression. The ~75% of patients with the highest ranking score were selected (for cohort details see[8]).

Based on literature, in IMI-APPROACH radiographic progression was pre-defined as: a reduction of minimum joint space width (minJSW) over time at a threshold of 0.3mm narrowing per year (i.e. ≥0.6mm decrease in the 2-year follow-up period)[9]. Pain progression (on a 0-100 scale; 100 means no pain and 0 means maximal pain) was pre-defined as: fast/significant pain increase and/or stable significant pain. *Fast pain increase:* Knee Injury and Osteoarthritis Outcome Score (KOOS) pain decrease between baseline and follow-up ≥10 points per year (i.e. ≥20 points decrease in the 2-year follow-up period) and final KOOS pain score ≤65 points. *Significant pain increase:* KOOS pain decrease between baseline and follow-up ≥5 points per year (i.e. ≥10 points decrease in the 2-year follow-up period) and final KOOS pain score ≤60 points. *Stable significant pain:* KOOS pain score ≤60 points during the whole study period[8, 10].

At baseline, IMI-APPROACH patients with a high S progression score were found to have a higher minJSW compared to patients with a low S progression score, providing an opportunity to show structural progression. Patients with a high P progression score reported more pain compared to patients with a low P progression score, and with that a smaller window to show pain progression. Although these baseline characteristics seem to be counter-intuitive, this combination provides potentially the best patient selection for treatment modalities that decrease pain (initially low KOOS score) and prevent, stop, or slow-down structural progression (initially large JSW)[11].

This present study evaluates the observed 2-year radiographic and pain progression within the IMI-APPROACH cohort in relation to the originally assigned S, and P progression score. The final aim was to determine if the originally designated S and P progression scores are able to distinguish between radiographic/pain progressors and non-progressors. If so, the selection procedure using these predicted progression score might be a first step to increase selection of radiographic and/or pain progressors in future clinical trials.

Methods

Patient selection

All patients of the IMI-APPROACH study (n=297 at baseline; mean age 66.5 years (SD 7.1), mean BMI 28.1 (SD 5.3), female/male ratio 230/67[8]) of whom sufficient follow-up data was available were included in the current analyses. The study is being conducted in compliance with the protocol, Good Clinical Practice (GCP), the Declaration of Helsinki, and the applicable ethical and legal regulatory requirements (for all countries involved). The study is registered under clinicaltrials.gov nr: NCT03883568. All patients have received oral and written information and provided written informed consent.

Evaluation of radiographic damage

For each participant an index knee was selected based on American College of Rheumatology clinical criteria for knee OA[12], using history and physical examination. If both knees fulfilled these criteria, the index knee was the most painful knee according to the participant. If both knees were equally painful, the right knee was chosen. Then, posterior-anterior weight bearing semi-flexed knee radiographs were obtained according to the protocol of Buckland-Wright[13]. Knee Image Digital Analysis (KIDA)[14] was used to determine minJSW.

Evaluation of pain

Pain was evaluated using the pain subscale of the KOOS questionnaire[15]. This score uses nine questions for pain, each scored on a 5-point Likert scale. A normalized score was calculated where 0 means maximal pain and 100 means no pain. Since the ML models used

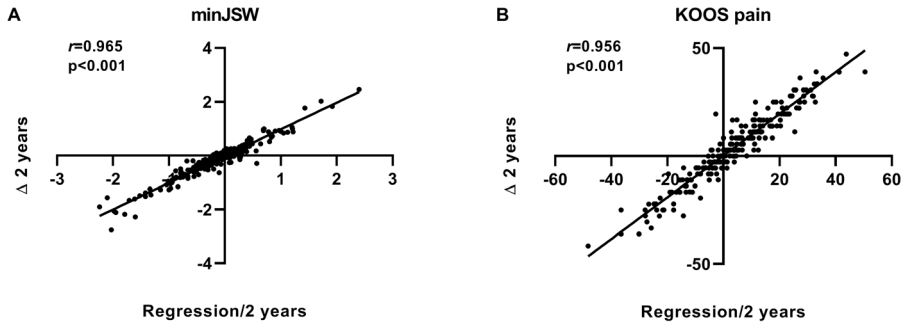
WOMAC pain[16] and the clinical study used KOOS pain, a sensitivity analysis was performed using the WOMAC pain scores deduced from the corresponding questions from the KOOS questionnaire as outcome.

Data acquisition

Radiographs and questionnaires were obtained at screening/baseline (BL) (M000), after 6±1 months (M006), after 12±2 months (M012), and after 24 -2/+6 months (M024). The larger time window for the M024 visit was allowed due to COVID-19 limitations. As the variation in scores due to variation in the actual timing of M000, M006, M012 and M024 were deemed negligible compared to other sources of variation (e.g. acquisition[17], pain perception) no corrections were made for this. All available KOOS pain data were used. For minJSW data, the change over time for all patients was visually checked by two observers as reference for quality of data acquisition; in case of doubtful data sets over time (e.g. unexpected variations or extreme changes over time), the radiographs from which the data were deduced were checked as time series per patient and individual patient-time point acquisitions were removed in case of incorrect image acquisition (n=18 images in total). Additionally, this check revealed one incorrect reading (n=1 image in total), that was removed. One patient was completely removed for analyses of structural progression because of doubtful image acquisitions at multiple time points.

Data is presented as a change score based on actual values (throughout the manuscript referred to as ΔminJSW/ΔKOOS pain; M024 minus M000) and as a change over two years based on a per patient fitted regression line over time using the observed BL, 6, 12 and 24 month values. Regression coefficients of these regression lines were multiplied by two to determine regression per two years (throughout the manuscript referred to as regression minJSW/regression KOOS pain). For ΔminJSW, only patients of which M000 and M024 minJSW data were present were included (n=224). For regression minJSW, only patients with minJSW available at no less than three of the four time points were included (n=266). Likewise, for ΔKOOS pain, only patients of which M000 and M024 KOOS pain data were present have been included (n=246). For regression KOOS pain, only patients with KOOS pain data available at M000, M024 and at least one other time point are included (n=246), since KOOS pain at M024 is included in the definition (different from the definition for radiographic progression). There was a good correlation between Δ and regression based change for both minJSW and KOOS pain, shown in fig. 1.

Figure 1. Correlations between absolute change and regression



Correlation between change in two years and regression over two years of (A) minJSW and (B) KOOS pain.

Statistical analysis

First, the number of actual observed radiographic progressors and pain progressors was determined for Δ and regression based change separately, and described using frequency and proportions. Secondly, the S progression scores of radiographic progressors and radiographic non-progressors, and P progression scores of pain progressors and pain non-progressors were compared using means and standard deviations (SD) and by plotting the histogram of the S and P scores of progressors and non-progressors. Differences in S and P progression scores between progressors and non-progressors were tested for statistical significance using Independent Samples T-tests. Thirdly, receiver operating characteristics (ROC) curves were constructed and the area under the ROC curves (AUC) were calculated to evaluate the discriminatory ability of the S and P progression score for radiographic and pain progression, respectively. Youden's indices (YI) were used to determine the optimal cut-offs for both progression scores. All analyses were performed using IBM SPSS Statistics version 26.0.0.1. P-values <0.05 were considered statistically significant.

Results

Observed numbers of radiographic and pain progressors in the IMI-APPROACH cohort

Table 1a shows the numbers of patients with ≥0.6 mm loss of minJSW during the 2-year follow-up period (radiographic progressors) and those with <0.6mm minJSW loss during the 2-year follow-up period (radiographic non-progressors).

Table 1a. Radiographic progressors

	Total	***Non-progressors***	***Progressors***
ΔminJSW	224	183 (81.7%)	41 (18.3%)
Regression minJSW	266	203 (76.3%)	63 (23.7%)

Radiographic progressors according to the definition described in the study protocol and above. The total cohort consisted of 297 patients; because of the COVID-19 pandemic a relatively large number of M024 visits were missed leaving 75% of patients for analyses using ΔminJSW and 90% for analyses using regression minJSW.
minJSW: minimum Joint Space Width.

Table 1b shows the numbers of patients fulfilling the criterion for *pain increase* (fast pain increase and significant pain increase combined) or *stable significant pain*, as well as both progression groups combined (pain progressors), and those not fulfilling any of the progression criteria (pain non-progressors).

Table 1b. Pain progressors

	Total	***Non-progressors***	***Progressors***		
				Pain increase	***Stable significant pain***
ΔKOOS pain	246	181 (73.5%)	65 (26.5%)	25 (38.5%)	40 (61.5%)
Regression KOOS pain	246	179 (72.7%)	67 (27.3%)	28 (41.8%)	39 (58.2%)

Pain progressors according to the definitions described in the study protocol and above. The total cohort consisted of 297 patients; because of the COVID-19 pandemic a relatively large number of M024 visits were missed leaving 83% of patients for analyses.
KOOS: Knee Injury and Osteoarthritis Outcome Score.

Table 1c combines the criteria for radiographic and pain progression to evaluate the final number of progressors within the IMI-APPROACH cohort.

Table 1c. Radiographic and/or pain progressors

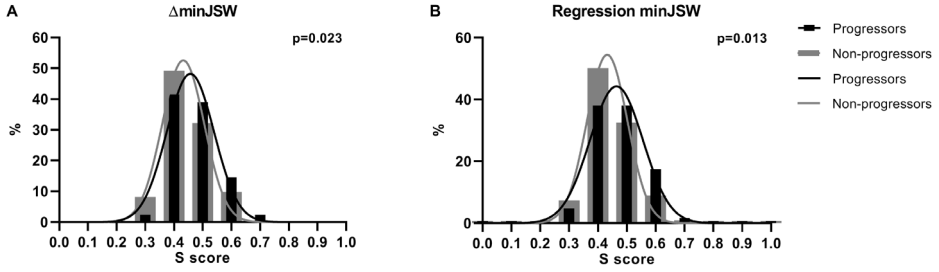
	Total	*Non-progressors*	*Radiographic progressors*	*Pain progressors*	*Radiographic + Pain progressors*
Δ	221	127 (57.5%)	31 (14.%)	54 (24.4%)	9 (4.1%)
Regression	242	130 (53.7%)	45 (18.6%)	57 (23.6%)	10 (4.1%)

Radiographic and/or pain progressors in the IMI-APPROACH cohort. The total cohort consisted of 297 patients; because of the COVID-19 pandemic a relatively large number of M024 visits were missed leaving 74% of patients for analyses using Δ and 81% for analyses using regression.

