Focal cartilage defects in the knee –A randomized controlled trial comparing autologous chondrocyte implantation with arthroscopic debridement

NCP – Chondrocyte implantation

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1. STUDY SYNOPSIS: NCP – Autologous Chondrocyte Implantation

Topic	Details
Protocol Title:	Focal cartilage defects in the knee – A randomized controlled trial comparing Autologous Chondrocyte Implantation with arthroscopic debridement
Study Type:	Clinical
Indication:	Compare the effect of Autologous Chondrocyte Implantation (ACI) with arthroscopic debridement (AD) in patients with symptomatic full thickness knee cartilage injuries larger than 2cm2.
Hypothesis Statement	Focal cartilage injuries in the knee might have devastating effect both in the short term and in the long term. Various surgical treatment options are available; with ACI established as a recognized treatment method for larger lesions. Meta-analysis and systematic reviews have required well-designed, long-term, multicenter studies to evaluate clinical outcomes of ACI with the use of a "no treatment" group as a control group. H0: There is no difference in KOOS QoL after ACI or AD from baseline to 24 months after surgery. H1: There is a difference in KOOS QoL after ACI or AD from baseline to 24 months after surgery.
Study Objectives:	Questionnaires: KOOS, Tegner score, Lysholm score, EQ-5D, VAS. Physical examination: range of motion and hop test. Radiology: x-ray and MRI of the knee. Primary aim: KOOS quality of life (QoL) subscore.
Study Design:	Prospective, single-blinded parallel-group bicenter study with 2 treatment arms.
Duration of Study Participation:	Approx. 36 months inclusion and 24 months follow up. In total 5 years.
Follow-up Period:	24 months. All will be invited to participate in late controls after 5 and 10 years.
Study Location:	2 Norwegian hospitals: Akershus University Hospital and Oslo University Hospital – Ullevål.
Number of Planned Subjects:	82 patients
Study Population:	Inclusion: age 18-50 years old, single symptomatic cartilage defect on femoral condyle or trochlea, defect size larger than 2

	cm2, defect ICRS grade 3-4, ligamentous stable knee, range of motion 5-105°, Lysholm score < 75 and informed consent. Exclusion: Osteoarthritis, rheumatoid or other systemic arthritis, malalignment > 5° measured on x-rays, BMI > 30, comorbidities that may influence surgery or rehabilitation, pregnancy, inability to complete questionnares or rehabilitation, serious alcohol or drug abuse, previous cartilage surgery to the chondral defect except OCD surgery.
Treatment Groups:	2 treatment groups with 41 patients in each group.
Visit Schedule:	3 months (± 2 weeks), 6 months (± 4 weeks), 12 months (± 6 weeks) and 24 months (± 8 weeks). All will be invited to participate in late controls after 5 and 10 years.
Safety Assessments:	If any unforeseen complication outside normal clinical practice occurs, the sponsor representative will be contacted as soon as possible with a parallel message to the local coordinators at the involved hospitals. During each follow up, there will be a case report form (CRF) regarding complications and safety.
Sample Collection:	A 5 mL venous blood sample will be drawn on the day of operation. The blood sample will be centrifuged before serum is pipetted in a sterile tube. The serum will be analyzed at Oslo University Hospital – Rikshospitalet on the cartilage biomarker microRNA-140 (miR-140). During the open chondrocyte implantation the excess cartilage debrided from the rim of the lesion will be sent for similar microRNA-140 (miR-140) analysis. (applicable for the 41 patients in the ACI arm only)
Overview of Statistical Plan:	If normal distribution, aims will be analyzed using linear mixed models (LMM), and the primary aim will be performed as a post hoc test for the LMM, similar to performing a two-sample t-test. If no normal distribution, analysis will be performed using Mann-Whitney U-test.
Sample Size Determination:	Detecting a difference of 10 in primary aim with 80% power using a standard deviation of 15. A $p < 0,05$ is statistically significant.

	This gives 37 patients in each group, adding 10% drop out meaning 41 patients in each group and 82 in total.
Interim Analysis:	No interim analysis will be done.
Ongoing Data Monitoring Plan:	Monitor at Akershus University Hospital.
Study Stopping Rules:	Inclusion of 82 patients.
End of Study:	The end of this study is 24 months after the last included patient.
Operational Risk Analysis:	Inability to include 82 patients in 3 years. May prolong the inclusion period, or add other including hospitals in the study.
Study Target Dates:	See table below.

Milestone	**Study Timeline: Target Date (Month/Year)**
IND Submitted (if appropriate)	October 2015
First Patient In (FPI)	March 2016
Last Patient In (LPI)	March 2019
Last Patient Out (LPO)	March 2021
Database Lock (DBL)	April 2021
First Look (FL)	May 2021
CSR Completed	December 2021

Tests and assessments	**Inclusion**	**Surgery**	**3 months**	**6 months**	**12 months**	**24 months**
Medical History	X		X	X	X	X
Physical Examination	X		X	X	X	X
Questionnaires	X		X	X	X	X
X-ray	X					X
Informed Consent	X					
Randomization and surgery		X				
Serum and cartilage sample		X				
MRI						X
Adverse Events			Monitor and record throughout the study			

Study Activities

2. SHORT SUMMARY

Focal cartilage injuries in the knee might have devastating effect both in the short term and in the long term due to the predisposition of early onset osteoarthritis. To this date, various surgical treatment options are available. Since its introduction in the late 1980s, autologous chondrocyte implantation has become a recognized treatment option for larger cartilage lesions in the knee. In this study we want to increase clinical and economic knowledge about autologous chondrocyte implantation compared to arthroscopic debridement in the short and long run.

3. INTRODUCTION

3.1. Background

The articular surfaces of joints are covered with hyaline cartilage, a unique tissue with extreme load shearing and low-friction properties. However, these characteristics come with a cost. It is aneural and avascular which explains its limited ability to self-repair[1-3]. Damage to the cartilage, which is common in young active adults, will therefore lead to permanent damage. These injuries are very common, with a reported prevalence of 12 % in the population [4]. Focal cartilage lesions predispose to development of early onset osteoarthritis, which in turn may lead to long rehabilitation periods and loss of function and time off work. Musculoskeletal problems are one of the major reasons for workers compensation and especially for younger patients this may lead to a significant reduced quality of life and loss of income [5-8]. Treatment of symptomatic cartilage injuries in the knee is therefore of particular interest and importance to the patient, the surgeon and the society [3, 9, 10].

The ideal treatment for isolated cartilage injuries aims at recreating a healthy hyaline-type tissue with similar strength and durability as the normal cartilage in the rest of the joint. Current surgical treatment options include debridement, microfracture, autologous osteochondral transplantation (mosaicplasty) and autologous chondrocyte implantation (ACI) [11-13]. Several studies conclude that microfracture is not effective for larger lesions [26, 27]. The purpose of arthroscopic debridement is to remove loose intraarticular tissue debris and inflammatory mediators down to the subchondral bone, but not through it [12]. ACI attempts to re-implant the patient's own chondrocytes over the defect to permit the chondrocytes to heal back onto the bone and mature into a hyaline-like cartilage. ACI have been directly compared to microfracture and mosaicplasty in randomized controlled trials [20, 35, 36]. The results indicate improved knee function in (carefully selected) patients [14], but studies have been criticized for methodological weaknesses [15]. The Cochrane database reported that there is insufficient evidence to conclude that ACI is superior to other treatment strategies for treating full thickness cartilage defect of the knee [19].