Differences in S/P progression score at baseline between radiographic/pain progressors and non-progressors after two years

The S progression scores of radiographic progressors and non-progressors are shown in figure 1. As anticipated, radiographic progressors were assigned on average a statistically significantly higher S progression score at inclusion, as compared to non-progressors (0.426±0.075 *vs* 0.396±0.076 for ΔminJSW, p=0.023; 0.427±0.085 *vs* 0.397±0.075 for regression minJSW, p=0.013).

Figure 1. S progression scores of radiographic progressors and non-progressors

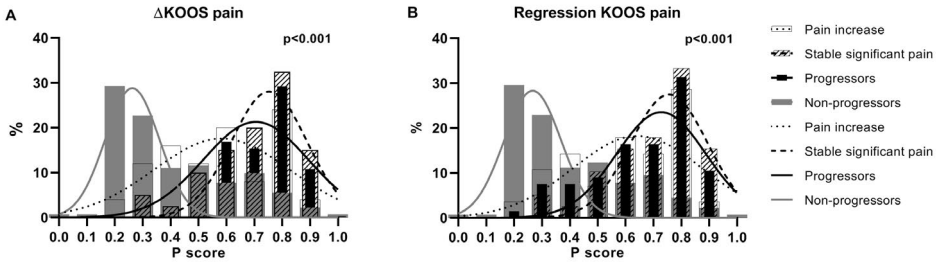


A. S progression scores for actual radiographic progressors (absolute decrease in two year ≥0.6mm, n=41) and non-progressors (n=183). B. S progression scores for actual radiographic progressors (regression of each patient ≥0.6mm/two year, n=63) and non-progressors (n=203).

The P progression scores of patients with *pain increase*, patients with *stable significant pain*, total progressors, and non-progressors are shown in figure 2. Mean P progression scores for pain progressors compared to pain non-progressors were 0.605±0.179 *vs* 0.359±0.201 for ΔKOOS pain and 0.612±0.175 *vs* 0.354±0.198 for regression KOOS pain, respectively (both p<0.001).

For patients with *pain increase* P progression scores were 0.529±0.197 for ΔKOOS pain and 0.555±0.192 for regression KOOS pain, respectively. For patients with *stable significant pain* mean P progression scores were 0.653±0.150 for ΔKOOS pain and 0.652±0.152 for regression KOOS pain, respectively (all p<0.001 as compared to non-progressors).

Figure 2. P progression scores of pain progressors and non-progressors

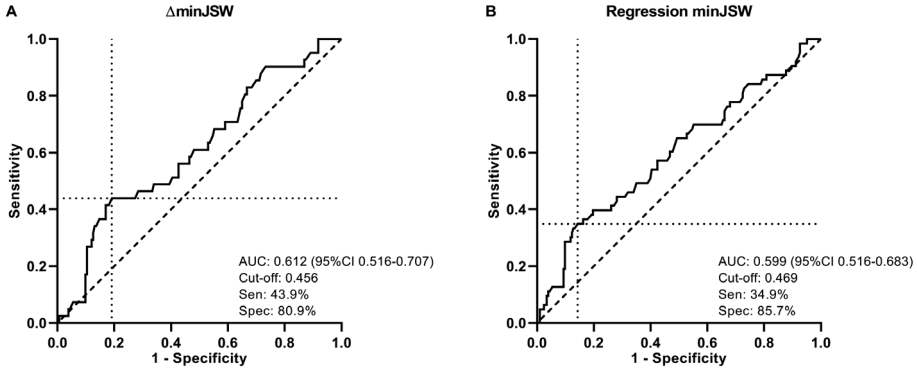


A. P progression scores for actual pain progressors (n=65; black) and non-progressors (n=181; grey), as well as for patients with pain increase (n=25; dotted) and patients with stable significant pain (n=40; dashed) using the absolute decrease during the 2-year follow-up period. B. P progression scores for actual pain progressors (n=67; black) and non-progressors (n=179; grey), as well as for patients with pain increase (n=28; dotted) and patients with stable significant pain (n=39; dashed) using the regression over two years of each individual patient.

Ability of S/P progression score to identify radiographic/pain progressors

ROC curves for the discrimination of progressors *vs* non-progressors by the S and P score are shown in figure 3 and 4 respectively. The AUC of 0.612 and 0.599 for ΔminJSW and regression minJSW, respectively, indicate that the S progression score is poorly able to distinguish radiographic progressors from radiographic non-progressors. At the optimal cut-off according to the YI the sensitivity and specificity were found to be 43.9% and 80.9% for ΔminJSW and 34.9% and 85.7% for regression minJSW, respectively.

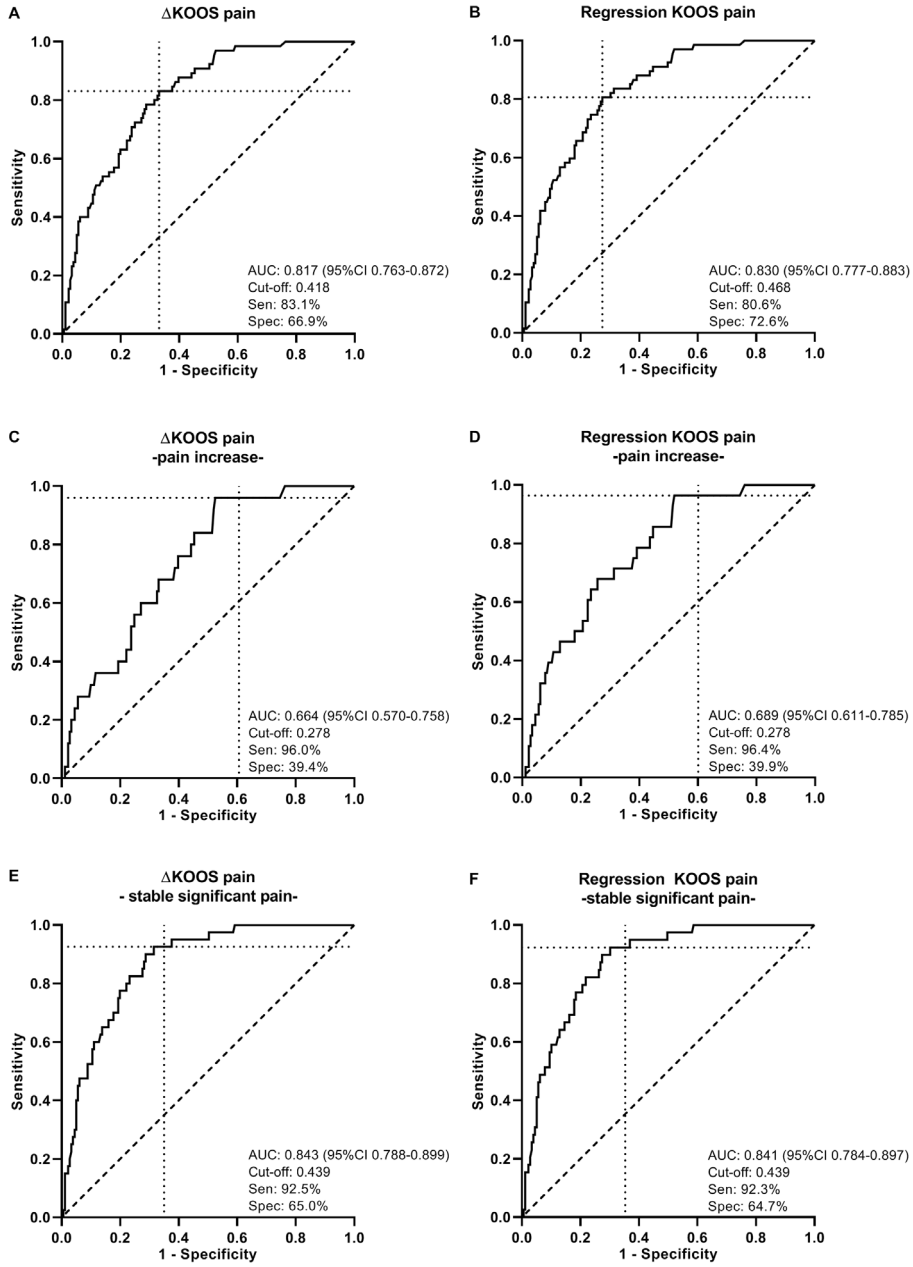
Figure 3. ROC-curves S progression score



ROC curves for ΔminJSW (A) and regression minJSW (B)
AUC: Area under the curve, Sen: Sensitivity, Spec: Specificity.

For the total group of pain progressors, AUC were 0.817 and 0.830 for ΔKOOS pain and regression KOOS pain, respectively, indicating that the P progression score is much better able to distinguish between pain progressors and pain non-progressors then the S progression score is for radiographic progressors and non-progressors. When separately analyzing patients with *pain increase* and patients with *stable significant pain*, AUC were 0.664 and 0.689 for patients with *pain increase*, and 0.843 and 0.841 for patients with *stable significant pain* for ΔKOOS pain and regression KOOS pain, respectively, indicating that the P progression score is better in identifying patients with *stable significant pain* compared to patients with *pain increase.* At the optimal cut-off according to the YI, sensitivity and specificity were found to be 83.1% and 66.9% for ΔKOOS pain, and 80.6% and 72.6% for regression KOOS pain, respectively.