No statistically significant differences have been found between the different surgical treatments. This supports the suggestion that the improvement might be a result of the post-operative rehabilitation rather than the surgery itself [16, 17]. Furthermore, inclusion in a clinical trial is known to improve symptoms by itself, known as the Hawthorne effect [18].

All studies on cartilage reconstructive surgery have put the patients through a strict, careful, intensive and prolonged rehabilitation following the procedure, which could contribute to the clinical improvement observed. Wondrasch and co-workers implemented an active rehabilitation program in 48 patients with focal cartilage damage to a weight-bearing area of the knee [17]. None of the patients had any form of surgery to their injury. After three months there was a statistical significant and clinical meaningful improvement in KOOS score, load progression and hop score, to the point where 31 (65%) of the patients declined further surgery for their cartilage lesion. This indicates that good results can be achieve with physiotherapy alone. Dozin and coworkers compared ACI versus mosaicplasty, where all candidates where evaluated arthroscopically with debridement of the lesion 6 months prior to definitive treatment [20]. All candidates completed an intensive rehabilitation protocol following the debridement, but before the cartilage surgery. This included non-weight bearing for two weeks and immediate active and passive physiotherapy. After two weeks isometric and proprioception exercises were introduced, as well as gradually strengthening exercises. 31 % of the candidates experienced substantial clinical improvement following the debridement and physiotherapy alone, and needed no further (surgical) treatment, questioning the need for cartilage treatment as a first port of call in such patients. The authors conclude that further randomized clinical trials are needed, where ACI should be compared to debridement alone. This is a missing link in our knowledge of cartilage reconstructive surgery and hampers further progression in cell therapy [20,33].

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3.2. Purpose of this study

The purpose of this trial is to compare autologous chondrocyte implantation (ACI) with arthroscopic debridement (AD) in symptomatic cartilage injuries larger than 2 cm^2 in patients aged 18-50 years old in regard to both subjective and objective variables at predefined times as described under “variables” in section 6.

4. RESEARCH DESIGN

The study is part of “The Norwegian Cartilage Project (NCP): A multidisciplinary Approach to Improve the Treatment of Injured Articular Cartilage”, which is a total of five studies regarding cartilage injuries in the knee, including clinical trials, register studies and basic research studies.

This study is a prospective, randomized, controlled study with 2 treatment arms (ACI and AD). The study will be conducted at Akershus University Hospital and Oslo University Hospital with multicenter inclusion contribution from other Norwegian institutions which together form the NCP network of hospitals: These hospitals are in addition to Akershus and Oslo University Hospitals: Kristiansund Hospital, Haukeland University Hospital, Ålesund Hospital, Diakonhjemmet Hospital and Buskerud Hospital.

Because one treatment arm consists of a two stage surgery, with a mini-open arthrotomy, while the other treatment arm is a single stage arthroscopy, it will not be possible to blind the patients as to what treatment they have received.

Inclusion and treatment will take place at Akershus University Hospital (Ahus) or Oslo University Hospital (Ullevål). .The follow up appointments will be performed at Ahus or at the local NCP affiliated hospital by an external reviewer connected to the NCP group. The follow up will be blinded so that the examinator is unaware of which treatment arm the patient belongs to. To achieve this, the patient is instructed not to reveal the nature of the treatment he/she has received, and an opaque elastic stocking is put over the knee to conceal the scars (which are different between the treatments). Follow up is planned to 2 years, but each participant will also be invited to clinical and radiological follow ups after 5 and 10 years.

4.1. Participants

The study will include a total of 82 patients, both men and non-pregnant women, with a full thickness or osteochondral defect in the weight bearing area of the femoral condyles or trochlea larger than 2 cm^2. The lesion must be symptomatic, with a Lysholm score less than 75 [34]. The inclusion criteria are based on the recommendations by Brittberg [22], and include patients aged 18 to 50 years old with a stable knee, good range of motion (ROM), normal alignment (less than 5° varus or valgus measured on hip-knee-ankle (HKA) angle images) and no sign of radiologically osteoarthritis classified after Kellgren-Lawrence [37]. The weight-bearing, fixed-flexion posteroanterior radiographs will be obtained with the SynaFlex X-ray positioning frame (Synarc) [38].

5. MATERIALS AND METHODS

All patients will be assessed clinically as well as radiologically prior to inclusion to avoid peroperative exclusions. The full thickness or osteochondral defect will be verified arthroscopically as a grade 3 or 4 lesion according to the International Cartilage Repair Society (ICRS) [39]. Still, some patients might not be included peroperatively based on arthroscopic findings such as osteoarthritis, less than 50% normal meniscus or inappropriate size of the lesion [40]. The definitive inclusion of the patient will be performed by the operating surgeon during the diagnostic arthroscopy, prior to randomization. Patients who will be excluded based on peroperative findings will receive appropriate treatment according to the standard of care at the local hospitals.

Exclusion criteria include general osteoarthritis, systemic arthritis, severe co-morbidities that may influence surgery or rehabilitation potential, significant alcohol or drug abuse, psychiatric disorders, language barriers, pregnancy, severe obesity (body mass index > 30) and previous surgery to the chondral defect such as previous microfracture (previous cruciate ligament reconstruction, fixation of osteochondritis dissecans lesions, alignment procedures (osteotomies) or meniscal surgeries are not exclusion criteria). Patients declining participation in the trial (and who fulfill the inclusion criteria) or withdrawing underway will receive appropriate treatment according to the standard of care at the local hospital.

Study Flow Chart

Inclusion
-Age 18-50 years old
-Single symptomatic cartilage defect on medial or lateral femoral condyle or trochlea
-Defect size larger than 2 cm2
-Defect ICRS grade 3-4
-Ligamentous stable knee
-Acceptable range of motion (minimum 5-105 °)
- Lysholm score < 75
-Informed consent
-Meniscus > 50 % intact

Exclusion
-Osteoarthritis, rheumatoid or other systemic arthritis (asked)
-Malalignment > 5° measured on HKA x-rays
-No radiological osteoarthritis
-Obesity (Body mass index > 30)
-Comorbidities that may influence surgery or rehabilitation
-Pregnancy
-Inability to complete questionnaires or rehabilitation
-Serious alcohol or drug abuse
- Previous cartilage surgery to the chondral defect except OCD surgery

RANDOMIZATION
during diagnostic

*Blood samples for healer/non-healer study taken during diagnostic

Autologous Chondrocyte Implantation
(Cartilage harvest during diagnostic arthroscopy)

Arthroscopic debridement

Chondrocyte Implantation
(2 weeks later)

Rehabilitation (3 phases)
Phase 1: Accomodation
Phase 2: Rehabilitation
Phase 3: Return to activity

Follow up
(3,6,12 and 24 months post op.)
KOOS
Tegner
Lysholm
VAS (pain)
EQ-5D
MRI, X-ray and hoptests at 24 months

The participants will also receive information about a parallel study (Healer Non-healer study) regarding obtaining venous blood samples, which also is part of the Norwegian Cartilage Project. For more information, see "Operative procedure – blood samples for parallel study" in section 5.4 and "Additional and secondary projects" in section 8.2.

Inclusion criteria	*Exclusion criteria*
-Age 18-50 years old -Single symptomatic cartilage defect on medial or lateral femoral condyle or trochlea -Defect size larger than 2 cm2 -Lesion graded ICRS 3-4 ->50% intact meniscus -Ligamentous stable knee -Acceptable range of motion (5-105°) -Lysholm score < 75 -Informed consent	-Osteoarthritis, rheumatoid or other systemic arthritis -Malalignment >5° measured on HKA images -No radiological osteoarthritis -Obesity (Body mass index > 30) -Comorbidities that may influence surgery or rehabilitation -Pregnancy -Inability to complete questionnaires or rehabilitation -Serious alcohol or drug abuse -Previous surgery to the chondral defect except OCD surgery

Inclusion and exclusion criteria

5.2. Randomization

The randomisation will be performed using an computer generator (randomization.org). 82 patient will be block randomized in pairs of six-eight (1:1) to treatment allocations. Each patient receives a patient number (1 through 82) on inclusion. Randomization will be printed in faded text and concealed in opaque numerically marked envelopes. The printing and concealing will be done by a person working at Akershus University Hospital, but is not involved in the study, to secure blinding. The randomization process occurs in the operation theatre during the arthroscopy after the operating surgeon has measured and graded the lesion to fulfill the inclusion criteria (see section "4.3 Operative procedure").

5.3. Operative procedure

In this study Autologous Chondrocyte Implantation (ACI) is compared with Arthroscopic Debridement (AD). All patients will undergo a diagnostic arthroscopy, and patients randomized to AD will have this procedure performed at the end of the diagnostic arthroscopy, while for the patients in the ACI group the chondrocyte implantation will take place two weeks later in a second stage operation as described below. The ACI technique is based on the technique described by Brittberg [23].

Diagnostic arthroscopy:

Three standard incisions are made (supralateral for the patella and medial and lateral for the patellar tendon). A thorough diagnostic arthroscopic examination is then done. Removal of loose bodies and other necessary intraarticular procedures are done first (meniscus, plica). The focal cartilage lesion is then measured using a standard 4-mm arthroscopic probe and ICRS graded [39, 40].

Then either arthroscopic debridement (AD) or autologous chondrocyte implantation (ACI) is done, depending on randomization.

Autologous Chondrocyte Implantation

ACI is a three stage procedure taking place at Akershus University Hospital and Oslo University Hospital (Ex-Vivo Laboratory) over a two week period.

Stage 1: Debridement and cartilage harvest.

Stage 1 includes a diagnostic arthroscopy with full inspection of the knee joint to ensure the inclusion criteria are fulfilled. Loose bodies are removed, any meniscal pathology is addressed. Inflamed synovium is debrided. The lesion is stabilized by debridement around the edges and down to the subchondral bone using a ring curette, but not through it, as described above. Cartilage biopsy is then taken from the non-weightbearing area of the medial aspect of the femoral notch. If this area does not contain enough healthy cartilage, the secondary donor site will be the non-weight bearing aspect of the lateral femoral condyle. Complications or donor site morbidity following arthroscopic harvesting of cartilage in this manner has not been described [22].

Stage 2: Chondrocyte growth in Ex Vivo Laboratory Rikshospitalet, Oslo University Hospital

The harvested cartilage is transported to the cell culture laboratory (Ex Vivo Laboratory at OUS, directed by Professor Jan Brinchmann) in a sterile tube containing 0.9 % NaCl. The cells undergo mechanical mincing and antibiotic washing, and isolation of the chondrocytes by overnight collagenase digestion. The chondrocytes are then cultured for two weeks and resuspended to a treatment density of 30 million cells/ml. The cells are then transported back to Ahus for implantation.

Stage 3: Implantation of chondrocytes two weeks after initial arthroscopy.

The chondrocyte implantation is performed under general or spinal anesthesia, and a tourniquet inflated to 300 mmHg to achieve a blood-less field is applied to the upper thigh. A mini-open arthrotomy (medial or lateral depending of the location of the lesion) is performed and the lesion is assessed. The lesion is curetted down to subchondral bone, but care is taken to avoid bleeding. The surrounding cartilage is debrided to healthy tissue, exposing the lesion to bare bone. The lesion is measured and a template of sterile aluminum foil is used to model the exact shape of the lesion, overcorrecting with 1-2 millimeters.

The template is then used to cut out a matching piece of collagen sheet (ChondroGide® (Geistlich Pharma, Switzerland)) which is used to contain the cells in the defect. The flap is sutured to the lesion with 6.0 resorbable stitches and sealed with fibrin glue, leaving and opening at the upper part for injection of the cells. Saline is injected to the cavity to check for leakage, then aspirated before the cells are slowly injected using a soft catheter. The last opening is then closed with a last stitch and fibrin glue. The knee is then closed in the standard manner, taking care to close the capsule with subcutaneous resorbable sutures, before closing the skin incision with nylon sutures.

Arthroscopic Debridement

The AD group will be subjected to a diagnostic arthroscopy with a full inspection of the knee joint to ensure the inclusion criteria are fulfilled. Lose bodies are removed, any meniscal pathology is addressed. Inflamed synovium is debrided. The lesion is stabilized by debridement around the edges and down to the subchondral bone using a ring curette, but not through it. Microfracture or any other cartilage treatment will not be performed.

No intra-articular local anesthetics will be used due to the possible harmful effect on cartilage [41-43].

All the operating surgeons will receive proper training in the operative procedure before study start.

5.4. Operative procedure – blood samples for parallel study

On the day of surgery, all patients will have a 5 mL venous blood sample drawn through a standard puncture of vena cubuti in the elbow using a 22 gauge needle. The fasting blood sample will be taken after 30 minutes of bedrest, to limit the influence of preceding physical activity. The blood sample is part of the healer – nonhealer study by professor Jan Brinchmann at Oslo University Hospital – Rikshospitalet. For more information see Section "7.2. Parallel study: "Healer / NonHealer"

5.5. Post-operative management

The two treatment groups will receive identical postoperative care. The patients are usually admitted to the hospital for up to 4 days. No intravenous prophylactic antibiotics is given, anti-thrombotic prophylaxis is given only when there is a risk of thromboembolic disease (such as previous deep vein thrombosis or pulmonary embolus, protein C or S deficiency, Leiden V mutation and use of contraceptive pills). All patients will be given a sick leave up to 2 weeks after the operation.

If a patient experiences complications during treatment such as wound infections, catching or locking etc. the patient will receive medical attention and follow-up according to the problem.

5.6. Rehabilitation protocol

The two treatment groups will receive identical rehabilitation protocol according to a modification of Wondrasch et al [17]. The study will have a designated study physiotherapist, Heidi A. Hanvold, leading the rehabilitation program along with the patients local physiotherapist.

Rehabilitation phases	Physiotherapy and activities	Objectives	Criteria for progression to next phase
Phase1: Accomodation	Education/coaching Ice, elevation and compression Isometric exercises Range of motion Gait training (no weight-bearing for two weeks)	Reduce pain and swelling Normalize range of motion Regain quadriceps control	No pain & swelling during activities of daily living (ADL) Flexion 90° Normalized quadriceps activity while walking (clinical evaluation by the physical therapist)
Phase2: Rehabilitation	Stationary bike cycling Progressive knee and hip resistance training Neuromuscular training	Recovery of full range of motion Normalize muscle strength Dynamic joint stability during ADL	Full range of motion No pain or swelling during and after training sessions Equally distributed weight on the lower limbs during weight-bearing exercises with no shift of the trunk (visually assessed by the physical therapist) Ability to stand on 1 limb on a flat surface for at lest 10 seconds
Phase 3: Return to activity	Knee and hip resistance training Neuromuscular training Cardiovascular training	Recovery of strength and neuromuscular control Return to activity/sport	Return to sport based on individual assessment

Rehabilitation protocol (identical for both groups).