Figure 4. ROC-curves P progression score



ROC curves for ΔKOOS pain and regression KOOS pain for total progressors (A+B), patients with pain increase (C+D), and patients with stable significant pain (E+F)

AUC: Area under the curve, Sen: Sensitivity, Spec: Specificity

YI were used to determine the optimal cut-off points of both progression scores, equally weighing false positives and false negatives, thereby giving the optimal combination of sensitivity and specificity. Table 2 (S score) and 3a-c (P score) provide alternative cut-off points, with corresponding sensitivity and specificity.

Table 2. Possible cut-off points for the S progression score

S score	***ΔminJSW***		***Regression minJSW***	
Cut-off	***Sensitivity***	***Specificity***	***Sensitivity***	***Specificity***
0.318	95.1%	10.9%	90.5%	10.3%
0.346	90.2%	24.6%	84.1%	23.2%
0.361	80.5%	33.9%	76.2%	33.0%
0.374	68.3%	44.8%	69.8%	41.9%
0.391	58.5%	53.0%	60.3%	52.2%
0.407	48.8%	62.3%	49.2%	61.1%
0.432	43.9%	72.7%	41.3%	72.9%
0.462	36.3%	83.1%	34.9%	83.7%
0.520	7.3%	90.7%	12.7%	91.1%

Cut-offs are based on percentile of the progression score. With a cut-off of 0.318 (10th percentile) 90% will be classified as progressor. With a cut-off of 0.520 (90th percentile) 10% will be classified as progressor etc.
minJSW: Minimum Joint Space Width

Table 3a. Possible cut-off points for the P progression score

P score	***ΔKOOS pain***		***Regression KOOS pain***	
Cut-off	***Sensitivity***	***Specificity***	***Sensitivity***	***Specificity***
0.158	100.0%	14.9%	100.0%	15.1%
0.198	98.5%	28.7%	98.5%	29.1%
0.240	96.9%	41.4%	97.0%	41.9%
0.300	90.8%	51.9%	91.0%	52.5%
0.407	83.1%	63.5%	83.6%	64.2%
0.492	73.8%	72.9%	76.1%	74.3%
0.580	56.9%	80.7%	59.7%	82.1%
0.663	49.2%	89.0%	50.7%	89.9%
0.742	26.2%	95.6%	25.4%	95.5%

Cut-offs are based on percentile of the progression score. With a cut-off of 0.158 (10th percentile) 90% will be classified as progressor. With a cut-off of 0.742 (90th percentile) 10% will be classified as progressor etc.
KOOS: Knee Injury and Osteoarthritis Outcome Score.

Table 3b. Possible cut-off points for the P progression score in patients with *pain increase*

P score	*ΔKOOS pain*		*Regression KOOS pain*	
Cut-off	*Sensitivity*	*Specificity*	*Sensitivity*	*Specificity*
0.158	100.0%	12.2%	100.0%	12.4%
0.198	96.0%	23.5%	96.4%	23.9%
0.240	96.0%	34.4%	96.4%	34.9%
0.300	84.0%	43.4%	85.7%	44.0%
0.407	68.0%	53.4%	71.4%	57.8%
0.492	60.0%	62.9%	67.9%	64.2%
0.580	36.0%	71.5%	46.4%	72.9%
0.663	32.0%	80.1%	39.3%	81.2%
0.742	24.0%	91.4%	21.4%	91.3%

Cut-offs are based on percentile of the progression score. With a cut-off of 0.158 (10th percentile) 90% will be classified as progressor. With a cut-off of 0.742 (90th percentile) 10% will be classified as progressor etc.
KOOS: Knee Injury and Osteoarthritis Outcome Score.

Table 3c. Possible cut-off points for the P progression score in patients with *stable significant pain*

P score	*ΔKOOS pain*		*Regression KOOS pain*	
Cut-off	*Sensitivity*	*Specificity*	*Sensitivity*	*Specificity*
0.158	100.0%	13.1%	100.0%	13.0%
0.198	100.0%	25.7%	100.0%	25.6%
0.240	97.5%	36.9%	97.4%	37.2%
0.300	95.0%	47.6%	94.9%	47.3%
0.407	92.5%	59.7%	92.3%	59.4%
0.492	82.5%	68.9%	82.1%	68.6%
0.580	70.0%	78.6%	69.2%	78.3%
0.663	60.0%	86.4%	59.0%	86.0%
0.742	27.5%	93.2%	28.2%	93.2%

Cut-offs are based on percentile of the progression score. With a cut-off of 0.158 (10th percentile) 90% will be classified as progressor. With a cut-off of 0.742 (90th percentile) 10% will be classified as progressor etc.
KOOS: Knee Injury and Osteoarthritis Outcome Score.

Discussion

Radiographic progressors in the IMI-APPROACH cohort showed statistically significantly higher S progression scores at inclusion, compared to radiographic non-progressors. Likewise, pain progressors showed statistically significantly higher P progression scores at inclusion compared to pain non-progressors, as did the two separate categories for pain progression; patients with pain increase and patients with stable significant pain. Nevertheless, the AUC for the S progression score was poor, showing that this score may not sufficiently predict

radiographic progression or non-progression. In contrast, the P progression score was found to be able to predict pain progression or non-progression.

For the selection procedure used in the IMI-APPROACH cohort, both progression scores were combined into one ranking score to include patients of both progression types. As such, the present study is not a validation of the original ranking. However, for clinical trials one might like to select mainly radiographic progressors (those with high S progression scores), or mainly pain progressors (those with high P progression scores). Therefore, in this study the value of the separate progression scores was evaluated, instead of using this combined (ranking) score.

The S and P progression scores are continuous variables, but cut-offs can be chosen to use for the selection of patients (e.g. in clinical trials). In this study, YI were used to determine the optimal cut-off points of both progression scores, equally weighing false positives and false negatives, thereby giving the optimal combination of sensitivity and specificity. The high specificity found for the S progression score means that most of the radiographic non-progressors indeed were assigned a S progression score below the cut-off value. However, the low sensitivity indicates that a minority of the radiographic progressors were assigned a S progression score above the cut-off value and the majority will be missed.

The S progression score showed high specificity, but low sensitivity for radiographic progression. The P progression score showed high sensitivity and specificity for pain progression. Nonetheless, for the identification of patients with *pain increase* specificity is low (<40%). As demonstrated by tables 2 and 3a-c, alternative cut-off points can be chosen to increase sensitivity or specificity (always at the expense of the other one), and with that increase the usefulness of both progression scores to select patients for clinical trials, depending on the goal of the study. We feel that this approach is generalizable as the patients were selected from multiple different European OA cohorts. Unfortunately, samples were too small to analyze the selection from each of these cohorts separately.

The sensitivity analysis using WOMAC pain score instead of KOOS pain score revealed that the number of total progressors remained approximately the same (70 *vs* 65 for Δ, and 64 *vs* 67 for regression). However, when using KOOS pain the majority of the pain progressors showed *stable significant pain*. In contrast, when using WOMAC pain the majority of the pain progressors showed *pain increase.* The explanation most likely lies in the number of questions used for the pain score. WOMAC pain is constructed out of five questions[16], while KOOS pain is constructed out of nine questions[15]. As a result, a higher score on one question will have more weight in the WOMAC pain score compared to the KOOS pain score, and with that a patient is more likely to fulfil the *pain increase* criterion. The total number of pain progressors (not separated in *pain increase* or *stable significant pain*) remains the same. AUC for pain progressors based on WOMAC pain scores were comparable to AUC for pain progressors based on KOOS pain scores (0.821 for ΔWOMAC pain *vs* 0.817 for ΔKOOS

pain and 0.817 for regression WOMAC pain *vs* 0.830 for regression KOOS pain). For patients with *pain increase* AUC were slightly better for WOMAC pain based progression (0.756 for ΔWOMAC pain and 0.731 for regression WOMAC pain) compared to KOOS pain based progression (AUC 0.664 for ΔKOOS pain and 0.689 for regression KOOS pain). For patients with *stable significant pain* AUC were slightly worse for WOMAC pain based progression compared to KOOS pain based progression (AUC were 0.790 and 0.823 for ΔWOMAC pain and regression WOMAC pain and 0.843 and 0.841 for ΔKOOS pain and regression KOOS pain, respectively).

The ML models constructing a S and P progression score for each individual, used for the initial selection of IMI-APPROACH patients, were trained on historical data from the Cohort Hip & Cohort Knee (CHECK)[3]. ~50% of IMI-APPROACH were recruited from the original CHECK cohort. For that reason, we also performed analyses including only patients recruited from CHECK (n=153) or excluding patients recruited from CHECK (n=144) to evaluate whether this influences (improves) our results. Using only patients recruited from CHECK, the portion of non-progressors was slightly higher (66.4% for Δ and 61.5% for regression) compared to the full IMI-APPROACH cohort (57.5% for Δ and 53.7% for regression). Excluding patients recruited from CHECK led to 46.5% non-progressors for Δ and 44.6% non-progressors for regression. For both groups, the ROC curves were comparable to the ROC curves including all IMI-APPROACH participants. AUC for ΔminJSW was 0.612 for all IMI-APPROACH patients, 0.583 when including only patients recruited from CHECK, and 0.640 when excluding patients from CHECK. For regression minJSW AUC was 0.599 for IMI-APPROACH, 0.612 for CHECK, and 0.592 when excluding CHECK patients, respectively. AUC for ΔKOOS pain was 0.817 for all IMI-APPROACH patients, 0.818 when including only patients recruited from CHECK, and 0.783 when excluding patients recruited from CHECK, and for regression KOOS pain AUC were 0.830, 0.828, and 0.801 for IMI-APPROACH, including only patients recruited from CHECK, and excluding CHECK patients, respectively. This indicates that the ML models, built on historical data of CHECK can be used for other OA cohorts as well.