The patients are admitted to the hospital for 2-4 days. The patients are seen by a physiotherapist and the surgeon day 1 after surgery to be instructed in range of motion

exercises and restrictions according to Phase 1. The patient is seen within two weeks by a local physiotherapist who has received information about the rehabilitation program, and who will follow the patient through the program, supervised by the Project Physiotherapist (Heidi A Hanvold).

The rehabilitation program is an active rehabilitation and education program divided into 3 phases: accommodation (1), rehabilitation (2) and return to activity (3). During the rehabilitation program the physical therapist focuses on explaining why the exercises are important and how the exercises should be performed and adjusted based on pain response and other symptoms. The rehabilitation program consists primarily of cardiovascular and knee/hip progressive resistance and neuromuscular training, including balance and plyometric exercises. The local physical therapist receive information about the rehabilitation program, including what kind of exercises the patients should perform.

To monitor the adherence to the rehabilitation program, all patients are asked to use training diaries to provide information about frequency, type of exercise, load progression and number of repetitions. In addition, the patients are asked to respond every second week to an online survey, with the questions; 1. Have you been to a supervised physical therapy session during the last two weeks?, 2. How many physical therapy sessions did you attend during the last two weeks?, 3. What kind of training/exercises have you performed during the physical therapy sessions?, and 4. What kind of activities have you performed during the last two weeks? All questions are followed by several predefined answers (closed answers), but also open answers are included to make comments if required. The online survey will continue as long as the patient is under the care of a physiotherapist, while the training diary will be continued to the end of the project (24 months) to estimate the amount of home exercise performed by the patient.

<u>Phase 1 - accomodation:</u>

Inpatient rehabilitation consists of placing the leg in a Continuous Passive Motion (CPM) machine within the range as tolerated due to pain and swelling, aiming to achieve 30-70° day 1 after surgery. The patients should use the machine for 6-8 hours every 24 hours. The physical therapist instructs the patients in exercises such as active dorsiflexion/ plantar flexion of the ankle to encourage lower extremity circulation and isometric contraction of the quadriceps, hamstrings, and gluteal musculature to maintain muscle tone and minimize muscle loss. This also includes abduction exercises for the hip. The patients remain non-weight bearing for two weeks, but are allowed touch-down weight-bearing (the foot or toes may touch the floor, but not support any weight) through the affected limb using crutches.

When discharged from the hospital, the patients are encouraged to continue range of motion exercises; flexion/extension of the knee 500 repetitions three times a day. In this phase, 2 to 3 supervised physical therapy sessions are scheduled for each patient. Interventions such as ice, compression, electrical muscle stimulation, muscle activation of the quadriceps, hamstrings, gastrocnemius and gluteal muscles and gait training is included. Swimming is allowed when the wounds are healed.

After two weeks, protected weight-bearing is carefully introduced within the pain threshold and gradually increased up to full weight-bearing (continues into phase 2). Crutches are used until the patient walks normal without limping.

Phase 2 - Rehabilitation:

The patients attends 2 or more supervised physical therapy sessions per week, in addition they perform 1 to 2 unsupervised training sessions per week. Cardiovascular training on a stationary bike and progressive knee and hip resistance training and neuromuscular training are performed in this phase. All strengthening exercises are performed with both the injured and uninjured limbs. When full weight-bearing is achieved, long distance walking with increasing distances is encouraged and cross-country skiing can be allowed.

Phase 3 – Return to activity/sport:

This phase is individualized according to the goals for each patient. The patients attend 1 or more supervised sessions per week, in addition they perform resistance training for a minimum of 2 and a maximum of 4 sessions per week. Cardiovascular and neuromuscular training can be performed daily. Where a return to sport is planned, it is important that sport-specific activities are included as functional progressions within the rehabilitation program.

6. VARIABLES

6.1. Demographics

Demographics to be collected at inclusion is age, gender, height (cm), weight (kg), BMI, injury mechanism (if any), previous medical history, current medication, smoking, social status, work status and nationality.

6.2. Primary endpoint

The Knee Injury and Osteoarthritis Outcome Score (KOOS) is a patient reported outcome measure validated to use in cartilage research studies and will enable comparison of our results with other reports. It assesses five domains; pain, symptoms, activity of daily living,

sport and recreational function and knee-related quality of life [44]. KOOS knee-related quality of life (QoL) subscore is the primary endpoint, where the primary aim is the difference in KOOS QoL subscore in the ACI group compared to the AD group at 2 years follow up. There will not be any interim analysis before 2 years follow up.

6.3. Secondary endpoints

A combination of self-explanatory questionnaires, clinical parameters, clinical hop tests and radiographs and Magnetic Resonance Imaging (MRI) will be used as secondary endpoints. The secondary aims will be the difference between the two treatment groups and within the group at predefined times as described below. The secondary aims are:

- KOOS score: all subscores except the knee-related QoL subscore which is the primary aim.
- Tegner score; To evaluate the level of physical activity.
- Lysholm score; A condition-specific outcome score containing eight domains; limp, locking, pain, stair-climbing, use of support, instability, swelling and squatting.
- EQ-5D; A generic measure of health status that provides a simple descriptive profile used in the clinical evaluation of health care. EQ-5D is also widely used by clinical researchers and recommended for use in cost-effectiveness analyses by the Washington Panel on Cost Effectiveness in Health and Medicine [45] and by the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) task force on good clinical practices: Randomized Clinical Trial-Cost-Effectiveness Analysis (RCT-CEA) [46].
- Visual analogue scale (VAS); A visual analogue scale for pain, where 0 represents no pain and 10 represents the worst pain imaginable.
- Range of motion (ROM) will be measured with a goniometer
- Costs; resource use related to the intervention, medication, rehabilitation, use of health care services and production loss.
- The patients will also provide information about work (return to work) physical activity and return to sport.

All outcome questionnaires will be completed by the patients before surgery (pre-operative or baseline values) and at the designated research follow-up appointments at 3 (±2 weeks), 6 (±4 weeks), 12 (±6 weeks), and 24 (±8 weeks) months.

At 24 months follow up, there will be 3 additional elements consisting of:

- Standing x-rays to evaluate any development of osteoarthritis.
- Magnetic Resonance Imaging (MRI); assessing the quality of the cartilage tissue, using a specific cartilageMRI technique, to assess the healing of the defect.
- A hop test, validated and described previously by Noyes, to assess clinical function [47].

We will also invite patients to attend a 5 year and 10 year follow up appointment. During these late controls, all the primary and secondary outcomes will be assessed for as described above.

7. HYPOTHESIS, STATISTICS ANALYSIS AND SAMPLE SIZE

7.1.Hypothesis for the primary aim

Null hypothesis: There is no difference in KOOS subscore quality of life following ACI or AD treatment of a symptomatic cartilage defect (>2 cm^2) in the weight bearing area of the knee 2 years after surgery.

The alternative hypothesis claims that differences between ACI and AD in KOOS quality of life subscore exist.

7.2.Statistical analysis

Demographic and clinical characteristics will be presented as means and standard deviations (SD) or frequencies and percentages, as appropriate. The normality of continuous data will be assessed by examining the histograms. If necessary, a suitable transformation will be considered to symmetrize the data.

Due to along follow-up period with 5 (7) measurement points, repeated observations will be available for each patient. A mixed model correctly adjusting for intra-patient correlations will be used to assess the trend in primary and secondary end-points. The model will contain random effects for intercepts and slopes, if significant. Fixed effects for time (likely non-linear) and group will be included together with the interaction between the two. The interaction will quantify possible differences between arms regarding time profiles and serve as an omnibus test. For continuous end-points, linear mixed model will be estimated, while generalized linear model will be fitted to dichotomous end-points. Relevant pairwise comparisons will be performed by deriving individual time point contrasts within each study arm. The results will be presented as estimated means of odds ratios together with the corresponding 95% confidence intervals (CI) and p-values. The estimates will further be adjusted for possible confounders such as age of the patient and severity of the cartilage lesion in the multivariate regression models.

The results with p-values below 0.05 will be considered statistically significant. Two-sided tests will be used. The data analysis will be conducted using SPSS v.22 (SPSS Inc, Chicago, Illinois) and SAS v 9.4.

7.3.Sample size

It has previously been shown that a change in subscore of 8-10 of KOOS QoL (quality of life) is clinically significant [48,49]. Therefore, for power analysis a difference in change of 10

between two treatment groups was assumed. A SD for change of 15 [32] was used. With the power of 80% and significance level of 5% the estimated minimum number of patients was 37 in each group. By adding 10% due to loss during follow-up, we therefore plan to include a total of 82 patients.

8. SECONDARY PROJECTS

8.1. Cost-effectiveness analysis

In addition to the prospective randomized trial, we will also conduct a treatment-cost analysis. By recording the cost of each treatment, need of sick leaves, cost of additional doctor's appointments and repeated surgery due to recurrence or complications, we will estimate the cost effectiveness and not only the functional results. This provides the necessary economic frame of reference from which to resolve treatment recommendations.

To assess the cost-effectiveness of the two treatment we need to estimate both health outcome and costs. The health outcome will be measured by means of EQ-5D, while costs will include costs in the health care sector and production loss. To estimate costs, we will include the cost of the interventions, which includes the use of other health care services (home care services, rehabilitation and institution) which will be registered for both groups. Standard methods in economic evaluation will be applied, and cost-effectiveness will be calculated by means of the incremental cost-effectiveness ratio, defined by the cost per incremental QALY. Further uncertainty will be displayed by applying the bootstrap method with 1000 replications to illustrate the variation in the patient population with regard to incremental health gain and cost.

8.2. Parallel study: "Healer-NonHealer"

Patients accepting enrollment, will also be asked participation in the "Healer-NonHealer" project run by Professor J. Brinchmann at Oslo University Hospital – Rikshospitalet. Patients will have a 5 mL venous blood sample drawn as described in Section "5.4 Operative procedure – blood samples for parallel study". The fasting blood sample will be taken after 30 minutes of bedrest, to limit the influence of preceding physical activity.

With this blood sample, we want to investigate the concentration of microRNA-140 (miR-140). MicroRNAs (miRNAs) are a class of endogenous and non-coding single-strand RNAs, and have been associated with various diseases [50, 51]. MiR-140 is cartilage specific, is the most prevalent miR in normal human cartilage and have shown to regulate homeostasis and development ofcartilage[52]. It has been found to play a significant role in cartilage pathogenesis [50, 53, 54]. Deletion of miR-140 in mice predisposed to osteoarthritis (OA) like changes [55], and decreased expression of miR-140 in OA cartilage may contribute to the abnormal gene expression pattern characteristic of OA [53, 55, 56].

Since miR-140 will be found in serum and not whole blood, the peripheral venous blood sample will be collected in sterile tubes without any anticoagulant. The tubes will be in a standing position for 40 minutes for clotting, before centrifuged at 3700 rpm for 7 minutes at 20 degrees Celsius. The serum will then be pipetted in sterile empty tubes and the remaining blood ingredients destroyed. The samples are frozen down using a slow freeze technique and stored at – 80 degrees at the biochemical department following the recommendations put forward by Chevillet et al [57]. All blood and serum sampling will be performed by a dedicated biochemist with study protocol training. The serum samples will within 3 weeks delivered to the Ex-Vivo Laboratory at Rikshospitalet, Oslo, for analysis. All samples will be transported frozen at – 80 degrees Celsius in an upright position.

Analysis will be performed at the Norwegian Center for Stem Cell Research, University of Oslo/Oslo University Hospital – Rikshospitalet. Total RNA and miRNA will be purified from serum using the miRNeasy mini kit following protocols from the manufacturer (Qiagen, Germantown, MD). cDNA synthesis and RT-qPCR will be performed following protocols from the manufacturer using the High Capacity cDNA Reverse Transcripton Kit (Applied Biosystems, Abingdon, U.K) [58]. Subsequently RT-qPCR will be performed using Taqman Gene or MicroRNA Expression assays and Taqman Universal PCR master mix with the 7300 Real-Time RT PCR system (Applied Biosystems). After analysis of miR-140, the remaining serum will be destroyed.

In addition, the patients randomized to the ACI group will be asked to donate the left over cartilage removed from the rim of the cartilage lesion during the preparation for the cartilage implantation (the second operation). Normally, this tissue is discarded, but in this case we will collect this (pathological) cartilage and send it to the Norwegian Center for Stem Cell Research for analysis similar to the one described above. The purpose is to detect biomarkers in the damaged cartilage that predicts good or poor outcome, as defined according to the primary and secondary outcome in this RCT. After analysis, the remaining cartilage will be destroyed.

9. INSTITUTIONS AND RESPONSIBLE INVESTIGATORS

9.1. Institutions

Inclusion and operation will be conducted at:

- Akershus University Hospital (Sykehusveien 25, 1478 Lørenskog)
- Oslo University Hospital – Ullevål (Kirkeveien 166, 0450 Oslo)

The postoperative follow-up will be performed at a designated research out-patient clinic at Akershus University Hospital.

Both hospitals will obtain venous blood samples from all included patients which is sent for analysis to Oslo University Hospital – Rikshospitalet (Sognsvannsveien 20, 0372 Oslo).For more see Section "7.2. Secondary Projects: Healer Non-healer".

9.2.Responsible investigators

- Professor Asbjørn Årøen (Head supervisor, Akershus University Hospital)
- Per-Henrik Randsborg, MD, PhD (local coordinator, Akershus University Hospital)
- Sverre Løken, MD, PhD (local coordinator Oslo University Hospital – Ullevål)
- Professor Jan Brinchmann (local coordinator Oslo University Hospital – Rikshospitalet)
- Heidi A. Hanvold (PT and NCP Research coordinator, Ahus).

10. ETHICS

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice and applicable regulatory requirements. Registration of patient data will be carried out in accordance with national personal data laws.

The investigator is responsible for giving the patients full and adequate verbal and written information about the nature, purpose, possible risk and benefit of the study, includingthe parallel project Healer Non-healer, the surgical methods, the possible complications and the scheduled follow-ups. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever she/he wants. This will not prejudice the patient's subsequent care. All patients will be given time to think between proposal and inclusion in the study. Documented informed consent must be obtained for all patients included in the study before they are registered in the study. This will be done in accordance with the national and local regulatory requirements. The investigator is responsible for obtaining signed informed consent.

A copy of the patient information and consent will be given to the patients. The signed and dated patient consent forms will be filed in the Investigator Site File binder and also scanned to be part of the patient's electronic medical record at the hospital.

The patient will be informed as soon as possible, if new information becomes available that

may affect willingness to participate in the study.