Unanticipated, 98 (33%) of the patients had a minJSW <2mm at M000, although this was described as an exclusion criterion in the study protocol[8]. To evaluate the influence of these 98 patients on the found results, we evaluated if these patients are indeed included in the radiographic non-progression group (since they may be restricted to fulfill the criterion for radiographic progression). The proportion of progressors and non-progressors only slightly differed after excluding these patients, indicating that the inclusion of these patients did not influence the final results of the main analyses. The ROC curves excluding patients with a minJSW at baseline <2mm are comparable to the ROC curves including all IMI-APPROACH participants (AUC 0.586 *vs* 0.612 for ΔminJSW, 0.610 *vs* 0.599 for regression minJSW, 0.840 *vs* 0.817 for ΔKOOS pain, 0.846 *vs* 0.830 for regression KOOS pain).

The recruitment procedure of IMI-APPROACH used a multi-step approach with the aim to decrease the number of included patients in the cohort that show neither radiographic, nor pain progression[18]. In an uninformed selection, not using this multi-step selection procedure, 61% was non-progressor. Evaluating the actual progression indicated that in the final selection, the observed number of non-progressors was 57.5% (for absolute progression) or 53.7% (for regression). So, the IMI-APPROACH selection process, based on ML models, indeed enriched the selection with OA progressors. Note in this respect that ~25% was deselected from inclusion in IMI-APPROACH based on the ML models.

In conclusion, the S and P progression scores as provided by the ML models developed and used for the selection of IMI-APPROACH patients were to some degree able to distinguish between progressors and non-progressors. Nevertheless, additional data should be used to adjust the models to improve the accuracy of the S and P progression scores so that, in future trials, the use of ML models might improve patient selection by increasing the number of radiographic and/or pain progressors. Depending on the goal of the trial and the nature of the study intervention, one can use a S or P progression score (or both) and adjust the cut-off point to select the most appropriate study population.

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12

13 Summary and general discussion

Summary

Part I: Current (pre-) clinical approach
An ideal disease modifying osteoarthritis drug (DMOAD) combines chondroprotective, anti-inflammatory and analgesic effects, all in one molecule. So far, no drugs are approved for this indication, although multiple drugs show promising results.

In **chapter 2** the DMOAD activity of celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor was evaluated. COX-2 has an important role in pain and inflammation. 172 patients waiting for a total knee arthroplasty (TKA) were assigned to four treatment groups (celecoxib 2dd200mg, celecoxib 2dd200 mg stopped three days before surgery, naproxen 3dd250mg, or no treatment) and treated for four weeks (until surgery). Effects on cartilage, synovial inflammation and clinical symptoms were evaluated. Celecoxib indeed decreased the amount of COX-2 in cartilage and in synovial tissue. However, no statistically significant effects were found on proteoglycan metabolism (a measure for cartilage metabolism), synovial inflammation, or WOMAC pain, function and stiffness scores. In **chapter 3**, cartilage and synovial tissue of twenty knee OA patients were collected during TKA surgery. A negative correlation was found between expression of the inflammatory cytokine granulocyte macrophage colony-stimulating factor (GM-CSF) in synovial tissue and reported knee pain in knee OA patients. As such, both studies could not support formerly reported results, where celecoxib did show DMOAD activity *in vitro*[1] and anti GM-CSF treatment showed analgesic effects in hand OA[2].

A fusion protein of two anti-inflammatory cytokines, interleukin-4 (IL-4) and IL-10, the IL4-10 fusion protein (FP) has been shown to have all three modalities of an ideal DMOAD[3-5]. To provide further insight in the interplay between cartilage damage, synovial inflammation, and pain, and to better understand the pathophysiological background behind the therapeutic effects of IL4-10 FP, in **chapter 4** a systematic review was conducted to describe the chondroprotective, anti-inflammatory, and analgesic effects of IL-4, IL-10, and the IL4-10 FP. The effects on cartilage are mainly positive for the separate cytokines, as well as for the IL4-10 FP. Although IL-4 and IL-10 are known as anti-inflammatory cytokines, both cytokines show pro-inflammatory effects as well. Nevertheless, for IL4-10 FP only anti-inflammatory results were found, indicating that possible pro-inflammatory effects of IL-10 can be reversed by IL-4 and the other way around. A limited number of studies reported on the analgesic effects of the separate cytokines or the IL4-10 FP but the results were promising at the level of pain mediators, as well as clinical pain.

Despite the promising results of IL4-10 FP on cartilage damage, synovial inflammation, and pain (the three pillars of a successful DMOAD), until now these three effects have never been studied all together in one OA model. Therefore, in **chapter 5** the DMOAD activity of a canine version of the IL4-10 FP was evaluated in the canine Groove model[6-8]. *In vitro*

this canine IL4-10 FP showed anti-inflammatory and chondroprotective effects on canine joint tissues. In the subsequent *in vivo* study, dogs were treated for ten weeks with weekly intra-articular injections with canine IL4-10 FP. During this period, Force Plate Analysis was used to determine joint loading as proxy measure for joint pain. After ten weeks, dogs were euthanized and cartilage and synovial tissue were collected to evaluate cartilage damage and synovial inflammation. *In vivo,* the canine IL4-10 FP showed analgesic and chondroprotective effects. The mild synovial inflammation in the model prevented conclusive evaluation of the third pillar, anti-inflammation. However, *in vitro* the anti-inflammatory activity was confirmed, so IL4-10 FP seems to fulfill all criteria for a successful DMOAD.

To better match the clinical population, where OA is more age-related and associated with obesity, in **chapter 6** the rat Groove model was combined with a high-fat diet to induce metabolic dysregulation[9,10]. Twenty rats were randomly assigned to intra-articular treatment with ten weekly injections of rat IL4-10 FP or Phosphate Buffered Saline. Again, cartilage damage, synovial inflammation and joint pain were evaluated. The analgesic and chondroprotective effects found in the canine study were confirmed in this rat study. Unfortunately, synovial inflammation was again not severe enough to properly evaluate the anti-inflammatory effects of IL4-10 FP.

Part II: First steps towards a new approach

To increase the chance for success in future OA trials a new approach is needed. A first step is to predict OA progression and subsequently define multiple OA phenotypes, with different disease characteristics. In **chapter 7** a new clinical OA cohort was described: APPROACH. This 2-year study included 297 knee OA patients, (pre-)selected from existing cohorts using machine learning (ML) models to display a high likelihood to show joint space width (JSW) loss and/or pain progression during the follow-up period. The study used multiple conventional and new markers to describe their disease process, leading to a large international database which can be used in the search for OA phenotypes.

When conducting a trial to evaluate whether a new treatment is able to slow down, or ideally stop the OA progression, you want to include patients who would have had progressed within the trial duration. All APPROACH patients were assigned a S progression score, reflecting the likelihood of showing radiographic progression, and a P progression score, reflecting the likelihood of showing pain progression. **Chapter 8** described the baseline characteristics (demographics, radiographic parameters and pain) of the APPROACH patients, in relation to their assigned S and P progression score. It turned out that patients with a high S progression score (e.g. a high likelihood of showing radiographic progression) have a larger minimum Joint Space Width (minJSW) at inclusion compared to patients with low S progression score, and with that a larger window to indeed show radiographic progression (i.e. reduction of minJSW) during the study period. On the other hand, patients with high P progression

scores already reported more pain compared to patients with low P progression scores, and with that have a small window to show pain progression.

In all probability, OA patients with not only nociceptive pain, but a more systemically induced pain as well, could be recognized as one or more distinct OA phenotype(s). **Chapter 9** studied one of these possible OA phenotypes: patients with a likely neuropathic pain (NP) component, associated with nerve damage. 24 patients with a likely NP component (painDETECT score ≥19) were matched on a 1:2 ratio to 48 patients without a likely NP component (painDETECT score ≤12). Pain, physical function and radiographic damage were compared between groups. Patients with a likely NP component, show less radiographic damage in their index knee but more physical limitations compared to patients without a likely NP component. This might be due to the presence of OA in other joints. Indeed, patients with a likely NP component reported more pain in other joints. However, radiographic damage in these joints did not differ from radiographic damage in patients without a likely NP component. Taken together, these results suggest that in patients with a likely NP component, pain and physical limitations are not a direct result of local joint damage and will most likely not respond to conventional OA treatments. In the future, these patients should be identified and offered a more personalized treatment for the NP component. For the same reason, these patients should be identified and excluded from DMOAD trials.

One of the new OA markers, used within the APPROACH clinical study is motion analysis using the GaitSmart® system. In **chapter 10** the relationship between GaitSmart® and radiographic OA was studied. As a first step, principal component analysis (PCA) was used to explore structure in relationships between multiple GaitSmart® parameters alone, and in addition to radiographic parameters and patient reported outcome measures (PROMs). It was found that GaitSmart® analysis provides additional information next to radiography (joint damage) and PROMs (clinical symptoms). Subsequently, the relationship between GaitSmart® and the presence and severity of radiographic OA was determined. A small relationship existed between GaitSmart® outcome parameters and radiographic damage, however on an individual patient level this is of limited value.

The relationship between GaitSmart® and physical function is somewhat better, as shown in **chapter 11**. Physical function comprises two aspects, objective function (the ability to perform a certain task, measured in an optical gait lab or with performance-based tests), and subjective function (the experience while performing a certain task, measured using PROMs). In this study, the relationship between GaitSmart® parameters and different aspects of physical function was evaluated. In addition, the ability of GaitSmart® to detect short-term changes in physical function was studied. It was demonstrated that GaitSmart® measures both aspects of physical function, and was able to detect short-term changes in objective function.