The study protocol, including the patient information and informed consent form to be used, must be approved by the regional ethics committee before enrolment of any patients into the study.

The investigator is responsible for informing the ethics committee of any serious and unexpected adverse events and/or major amendments to the protocol as per national requirements.

The trial will be registered on https://clinicaltrials.govand www.helseforsk.no before inclusion of the first patient, and the trial is subject to pre-approval by the regional ethical committee.

11. QUALITY-SECURITY CONTROL/SAFE STORAGE OF SENSITIVE DATA

The study will be monitored by an employee (N. Weldingh) with formal monitoring qualifications at Akershus University Hospital. Authorized representatives of a Competent Authority and Ethics Committee may visit the centre to perform inspections, including source data verification. Likewise the representatives from sponsor may visit the center to perform an audit. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (ICH GCP), and any applicable regulatory requirements. The principal investigator will ensure that the inspectors and auditors will be provided with access to source data/documents.

All data is primarily registered on paper in form of Clinical Research Forms (CRFs). These CRFs include the validated patient reported outcome measures scores (PROMS) such as KOOS, Lysholm Score, Tegner score and EQ5D, as well as baseline information such as age, sex, injury mechanism, work status and the VAS score for pain. They will also include inclusion and exclusion criteria. The CRFs are stored in a designated room that is locked in the department's doctor's office. This room is only available to the personal treating the patient. A designated research coordinator, H Hanvold, PT, will continuously follow up the patients to make sure the patients are completing their training diary. At the patient's appointment, the treating doctor secures that the patient completes all the questionnaires. Objective measurements and any complications are recorded. The CRF data is then transferred digitally to a secure investigation server allocated at the University of Oslo (Services for Sensitive Data, SSD). Sensitive data will be anonymized and kept in a secure coded database and is traceable by a code that is kept safe and accessible only by the prime investigators. It will not be possible to identify patients in the results of the study when these are published, and data will be stored for 10 years after inclusion.

12. RISK ASSESSMENT

Some patients may find it unpleasant when asked about demographic information. The treatment of choice in this patient population is not established, but standard clinical care include both arthroscopic debridement (AD) and autologous chondrocyte implantation (ACI). There are no additional risks of this study other than the potential risks of standard clinical practice.

Potential risk of AD is rare with a frequency < 1% and include infection, deep vein thrombosis (DVT), arthralgia, headache, joint effusion/swelling, nasopharyngitis.

Potential risk of ACI is rare, but slightly higher than for AD, as this treatment involves a second operation with a mini-opening of the knee. Four major complications requiring surgery following ACI have been identified [25]:

- Malfusion of the repair tissue to the bed of the lesion (3.8 %)
- Delamination of healthy cartilage near lesion (2.8 %)
- Hyperthrophy of the repair tissue (> 2 %)
- Insufficient degenerative tissue (> 2 %)

Other complications are rare and occurs in < 1 % of patients, such as wound break down, infection, DVT and joint stiffness .

Both treatments have a risk of failure, meaning that the pain and stiffness in the knee does not improve despite surgery, and further surgery might be required.

13. COST AND FINANCING PLAN

13.1. Cost

All follow ups up to 12 months are standard practice. Outside standard, the 24 month follow up will generate extra costs. X-ray and MRI will cost approximately 1000 Norwegian kroner (NOK) per patients in total. This gives a cost of 82 000 NOK outside standard clinical practice, in addition to the cost of running the research outpatient-clinic. The cost of chondrocyte production at Ex Vivo Laboratory is estimated at 35.000 NOK per patient. The ChondroGide® (Geistlich Pharma, Switzerland) membrane used during chondrocyte implantation costs 7500 NOK.

13.2. Financing plan

The study is funded by research grants already awarded NCP from the Regional Health Authority (Regionale Helseforetak, tverregionale midler).

14. TIME SCHEDULE AND PUBLICATION PLAN

14.1 Time Schedule

The inclusion will start in the first quarter of 2016 and is expected due the first quarter of 2019. Follow up is 2 years. Data collection is estimated to 6 months and data analysis is estimated to 6 months. Publication is expected 6 months after first submission.

14.2 Publication plan

The results will be published in peer reviewed orthopaedic journals, as well as presented to the orthopaedic community at national and international scientific meetings. They will also be actively communicated to the community by using the hospital web page with publication of the studies.

Upon study completion and finalization of the study report the results of this study will be submitted for publication and/or posted in a publicly assessable database of clinical study results.

The results of this study will also be submitted to the Competent Authority and the Ethics Committee according to EU and national regulations.

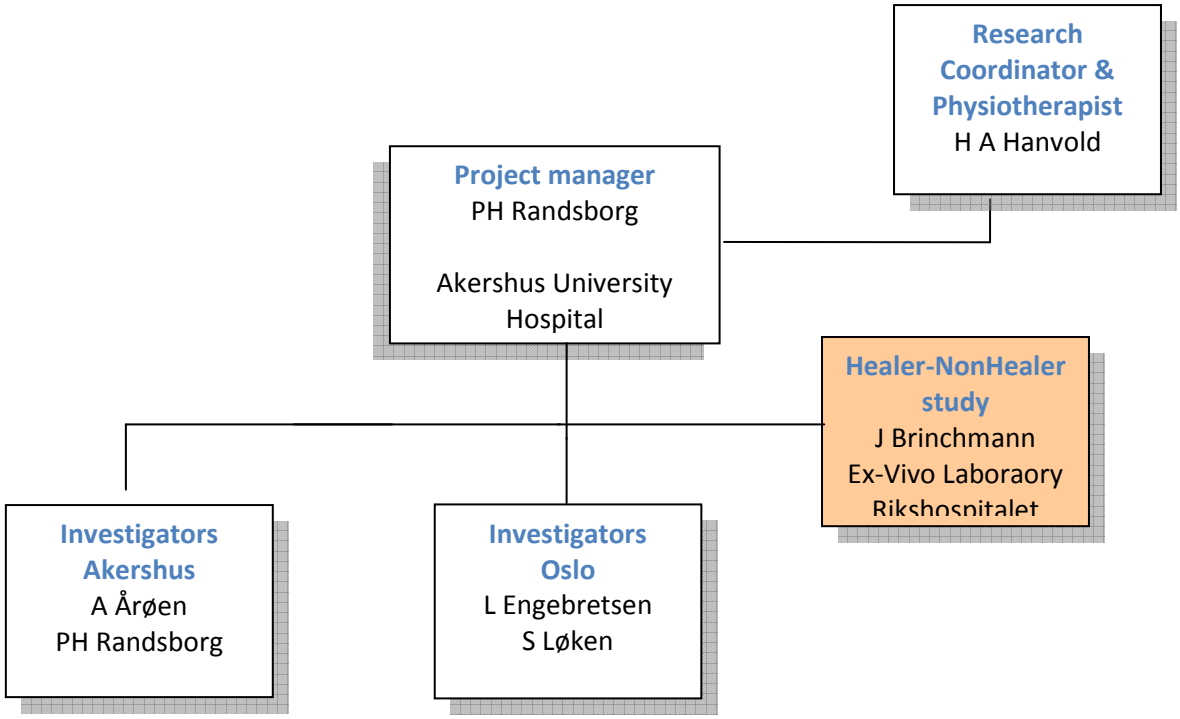
All personnel who have contributed significantly with the planning and performance of the study (Vancouver convention 1988) may be included in the list of authors.

The study will result in at least the following publications:

1. Autologous Chondrocyte Implantation versus Arthroscopic Debridement for the treatment of focal cartilage lesions of the weight bearing area of the knee. A randomized controlled trial – results at 24 months.
2. Cost related to Autologous Chondrocyte Implantation and Arthroscopic Debridement of cartilage lesions in the knee; considering the costs of surgery, hospitalization and follow-up, sick-leave and the need for secondary surgery.
3. Autologous Chondrocyte Implantation versus Arthroscopic debridement for the treatment of focal cartilage lesions of the weight bearing area of the knee. A randomized controlled trial – results at 5 years.

15. STUDY ORGANIZATION

Study organization



16. REFERENCES

1. Frisbie DD, O.J., Southwood L, Trotter GW, Rodkey WG, Steadman JR, Goodnight JL, McIlwraith CW, *Early events in cartilage repair after subchondral bone microfracture.* Clin Orthop Relat Res, 2003. **407**: p. 215-27.
2. Ossendorf C1, K.C., Kreuz PC, Burmester GR, Sittinger M, Erggelet C., *Treatment of posttraumatic and focal osteoarthritic cartilage defects of the knee with autologous polymer-based three-dimensional chondrocyte grafts: 2-year clinical results.* Arthritis Res Ther, 2007. **9**(2): p. R41.
3. Bedi A, F.B., Williams RJ 3rd., *Management of articular cartilage defects of the knee.* J Bone Joint Surg Am, 2010. **92**(4): p. 994-1009.
4. Basad E, I.B., Bachmann G, Stürz H, Steinmeyer J *Matrix-induced autologous chondrocyte implantation versus microfracture in the treatment of cartilage defects of the knee: a 2-year randomised study* Knee Surgery, Sports Traumatology, Arthroscopy, 2010. **18**(4): p. 519-527.
5. Messner K, G.J., *Cartilage repair. A critical review.* Acta Orthop Scand, 1996. **67**(5): p. 523-9.
6. Løken S, H.S., Holme I, Engebretsen L, Årøen A, *6-Year follow-up of 84 patients with cartilage defects in the knee. Knee scores improved but recovery was incomplete.* Acta Orthop, 2010. **81**(5): p. 611-618.
7. Minas T, N.S., *Current concepts in the treatment of articular cartilage defects.* Orthopedics, 1997. **20**: p. 525-538.
8. Prakash D, L.D., *Natural progression of osteo-chondral defect in the femoral condyle.* Knee 2002. **9**(1): p. 7-10.

9. Steadman JR, H.C., Briggs KK, Matheny LM, James EW, Guillet A., *Outcomes after knee microfracture of chondral defects in alpine ski racers.* J Knee Surg, 2014. **27**(5): p. 407-410.
10. Goyal D, K.S., Lee EH, Hui JH, *Evidence-based status of microfracture technique: a systematic review of level I and II studies.* Arthroscopy, 2013. **29**(9): p. 1579-88.
11. Madry H, G.U., Knutsen G, *Cartilage Repair and Joint Preservation.* Deutsches Ärzteblatt International, 2011. **108**(40): p. 669-77.
12. Özmeriç A, A.K., Aydoğan NH, *Treatment for cartilage injuries of the knee with a new treatment algorithm.* World Journal of Orthopedics, 2014. **18**(5): p. 677-684.
13. Farr J, C.B., Dhawan A, Kercher J, Sherman S, *Clinical Cartilage Restoration. Evolution and Overview.* Clin Orthop Relat Res, 2011. **469**: p. 2696-2705.
14. Oussedik S, T.K., Parker D, *Treatment of Articular Cartilage Lesions of the Knee by Microfracture or Autologous Chondrocyte Implantation: A Systematic Review* Arthroscopy, 2015. **31**(4).
15. Jakobsen RB, E.L., Slauterbeck JR, *An Analysis of the Quality of Cartilage Repair Studies.* J Bone Joint Surg Am, 2005. **87**(10): p. 2232-2239.
16. Hurst JM, S.J., O´Brien L, Rodkey WG, Briggs KK, *Rehabilitation Following Microfracture for Chondral Injury in the Knee.* Clin J Sport Med, 2010. **29**: p. 257-265.
17. Wondrasch B, Aroen A, Røtterud JH, Høysveen T, Bølstad K, Risberg MA, *The Feasibility of a 3-Month Active Rehabilitation Program for Patients With Knee Full-Thickness Articular Cartilage Lesions: The Oslo Cartilage Active Rehabilitation and Education Study.* Journal of orthopaedic & sports physical therapy, 2013. **43**(5): p. 310-324.
18. McCambridge J, W.J., Elbourne DR, *Systematic review of the Hawthorne effect: New concepts are needed to study research participation effects.* J Clin Epidemiol. , 2014. **67**(3): p. 267-277.
19. Vasiliadis and Wasiak, Autologous Chondrocyte Implantation for full thickness articular cartiage defects of the knee. Cochrane report, 2010
20. Dozin B, Malpeli M, Cancedda R, Bruzzi P, Calcagno S, Molfetta L, Priano F, Kon E, Marcacci M, *Comparative evaluation of autologous chondrocyte implantation and mosaicplaty - a multicentered randomized clinical trial.* Clin J Sport Med 2005. **15**: p. 220-226.
21. Bekkers JEJ, I.M., Saris DBF, *Treatment selection in articular cartilage lesions of the knee: a systematic review.* Am J Sports Med, 2009. **37**: p. 148S-155S.
22. Peterson L, Vasiliadis HS; International Experience with Autologous Chondrocyte Implantation, Chapter 22. In: Insall & Scott Surgery of the Knee, Fifth Edition, 2012 Elsevier Churchill Livingstone, p. 163-177
23. Brittberg M, Lindahl A, Nilsson A et al: *Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation.* 1994. N Engl J Med **331**: p. 889-895.
24. Blevins FT, S.J., Rodrigo JJ, Silliman J, *Treatment of Articular Cartilage Defects in Athletes: An Analysis of Functional Outcome and Lesion Appearance.* Orthopedics, 1998. **21**(761-768).
25. Niemeyer P, Pestka J, Kreuz P et al, *Characteristic Complicationsafter ACI of the Knee Joint AJSM* 2008. **36** p. 2091-2099.
26. Mithoefer K, M.T., Williams RJ, Kreuz PC, Mandelbaum BR, *Clinical Efficacy of the Microfracture Technique for Articular Cartilage Repair in the Knee. An Evidence-Based Systematic Analysis.* Am J Sports Med, 2009. **37**(10).
27. Behery O, S.R., Harris JD, Flanigan DC, *Treatment of Cartilage Defects of the Knee: Expanding on the Existing Algorithm.* Clin J Sport Med, 2014. **24**: p. 21-30.
28. Gudas R, S.E., Monastyreckiene E, Pranys D, Kalesinskas RJ, *Osteochondral autologous transplantation versus microfracture for the treatment of articular cartilage defects in the knee joint in athletes.* Knee Surg Sports Traumatol Arthrosc, 2006. **14**: p. 834-842.
29. Miller BS, S.J., Briggs KK, Rodrigo JJ, Rodkey WG, *Patient satisfaction and outcome after microfracture of the degenerative knee.* J Knee Surg, 2004. **17**(1): p. 13-17.
30. Asik M, C.F., Sen C, Erdil M, Atalar A, *The microfracture technique for the treatment of full-thickness articular cartilage lesions of the knee: midterm results.* Arthroscopy, 2008. **24**: p. 1214-1220.