Finally, the actual two-year progression of the APPROACH patients was evaluated in **chapter 12**. Structural progression was defined as >0.6mm JSW loss in two years. Pain progression was defined as *fast pain increase:* Knee Injury and Osteoarthritis Outcome Score (KOOS) pain decrease between baseline and follow-up ≥10 points per year (i.e. ≥20 points decrease in the 2-year follow-up period) and final KOOS pain score ≤65 points, s*ignificant pain increase:* KOOS pain decrease between baseline and follow-up ≥5 points per year (i.e. ≥10 points decrease in the 2-year follow-up period) and final KOOS pain score ≤60 points, or s*table significant pain:* KOOS pain score ≤60 points during the whole study period. The observed progression was evaluated in relation to the originally assigned S progression score and P progression score. Patients who fulfilled the criterion for structural progression were assigned higher S progression scores at baseline. Likewise, patients who fulfilled one of the criteria for pain progression were assigned higher P progression scores at baseline. Nevertheless, the area under the curve (AUC) for the S progression score was poor, showing that this score may not sufficiently distinguish between radiographic progressors and non-progressors. In contrast, the P progression score was found to be able to distinguish between pain progressors and non-progressors.

General discussion

The work described in this thesis seems to comprise two independent subjects. However, when placing it in perspective, the two are more intertwingled than at first glance appears.

OA pain is not (simply) the result of local joint damage

The idea of OA being a disease of the whole joint in contrast to only cartilage degeneration is more and more appreciated[11]. The results described in this thesis might even take things a step further, implying OA pain is not simply the result of local joint damage, but involves systemically/centrally induced pathology as well.

The use of NSAIDs or selective COX-2 inhibitors is recommended as the first pharmacological step in OA pain management[12-14]. However, confirmed reduction of intra-articular COX-2 after oral celecoxib treatment did on average not result in clinical benefits **(chapter 2)**. A possible explanation for the lack of an effect is the inclusion of patients on the waiting list for a total knee arthroplasty (TKA), considered having end-stage knee OA. This may be a phase of the disease in which a disease modifying effect by a drug is not optimal anymore. Although treatment duration was short, a local reduction of COX-2 was found, indicating bioavailability. This corroborates the, in a previous conducted pilot study found beneficial effects after four weeks of treatment[1]. The lack of a clear analgesic effect, despite local COX-2 inhibition, might result from a more systemically induced pain component, insufficiently sensitive to the drug (e.g. a neuropathic pain component).

In the study described in **chapter 3**, a negative correlation was found between local expression of GM-CSF and clinical OA pain. This was not directly anticipated, since previous studies showed that monoclonal antibodies (mAb) against GM-CSF suppressed pain in a collagen-induced OA mice model[15], and anti-GM-CSF treatment reduced pain in inflammatory hand OA[2]. These results might also be explained by the centrally/systemically mediated pain mechanism present in OA. This is supported by the observation that intrathecally administered mAb against GM-CSF reduced pain in a mouse model for neuropathic pain[16].

In **chapter 5 and 6** analgesic effects were found after local (intra-articular) treatment with IL4-10 FP. It was previously shown that in a mice model where carrageen was injected in the hind paw to induce persistent inflammatory pain intrathecally injected IL4-10 FP was analgesic as well[17]. This again indicates that pain is not only the result of local joint pathology but also the result of systemic pathology.

In **chapter 9** (part II of this thesis) we identified an OA phenotype with a likely NP component. Compared to OA patients without this likely NP component, these patients show less radiographic damage in their index knee, but more functional limitations. Besides, they report more pain in other joints than their index knee, while the OA grade of other joints determined on CT did not differ from those of OA patients without a likely neuropathic pain component. Clearly, a more central/systemic pain component is dominating in this specific subgroup of OA patients.

Together, these results indicate that OA pain is heterogeneous and complex, varying between individuals, and not always simply related to joint tissue damage. Next to joint nociception, peripheral and central sensitization play a role as well[18,19]. These individual differences between OA patients should be considered when treating OA pain, and with that a more personalized approach is warranted in future OA research.

Further development of IL4-10 FP as disease modifying osteoarthritis drug

Taking the results described in this thesis and previous studies together, the IL4-10 FP seems to fulfill all requirements for a potential DMOAD. However, additional steps need to be made to enable this novel molecule to enter the market as a DMOAD.

In vitro, IL4-10 FP had anti-inflammatory effects on OA cartilage and synovial tissue[5], but also in case of blood-induced cartilage damage (as in hemophilic arthropathy)[3,4], which in general shows more inflammation compared to OA degenerative cartilage. In the canine and rat OA Groove model used in **chapter 5 and 6** only limited inflammation was present, which is in line with the original intention for designing these models[6,9] but at the same time hinders proper evaluation of anti-inflammatory effects of IL4-10 FP in these models. Consequently, there is still a need to explore chondroprotective and analgesic effects in an *in vivo* OA model with evident inflammation. Moreover, future studies should be sufficiently powered, a limitation of all presently described pre-clinical studies.

Skipping a few steps in the development towards an FDA/EMA approved drug, looking into the future, the clinical application of the IL4-10 FP entails some challenges. Although the IL4-10 FP also acts directly on cartilage, and with that is expected to be effective in multiple OA phenotypes, it seems obvious for future trials to focus on an inflammatory OA phenotype. Previous DMOAD trials have focused on inflammatory OA, which responds better to DMOAD treatment compared to other phenotypes[20]. Since the IL4-10 FP is composed of two inflammation controlling cytokines, an inflammatory OA phenotype seems to be the most relevant candidate for IL4-10 FP treatment in clinical trials. In line with this, for future animal studies, a model representing inflammatory OA should be evaluated next to the surgically induced OA we used, mimicking a more post-traumatic degenerative OA[20]. Identification of different OA phenotypes is therefore key to design proper personalized (pre-)clinical trials.

Another issue which needs to be resolved for clinical use is the rapid clearance from the joint cavity. In a clinical setting, weekly intra-articular injection is not feasible for years of treatment. Increasing duration of action after intra-articular injection is key for future research. Hydrogels, liposomes, nanoparticles and microparticles have all been tested for this purpose[21]. Intra-articular delivery of celecoxib using a hydrogel showed that after four weeks celecoxib could be detected in synovial fluid, but still more than 90% was cleared by day 7[22]. Liposomes can provide controlled release of drugs, but have limited long-term stability, especially in OA joints[21]. Nanoparticles can be retained in the joint for a few weeks, and microparticles can still be found a few months after intra-articular injection[23]. Ideally, the release of intra-articular treatment from one of these delivery systems, should be controlled by disease activity, where more severe disease leads to higher levels of treatment released from the delivery system. A recent study developed microspheres releasing IL-4 or IL-10 in response to collagenase activity (an active component in cartilage degeneration). The collagenase-mediated degradation of microspheres led to controlled release of IL-4 or IL-10, increasing their half-life and reducing their wash-out during periods with low disease activity[24]. Nevertheless, future research on usable drug delivery vehicles, especially in the clinical setting is still needed[21].

Lastly, intra-articular injections are not usable in case of OA in smaller joints and/or polyarthritis. For these phenotypes, systemic applications should be a topic of feature research. In this case, finding balance between sufficient local bioavailability and systemic overload (toxicity) will become key.

As such, further development of IL4-10 FP as a DMOAD should consider different OA phenotypes (e.g. inflammatory *vs* post-traumatic, locally *vs* systemically induced pain, and monoarthritis *vs* polyarthritis), enabling a more personalized approach for these specific phenotypes, and with that a more successful marketing of this potential new DMOAD. To achieve this, more different pre-clinical models are needed. Nevertheless, to

demonstrate short-term analgesic, chondroprotective, and anti-inflammatory properties in a fist-man-study, the first step could be a simple proof of concept study as used for celecoxib in **chapter 2.** This set-up provides the opportunity to combine *in vivo* evaluation of analgesic effects in humans, and *ex vivo* analysis of cartilage and synovial tissue/fluid after *in vivo* drug administration. If such a study shows promising results in a heterogeneous group of OA patients, the next step should be to evaluate efficacy in the most relevant OA phenotype.

APPROACH cohort

The final goal of the APPROACH study **(chapter 7)** is to predict disease progression to improve clinical OA trial design and describe different OA phenotypes as a first step to a more personalized approach, of which the relevance is obvious.

The cohort includes 297 patients with a high likelihood for structural and/or pain progression, followed for two years, providing a valuable database for future research **(chapter 7)**. The initial combination of a high minimum JSW (limited structural tissue damage) and high pain scores provides an optimal patient selection for treatment modalities that decrease pain and prevent, stop, or slow-down structurally progression **(chapter 8)**.

A high S score was assigned to those with a high likelihood to show structural (tissue damage) progression. Unfortunately, the S progression score was poorly able to distinguish structural progressors from structural non-progressors (AUC 0.612). In contrast, the P progression score (designated to those with a high likelihood to show pain progression or stable significant pain) can to a certain extend distinguish pain progressors from pain non-progressors (AUC 0.817). However, there is a clear difference between patients with *pain increase* (AUC 0.664) and patients with *stable significant pain* (0.843) **(chapter 12)**. This indicates that these two groups of 'pain progressors' might be considered as separate OA pain phenotypes, each needing their own personalized approach. Treatment of patients with a high likelihood for pain progression should be focused on preventing further pain development, while in patients with stable significant pain treatment should be focused on reducing pain or coping with chronic pain. Specially in OA patients with stable significant pain the presence of a neuropathic pain component should be considered. Of the nineteen patients with a neuropathic pain component **(chapter 9)** that had a KOOS pain score at baseline and after two years, ten patients showed stable significant pain while only two showed pain increase.

Despite the unicity of the APPROACH cohort, one should consider its representability for the general world-wide OA population. 68% (203 out of 297) of the patients included in this international study were included in the Netherlands. We found important differences in baseline demographic and clinical characteristics between patients in the different centers **(chapter 7)**, indicating that the findings in one country are not always directly translatable to other countries. However, these differences may also be explained by the

different in- and exclusion criteria of the existing cohorts patients belonged to. In addition, the APPROACH cohort includes patients with a high likelihood for structural and/or pain progression, while this is not the case in the general OA population.