31. de Windt, T., Bekkers JEJ, Creemers LB, Dhert WJA, Saris DBF, *Patient Profiling in Cartilage Regeneration - Prognostic Factors Determining Success of Treatment for Cartilage Defects.* Am J Sports Med, 2009. **37**: p. 58S-62S.
32. http://www.koos.nu/koosfaq.html#mic
33. Mundi R, B.A., Chow L, Crouch S, Simunovic N, Sibilsky Enselman E, Ayeni OR., *Cartilage Restoration of the Knee: A Systematic Review and Meta-Analysis of Level 1 Studies.* Am J Sports Med, 2015. **XX**(X).
34. Collins N, M.D., Felson DT, Crossley KM, Roos EM, *Measures of Knee Function.* Arthritis Care & Research, 2011. **63**(11): p. S208-S228.
35. Saris D, P.A., Widuchowski W, Bertrand-Marchand M, Caron J, Drogset JO, Emans P, Podskubka A, Tsuchida A, Kili S, Levine D, Brittberg M, *Matrix-Applied Characterized Autologous Cultured Chondrocytes Versus Microfracture: Two-Year Follow-up of a Prospective Randomized Trial.* Am J Sports Med, 2014. **42**(6): p. 1384-94.
36. Ulstein S, Å.A., Røtterud JH, Løken S, Engebretsen L, Heir S, *Microfracture technique versus osteochondral autologous transplantation mosaicplasty in patients with articular chondral lesions of the knee: a prospective randomized trial with long-term follow-up.* Knee Surg Sports Traumatol Arthrosc, 2014. **22**(6): p. 1207-15.
37. Kellgren JH, L.J., *Radiological assessment of osteo-arthrosis.* Ann Rheum Dis, 1957. **16**(4): p. 494-502.
38. Peterfy C, L.J., Zaim S, Duryea J, Lynch J, Miaux Y, Yu W, Genant HK, *Comparison of fixed-flexion positioning with fluoroscopic semi-flexed positioning for quantifying radiographic joint-space width in the knee: test-retest reproducibility.* Skeletal Radiol, 2003. **32**(3): p. 128-32.
39. Brittberg M, W.C., *Evaluation of cartilage injuries and repair.* J Bone Joint Surg Am, 2003. **85**(-A(Suppl 2)): p. 58-69.
40. Årøen A, R.J., Sivertsen EA, *Agreement in Arthroscopic and Arthrotomy Assessment of Full-Thickness Articular Cartilage Lesions of the Knee in a Clinical Setting in 33 Consecutive Patients.* Cartilage, 2013. **4**(3): p. 214-218.
41. Ravnihar K, B.A., Drobnič M, *Effect of intra-articular local anesthesia on articular cartilage in the knee.* Arthroscopy, 2014. **30**(5): p. 607-12.
42. Chu CR, C.C., Chu CT, Szczodry M, Seshadri V, Karpie JC, Cieslak KM, Pringle EK, *In vivo effects of single intra-articular injection of 0.5% bupivacaine on articular cartilage.* J Bone Joint Surg Am, 2010. **92**(3): p. 599-608.
43. Lo IK, S.P., Chung M, Liang S, Boorman RB, Thornton GM, Rattner JB, Muldrew K, *Local anesthetics induce chondrocyte death in bovine articular cartilage disks in a dose- and duration-dependent manner.* Arthroscopy, 2009. **25**(7): p. 707-15.
44. Roos EM, R.H., Lohmander LS, Ekdahl C, Beynnon BD, *Knee Injury and Osteoarthritis Outcome Score (KOOS) - development of a self-administered outcome measure.* J Orthop Sports Phys Ther, 1998. **28**(2): p. 88-96.
45. Rabin R, d.C.F., *EQ-5D: a measure of health status from the EuroQol Group.* Ann Med., 2001. **33**(5): p. 337-43.
46. Ramsey S, W.R., Briggs A, Brown R, Buxton M, Chawla A, Cook J, Glick H, Liljas B, Petitti D, Reed S, *Good Research Practices for Cost-Effectiveness Analysis Alongside Clinical Trials: The ISPOR RCT-CEA Task Force Report.* Value In Health, 2005. **8**(5): p. 512-533.
47. Noyes FR, Barber SD, Mangine RE. Abnormal lower limb symmetry determined by function hop tests after anterior cruciate ligament rupture. *Am J Sports Med.* 1991;19(5):513-518.

48. Engelhart L, N.L., Lewis S, Mordin M, Demuro-Mercon C, Uddin S, McLeod L, Cole B, Farr J, *Validation of the Knee Injury and Osteoarthritis Outcome Score subscales for patients with articular cartilage lesions of the knee.* Am J Sports Med, 2012. **40**(10): p. 2264-2272.
49. Roos EM, T.-L.S., *Knee injury and Osteoarthritis Outcome Score (KOOS) - validation and comparison to the WOMAC in total knee replacement.* Health and Quality of Live Outcomes, 2003. **1**: p. 1-10.
50. Zhang R, M.J., Yao J, *Molecular mechanisms of the cartilage-spesific microRNA-140 in osteoarthritis.* Inflamm Res., 2013. **62**(10): p. 871-7.
51. Chevillet JR, L.I., Briggs HA, He Y, Wang K, *Issues and Prospects of microRNA-Based Biomarkers in Blood and Other Body Fluids.* Molecules, 2014. **19**: p. 6080-6105.
52. Karlsen TA, S.A., Brinchmann JE, *Human Primary Articular Chondrocytes, Chondroblasts-Like Cells, and Dedifferentiated Chondrocytes: Differences in Gene, MicroRNA, and Protein Expression and Phenotype.* Tissue Engineering, 2011. **17**(2): p. 219-227.
53. Miyaki S, N.T., Otsuki S, Grogan SP, Higashiyama R, Inoue A, Kato Y, Sato T, Lotz MK, Asahara H, *MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses.* Arthritis Rheum, 2009. **60**(9): p. 2723-30.
54. Tardif G, H.D., Pelletier J-P, Duval N, Martel-Pelletier J, *Regulation of the IGFBP-5 and MMP-13 genes by the microRNAs miR-140 and miR-27a in human osteoarthritic chondrocytes.* BMC Musculoskelet Disord, 2009. **10**: p. 148.
55. Miyaki S, S.T., Inoue A, Otsuki S, Ito Y, Yokoyama S, Kato Y, Takemoto F, Nakasa T, Yamashita S, Takada S, Lotz MK, Ueno-Kudo H, Asahara H, *MicroRNA-140 plays dual roles in both cartilage development and homeostasis.* Genes Dev., 2010. **24**(11): p. 1173-85.
56. Iliopoulos D, M.K., Oikonomou P, Tsezou A, *Integrative MicroRNA and Proteomic Approaches Identify Novel Osteoarthritis Genes and Their Collaborative Metabolic and Inflammatory Networks.* Plos One, 2008. **3**(11): p. e3740.
57. Chevillet JR, L.I., Briggs HA, He Y, Wang K, *Issus and Prospects of microRNA-Based Biomarkers in Blood and Other Body Fluids.* Molecules, 2014. **19**: p. 6080-6105.
58. Jakobsen RB, A.S., FP Reinholt and JE Brinchmann, *Chondrogenesis in a hyaluronic acid scaffold: comparison between chondrocytes and MSC from bone marrow and adipose tissue.* Knee Surg Sports Traumatol Arthrosc, 2010. **18**: p. 1407-1416.