55% (133 out of 242) of the patients that finished the study were included in Utrecht. Utrecht selected their patients from CHECK, a knee and hip OA study which started in 2002. The fast progressors of CHECK (showing progression within two years, the follow-up period of the APPROACH cohort study) most likely had a joint arthroplasty before they could be selected for the APPROACH cohort. One can question whether the CKECK patients who were eligible for APPROACH after more than ten years of follow-up (those who did not or hardly progress during CHECK), are still representative for the general OA population, and more specifically for the fast progressing OA population as was the quest in the APPROACH study. According to the idea of inertia, knees who were stable before (patients from CHECK) are more likely to remain unchanged[25], suggesting that the actual progression of CHECK patients within the APPROACH cohort might be limited. Nevertheless, a sensitivity analysis described in **chapter 12**, using only patients included from CHECK showed that relatively more patients fulfilled the criterion for structural progression (19.4%), compared to either those included from the other cohorts(17.0%), or to the whole APPROACH cohort (18.3%).

Compared to CHECK, APPROACH uses more sophisticated disease markers (e.g. different MRI sequences, HandScan, GaitSmart®, multiple biochemical markers at several different omic-platforms) in addition to the conventional questionnaires, clinical assessment and conventional radiographs used in CHECK[26]. So, irrespective of all limitations, the APPROACH study enables further detailed evaluation of different OA (progression) phenotypes, which is essential for the design of successful clinical trials, leading to a more personalized approach for specific OA phenotypes.

GaitSmart® motion analysis as OA parameter

Multiple studies describe an association between joint loading and OA progression[27-29]. Such studies use 3D gait analysis in an optical gait lab, which is not easily applicable in large clinical trials or everyday clinical practice. In APPROACH, the GaitSmart® motion analysis system was used to evaluate gait characteristics. In this thesis a first attempt was made to evaluate the use of GaitSmart® within the OA field.

Importantly, we were able to demonstrate that GaitSmart® gives additional information about OA disease status, next to radiographic parameters and PROMs **(chapter 10)**. It improved the prediction of having radiographic OA (KL grade≥2) in at least one knee, but the discriminatory value is still limited on individual patient level **(chapter 10)**. The strength of GaitSmart® lies in its easy use compared to analysis in an optical gait lab. It can be used anywhere and takes about 10-15 minutes. Moreover, we could demonstrate that GaitSmart® combines evaluation of self-reported and performance-based function, with

the ability to detect short-term changes in performance-based function **(chapter 11)**. As such, this motion analysis system adds to the selection of specific phenotypes and potential more personalized therapy.

Despite its limited value in the diagnosis of radiographic OA, multiple applications of GaitSmart® during the OA disease process could be considered and should be explored in the future. Starting early in the OA process, with the prevention of disease progression, patients with mild OA adopt a strategy of gait compensation, lowering the load on the affected compartment, reducing the risk for progression. In contrast, patients with more severe OA are unable to lower the load on the affected compartment, increasing the risk of disease progression[30]. GaitSmart® might be used to detect altered joint biomechanics in early OA, and as a guide to adapt the gait pattern, assisting this natural compensation strategy and thereby possibly slow down disease progression and postpone surgery. Recently, it was demonstrated that gait risk factors for disease progression differ between non-traumatic and post-traumatic OA[31,32], and even between different types of post-traumatic ankle OA[33]. So, as for testing possible DMOADs like the IL4-10 FP, pain medication like anti-GM-CSF therapy, or COX-2 inhibitors, for studies evaluating interventions that adjust gait and loading (e.g. gait adaptation or tibial osteotomy) it is also important to consider the differences between multiple OA phenotypes. Using motion analysis like the GaitSmart® system to distinguish different OA phenotypes, and assess and adapt someone's gait is a textbook example of a personalized approach in OA patients. Evaluating the follow-up data of the APPROACH study will be of relevance to determine how GaitSmart® motion analysis could optimally serve as a non-invasive and easily applicable parameter to assess gait in knee OA patients.

In conclusion, the work described in this thesis contributes to the search for a successful DMOAD. In contrast to celecoxib, IL4-10 FP showed the characteristics that an ideal DMOAD should comprise. Future studies should focus on identification of OA phenotypes as guide for a more personalized approach, increasing the chances for successful clinical trials. The APPROACH database will be of direct help to identify and describe multiple OA phenotypes that may benefit from personalized treatments, ideally combining analgesic, chondroprotective, and anti-inflammatory effects.

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ADDENDUM

Addendum

Introductie

Artrose is een degeneratieve gewrichtsaandoening die leidt tot pijn, stijfheid en beperkte functie. Ongeveer 10% van de mensen in de westerse wereld lijdt aan artrose en daarmee is de wereldwijde ziektelast van artrose groot. Daarbij zal deze alleen nog maar toenemen door het ouder en zwaarder worden van de populatie, twee belangrijke risicofatoren voor het ontwikkelen van artrose. Ondanks de grote ziektelast en kosten die dit met zich meebrengt, is er nog steeds geen geneesmiddel beschikbaar. De huidige behandeling is voornamelijk symptomatisch en bestaat uit pijnstilling, fysiotherapie en uiteindelijk een (gewrichtsvervangende) operatie.

Met het toenemen van de ziektelast neemt ook de behoefte toe aan behandelingen die artrose genezen. Waar artrose voorheen vooral gezien werd als slijtage van kraakbeen, wordt er nu steeds meer naar gekeken als een ziekte waarbij verschillende weefsels van een gewricht aangedaan zijn en waarbij ontsteking een belangrijke rol speelt. Idealiter geeft een artrosebehandeling niet alleen pijnverlichting, maar zorgt dezelfde behandeling ook voor vertraging of zelfs omkering van de kraakbeenschade en vermindering van ontsteking. Er wordt al enige tijd gewerkt aan specifieke artrosemedicatie. Zogehetend disease modifying osteoarthritis drugs, kortweg DMOADs, moeten idealiter dus drie eigenschappen bezitten om toegevoegde waarde te hebben in de kliniek: pijnstillend, ontstekingsremmend en kraakbeen beschermend.

Deel I: Huidige (pre-)klinische benadering

Celecoxib is een pijnstiller en ontstekingsremmer die vaak wordt voorgeschreven bij artrose. Celecoxib remt cyclooxygenase-2 (COX-2), een belangrijke factor bij het ontstaan van pijn en ontsteking. Eerder laboratoriumonderzoek heeft aangetoond dat celecoxib mogelijk ook kraakbeen beschermend werkt. In **hoofdstuk 2** is onderzocht of celecoxib deze DMOAD effecten ook heeft bij mensen met knieartrose. 172 patiënten die op de wachtlijst stonden voor een knieprothese werden verdeeld in vier behandelgroepen (celecoxib 2dd200mg; celecoxib 2dd200mg die drie dagen voor de operatie gestopt werd; naproxen 3dd250mg; of geen behandeling). De effecten op kraakbeen, ontsteking en klinische symptomen werden bestudeerd. Celecoxib zorgde inderdaad voor een verlaging van COX-2 in zowel kraakbeen als synoviaal weefsel, de binnenste laag van het gewrichtskapsel, wat aantoont dat celecoxib het gewricht daadwerkelijk bereikt heeft. Ondanks deze lokale effecten werden er geen verschillen gevonden in het metabolisme van proteoglycanen (een belangrijk bestanddeel van kraakbeen en daarmee een maat voor de beschermende werking op kraakbeen), ontsteking en pijn. De eerder gevonden DMOAD effecten van celecoxib in *in vitro* laboratoriumonderzoek[1] konden *in vivo* dus niet bevestigd worden.

Granulocyte macrophage colony-stimulating factor (GM-CSF) speelt een belangrijke rol bij de regulering van pijn bij artrose. Recent onderzoek heeft aangetoond dat remming van GM-CSF mogelijk gunstige effecten heeft bij artrose[2]. **Hoofdstuk 3** beschrijft een studie waarbij is gekeken naar de relatie tussen de aanwezigheid van GM-CSF in het gewricht en gerapporteerde pijn. Van twintig patiënten is kraakbeen en synoviaal weefsel verzameld tijdens het plaatsen van een knieprothese. Hoewel een positieve relatie werd verwacht, werd juist een negatieve relatie gevonden tussen de lokale aanwezigheid van GM-CSF en pijn, wat aangeeft dat de oorzaak van pijn complexer is dan simpelweg een verhoging van GM-CSF in het gewricht.

Recent is door onze onderzoeksgroep een fusie-eiwit ontwikkeld waarbij twee anti-inflammatoire cytokines, interleukine-4 (IL-4) en interleukine-10 (IL-10) gecombineerd zijn in één molecuul, het IL4-10 FP. Eerdere onderzoeken lieten zien dat het IL4-10 FP over alle drie de aspecten van een DMOAD lijkt te beschikken[3-5]. Om meer inzicht te krijgen in de samenwerking tussen kraakbeen, ontsteking en pijn, en de onderliggende werkingsmechanismen van IL-4, IL-10 en IL4-10 FP beter te begrijpen, is in **hoofdstuk 4** de bestaande literatuur in een review beschreven. Er werd specifiek gekeken naar wat er al bekend is over de effecten op kraakbeen, ontsteking en pijn. Er werden nagenoeg alleen positieve effecten op kraakbeen gevonden voor zowel IL-4 als IL-10 en het IL4-10 FP. Hoewel beide cytokines bekend staan als zijnde ontstekingsremmend, laten ze in sommige studies ook zien ontsteking te kunnen stimuleren. Voor IL4-10 FP worden echter alleen ontstekingsremmende effecten gevonden, een teken dat mogelijke stimulerende effecten van IL-10 geremd worden door de ontstekingsremmende effecten van IL-4 en vice versa. Wat betreft de pijnstillende werking worden slechts enkele, maar veelbelovende resultaten gevonden, zowel op de remming van pijnmediatoren als op het verminderen van klinische pijn.

Ondanks dat het IL4-10 FP veelbelovende resultaten laat zien op kraakbeen, ontsteking en pijn (de drie pijlers van een succesvolle DMOAD) is het tot op heden nog niet gelukt om alle drie de effecten tegelijk in hetzelfde (*in vivo*) artrosemodel aan te tonen. Daarom is in **hoofdstuk 5** specifiek gekeken naar de DMOAD activiteit van IL4-10 FP in het honden Groove model voor artrose[6-8]. Nadat artrose is aangebracht in een van de achterpoten, werden de honden tien weken lang behandeld met een wekelijkse injectie met IL4-10 FP in het gewricht. Tijdens de behandelperiode werd, als maat voor gewrichtspijn, de mate van belasting van de gewrichten gemeten met behulp van een drukgevoelige plaat in de grond (Force Plate Analysis). Na tien weken werd kraakbeen en synoviaal weefsel verzameld om kraakbeenschade en ontsteking te bestuderen. Er werd zowel een pijnstillend (meer belasting van het gewricht) als een kraakbeen beschermend effect gevonden. Het hondenmodel leidde slechts tot milde ontsteking, waardoor de ontstekingsremmende effecten *in vivo* niet goed geëvalueerd konden worden. Echter, *in vitro* werd wel een ontstekingsremmend effect gevonden.

Addendum

DMOADs worden vaak getest op jonge dieren met een normaal gewicht waarbij na het aanbrengen van artrose meteen wordt gestart met behandeling. Dit komt echter niet overeen met de huidige realiteit waarin artrose meer gerelateerd is aan een hoge leeftijd en obesitas. In **hoofdstuk 6** is een studie beschreven die gebruik maakte van een diermodel dat artrose combineert met obesitas. In ratten werd kraakbeenschade (Groove model) gecombineerd met een dieet met een hoog vet gehalte (waardoor snel obesitas ontstaat) om de effecten van IL4-10 FP in een meer klinisch relevant model te testen[9,10]. Twintig ratten werden willekeurig ingedeeld in twee groepen. De behandelgroep kreeg tien wekelijkse injecties in het gewricht met IL4-10 FP en de controlegroep kreeg tien wekelijkse injecties in het gewricht met een zoutoplossing. Ook in deze studie werden de effecten op kraakbeen, ontsteking en pijn bestudeerd. In deze rattenstudie konden de eerder genoemde kraakbeen beschermende en pijnstillende effecten bevestigd worden. Helaas bleek ook in dit model de ontsteking niet ernstig genoeg om de remmende effecten van IL4-10 FP op ontsteking goed te kunnen bestuderen. Deze en voorgaande resultaten laten zien dat de combinatie van IL-4 en IL-10 in één molecuul (IL4-10 FP) de potentie heeft om te voldoen aan alle eisen voor een DMOAD.

Deel II: Eerste stappen naar een nieuwe benadering

Ondanks vele pogingen is er nog steeds geen genezende behandeling voor artrose. Een mogelijke verklaring hiervoor ligt in de huidige benadering van mensen met klachten van artrose. Op dit moment wordt artrose gezien als één ziekte en worden alle artrosepatiënten op dezelfde manier behandeld: pijnstillers, fysiotherapie, en indien deze conservatieve behandeling faalt, een (gewrichtsvervangende) operatie. Dit terwijl de oorzaak van artrose erg kan verschillen tussen patiënten. Zo kan iemand artrose hebben als gevolg van een ongeluk in het verleden (bijvoorbeeld een gescheurde kruisband), omdat iemand zijn/haar gewricht al tachtig jaar gebruikt, omdat iemand een onderliggende ziekte heeft waardoor er vaak ontstekingen van een gewricht zijn geweest, etc. Er zijn dus verschillende onderliggende oorzaken (fenotypes) die uiteindelijk leiden tot hetzelfde resultaat: artrose.

Ook bij het selecteren van deelnemers aan klinisch onderzoek wordt vaak geen onderscheid gemaakt tussen deze verschillende oorzaken van artrose. Dit leidt tot een te gevarieerde onderzoekspopulatie. Op groepsniveau kan de uitkomst dan zijn dat de onderzochte behandeling geen effect heeft; een behandeling die zich richt op een onderliggende ontsteking zal immers minder effect hebben bij de patiënt met artrose als gevolg van een kruisbandletsel, terwijl dezelfde behandeling voor iemand met een ontstekingsziekte als onderliggende oorzaak van artrose wél het gewenste resultaat kan hebben. Het is dus zowel voor de kwaliteit van klinisch onderzoek naar nieuwe behandelingen voor artrose als in de zorg voor mensen met artrose belangrijk om onderscheid te kunnen maken tussen de verschillende fenotypes van artrose.

In **hoofdstuk 7** wordt daarom een nieuw artrosecohort beschreven, met als doel het voorspellen van ziekteprogressie en vervolgens het definiëren van verschillende fenotypes van artrose: APPROACH. In deze 2-jaar durende studie met 297 artrosepatiënten wordt gebruik gemaakt van veel conventionele én nieuwe markers om het artroseproces in kaart te brengen. Op die manier ontstaat er een grote internationale database die gebruikt kan worden om op zoek te gaan naar de verschillende fenotypes van artrose.

Voor de selectie van patiënten voor APPROACH is gebruik gemaakt van machine learning modellen om patiënten te selecteren die de grootste kans hebben op snelle verslechtering (progressie) van hun artrose. Immers, als je in een trial wilt aantonen dat een nieuwe behandeling het artroseproces vertraagt, dan wel stopzet, moet je wel zeker weten dat de onderzochte populatie zonder behandeling progressie zou hebben getoond. Alle patiënten in APPROACH kregen een S progressie score, die voorspelt hoe groot de kans is op progressie van weefselschade (structurele schade) en een P progressie score, die voorspelt hoe groot de kans is op pijn progressie. **Hoofdstuk 8** beschrijft de karakteristieken van de APPROACH patiënten in relatie met de toegewezen S en P progressie scores. Patiënten met een hoge toegewezen S progressie score hebben ten tijde van inclusie een grotere minimum Joint Space Width (minJSW; minder schade) dan patiënten met een lage S progressie score en daarmee inderdaad meer ruimte om structurele progressie te laten zien. Patiënten met een hoge toegewezen P progressie score hebben daarentegen al meer pijn dan mensen met een lage P progressie score en daarmee minder ruimte om ook daadwerkelijke pijn progressie te laten zien. Desalniettemin vormt de APPROACH populatie met een grote minJSW en veel pijn mogelijk de ideale populatie om behandelingen te testen die structurele schade afremmen/stoppen/herstellen en pijn verminderen.

De bovengenoemde discrepantie tussen een positief effect van celecoxib op de lokale hoeveelheid COX-2 en de afwezigheid van DMOAD effecten, in combinatie met de negatieve relatie tussen lokale GM-CSF expressie en kniepijn, suggereert dat artrosepijn niet alleen wordt veroorzaakt door lokale schade (zogeheten nociceptieve pijn) maar ook andere, meer systemische oorzaken heeft. Dit wordt ondersteund door de zwakke relatie die bestaat tussen gewrichtsschade die te zien is op een röntgenfoto en de hoeveelheid pijn die patiënten ervaren[11].

Hoogstwaarschijnlijk vormen artrosepatiënten die naast nociceptieve pijn (pijn veroorzaakt door schade aan het gewricht) ook een meer systemisch veroorzaakte pijn ervaren specifieke fenotypes van artrose. In **hoofdstuk 9** is een van deze fenotypes onderzocht: patiënten waarbij hoogstwaarschijnlijk een neuropathische pijn component een rol speelt. Neuropathische pijn is pijn die veroorzaakt wordt door zenuwschade. Er werd gebruikt gemaakt van een vragenlijst die met grote betrouwbaarheid onderscheid kan maken tussen neuropathische en nociceptieve pijn. 24 patiënten met een neuropathische pijn component zijn vergeleken met 48 patiënten die eenzelfde pijnscore in hun knie aangaven,

maar géén neuropathische pijn component hebben. Er is gekeken naar verschillen in pijn, lichamelijke functie en gewrichtsschade. Patiënten waarbij hoogstwaarschijnlijk sprake is van een neuropathische pijn component hebben minder lokale schade in hun knie, maar wel een slechtere functie. Deze verslechterde functie zou ook verklaard kunnen worden door de aanwezigheid van artrose in andere gewrichten. Patiënten met een neuropathische pijn component gaven namelijk meer pijn aan in hun andere gewrichten. Echter, de gewrichtsschade in deze gewrichten was vergelijkbaar tussen beide groepen. Dit suggereert dat bij patiënten waar een neuropathische pijn component een rol lijkt te spelen, functiebeperking en pijn niet veroorzaakt worden door de gevonden gewrichtsschade. Dit kan betekenen dat deze patiënten ook niet, of slechter, reageren op de huidige beschikbare behandelingen. In de toekomst zouden deze patiënten geïdentificeerd moeten worden, zodat ze een meer gerichte behandeling voor hun neuropathische pijn kunnen krijgen. Om diezelfde reden is het wenselijk om deze patiënten te identificeren en niet mee te nemen in onderzoeken met DMOADs.

Een van de nieuwe biomarkers van artrose die gebruikt is in APPROACH is bewegingsanalyse met behulp van het GaitSmart® systeem. GaitSmart® maakt gebruik van zes sensoren die op de benen van een patiënt worden geplaatst. Vervolgens wordt iemand gevraagd om 15-20 meter te lopen. Daarna worden de sensoren verwijderd en aan de laptop gekoppeld. De software analyseert de gegevens en laat vervolgens het resultaat zien. Een GaitSmart® resultaat bevat ongeveer 20-30 parameters die informatie geven over verschillende aspecten van iemands looppatroon (beweging in de gewrichten, zijwaartse beweging, snelheid, etc.). **Hoofdstuk 10** beschrijft de relatie tussen GaitSmart® en radiologische schade. Als eerste stap werd Principal component analysis gebruikt om structuur te vinden in alle GaitSmart® parameters en te bestuderen of ze hetzelfde of iets anders meten dan röntgenfoto's en patient reported outcome measures (PROMs; vragenlijsten). Er werd aangetoond dat de uitslag van een GaitSmart® analyse, naast een röntgenfoto (gewrichtsschade) en PROMs, extra informatie geeft over iemands artrose. Vervolgens werd de relatie tussen GaitSmart® en aanwezigheid en ernst van radiologische schade bestudeerd. Op groepsniveau werd een kleine relatie gevonden tussen de uitkomsten van een GaitSmart® analyse en de schade die te zien is op röntgenfoto's. Echter, op individueel patiëntniveau heeft dit weinig toegevoegde waarde.

Hoofdstuk 11 laat zien dat de relatie tussen GaitSmart® en lichamelijke functie wat beter is dan die tussen GaitSmart® en radiologische schade. Iemands lichamelijke functie behelst twee aspecten, namelijk hoe iemand zijn of haar functie waardeert (subjectief, te meten met vragenlijsten) of iemands feitelijke functie (objectief, te meten in een professioneel bewegingslaboratorium of met simpele fysieke testen). In deze studie werd gekeken naar de relatie tussen GaitSmart® en de verschillende aspecten van lichamelijke functie en werd bestudeerd of GaitSmart® in staat is om veranderingen in functie op korte termijn waar te nemen. Met behulp van het GaitSmart® systeem is het mogelijk om beide aspecten van

lichamelijke functie te meten. Daarnaast kan het systeem ook korte termijn veranderingen in objectieve functie meten. Het voordeel van GaitSmart®, ten opzichte van analyse in een professioneel bewegingslaboratorium, is dat het een relatief simpele procedure is die slechts 10-15 minuten in beslag neemt en overal uitgevoerd kan worden. De combinatie van vragenlijsten en simpele fysieke testen is ook snel uit te voeren, maar is minder objectief (vragenlijsten) en minder nauwkeurig (simpele fysieke testen) dan een analyse met GaitSmart®.

Ten slotte wordt in **hoofdstuk 12** de progressie van de APPROACH patiënten tijdens de studieduur beschreven, in relatie met hun in het begin van de studie toegewezen S en P progressie score. Structurele progressie is gedefinieerd als een afname van de minJSW van >0.6mm. Pijn progressie is gedefinieerd als *snelle toename van pijn*: Knee Injury and Osteoarthritis Outcome Score (KOOS) pijn afname tussen start en eind van de studie ≥10 punten per jaar (m.a.w. ≥20 punten afname tijdens de tweejarige studie) en een uiteindelijke KOOS pijn score ≤65 punten, *significante toename van pijn*: KOOS pijn afname tussen begin en eind van de studie van ≥5 punten per jaar (m.a.w. ≥10 punten afname tijdens de tweejarige studie) en een uiteindelijke KOOS pijn score ≤60 punten of *stabiele ernstige pijn*: KOOS pijn score ≤60 punten tijdens de hele studie periode. De patiënten die voldeden aan de definitie van structurele progressie hebben in het begin gemiddeld een hogere S progressie score toegewezen gekregen dan mensen die niet voldeden aan de definitie van structurele progressie. Hetzelfde geldt voor mensen die voldeden aan de definitie van pijn progressie; zij hebben in het begin een hogere P progressie score toegewezen gekregen dan mensen die niet voldeden aan de definitie van pijn progressie. Desondanks is de voorspellende waarde van de S progressie score voor het daadwerkelijk hebben van structurele progressie in de komende twee jaar matig. De P progressie score daarentegen kan wel voorspellen of iemand in de komende twee jaar pijn progressie zal laten zien. Meer onderzoek is nodig om de uiteindelijk waarde van het gebruik van machine learning modellen voor de selectie van patiënten voor klinisch onderzoek te evalueren en optimaliseren.

Conclusie

Dit proefschrift draagt bij aan de zoektocht naar een succesvolle DMOAD. In tegenstelling tot celecoxib liet het IL4-10 FP alle eigenschappen zien die een ideale DMOAD zou moeten bezitten. Toekomstig onderzoek zal zich moeten richten op het identificeren van verschillende fenotypes van artrose die als leidraad kunnen dienen voor een meer persoonlijke benadering. Dit verhoogt de kans op goede uitkomsten in klinisch onderzoek naar nieuwe behandelingen met effecten op kraakbeenschade, ontsteking en pijn. De APPROACH database kan gebruikt worden voor het identificeren én beschrijven van deze verschillende fenotypes.

Addendum

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Addendum

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'Maar ook geen échte wetenschapper hè?' 'Nee ook niet'
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Addendum

Collega's van H3 en het lab. Anne, als frisse wind in het lab en in het APPROACH-team heb je me door vele avonden polikliniek en MRI gesleept. Arno, jij bent altijd vrolijk en dat werkt inspirerend. Astrid, jij hebt het talent om in alles en iedereen het goede te zien, daar kunnen velen, inclusief ikzelf, nog wat van leren. Goran, hopelijk ondervindt je bij je onderzoek minder tegenslag dan bij het verbouwen. Jelena, ik doe het gewoon in het Nederlands, altijd druk, altijd aan de telefoon maar altijd beschikbaar voor overleg of een koffie. Marianne, wat ontzettend vet dat je het avontuur aangaat in Lissabon, veel succes en plezier. Marion, bedankt voor al je wijze woorden over experimenten maar ook over het leven. Matthijs, fijn om iemand te hebben om frustraties rondom klinische studies mee te delen. Gelukkig loopt TOPIRA inmiddels goed, hou vol. Maxime, stille wateren hebben diepe gronden, of misschien is het je Twentse bloed maar wat kan jij verrassend uit de hoek komen. Mylène, fijn om frustraties rondom APPROACH met je te kunnen delen, gedeelde smart is halve smart. In mijn ogen was jij van alle collega's de enige echt goede wetenschapper. Ze mogen blij zijn dat je als post-doc bent gebleven, heel veel succes! Nadia, ik heb bewondering voor hoe jij ondanks enkele tegenslagen je promotietraject zeer succesvol hebt weten af te ronden, dat werkte inspirerend! Ik weet zeker dat je een fantastische reumatoloog wordt. Tammo, text-mining Tammo, jij wist altijd over elk onderwerp wel iets te zeggen. Thijmen, het heeft even tijd nodig om je te leren kennen maar dan heb je ook wat. Dank voor al je adviezen in de tijd dat we collega's waren, maar ook nog daarna.

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Addendum

List of publications

Van Helvoort EM, Coeleveld K, de Boer TN, Huisman AM, Polak AA, et al. Lack of a Clear Disease Modifying Activity of Celecoxib Treatment of End-Stage Knee Osteoarthritis: A Randomized Observer Blinded Clinical Trial.
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RMD Open. 2021;7(3):e001759. doi: 10.1136/rmdopen-2021-001759.

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RMD Open. 2021;7(3);e002025. doi: 10.1136/rmdopen-2021-002025.

Addendum

List of conference abstracts

Lack of a clear disease modifying activity of celecoxib in treatment of end-stage knee osteoarthritis: a randomized observer blinded clinical trial (chapter 2)
Poster presentation at OARSI congress 2017 and EULAR congress 2017

Effects of the human IL4-10 fusion protein in the canine Groove model of osteoarthritis
Poster presentation at OARSI congress 2017 and EULAR congress 2017

Canine IL-4-10 fusion protein provides disease modifying activity in a canine model of OA: an exploratory study (chapter 4)
Oral presentation at EULAR congress 2018 and NVR congress 2018; Poster presentation at OARSI congress 2018

Cohort profile: The IMI-APPROACH study: A 2-year, European, cohort study to describe, validate, and predict phenotypes of osteoarthritis using clinical, imaging, and biochemical markers (chapter 6)
Oral presentation at NVR congress 2018; Poster presentation at OARSI congress 2018 and IMI 10th anniversary scientific symposium 2018

Baseline clinical characteristics of the IMI-APPROACH knee OA cohort (chapter 7)
Poster presentation at OARSI congress 2020

The relationship between motion, using the GaitSmart® system, and radiographic knee OA: an explorative analysis in the IMI-APPROACH cohort (chapter 10)
Oral presentation at OARSI virtual congress 2021 - one of the highest rated abstracts by Young Investigators

Addendum

Curriculum Vitae

Eefje Martine van Helvoort was born January 11th 1991 in Oss, The Netherlands and grew up in Heesch together with her parents and twin sister. In 2009 she graduated from secondary school (gymnasium) at the Titus Brandsmalyceum in Oss, after which she started her Medicine study at Radboud University Nijmegen. During her study she developed special interest in the musculoskeletal system. In her fourth year she did a research internship at the department of Rheumatology & Clinical Immunology of the University Medical Center (UMC) Utrecht. After obtaining her medical degree in 2016, she started as a researcher at this same department. In January 2017 she continued as a PhD candidate under supervision of prof. dr. F.P.J.G. Lafeber, dr. S.C. Mastbergen and dr. N. Eijkelkamp. During her PhD she combined translational research (part I of this thesis) with the coordination and performance of an international multi-center clinical study (www.approachproject.eu), of which the first results are described in part II of this thesis. Her work during these years thus far led to eight peer reviewed publications, and three manuscript submissions. Her work on the GaitSmart® motion analysis system as new marker for osteoarthritis was ranked amongst the highest rated abstracts from young investigators at OARSI 2021.