DETAILED STATISTICAL ANALYSIS PLAN (SAP)

1.0 ADMINISTRATIVE INFORMATION

1.1 TITLE, REGISTRATION, VERSIONS AND REVISIONS

Full study title	Musculoskeletal function in anterior cruciate ligament reconstructed individuals with and without knee pain.
Acronym	MIRAKOS
Local project number	APPI2-PT-2020-02
Ethics committee number	H-20060332
Study protocol version	1.2 (6 December 2021)
SAP version	1.0 (23 February 2022)

SAP revision history

Version #	Issue date	Amendment

SAP revision justification	-
SAP revision timing	-

1.2 ROLES AND RESPONSIBILITY

Author	Tine Alkjær[1,2]
Statistician	Marius Henriksen[2]
Principle investigator	Tine Alkjær[1,2]
Contributors and roles	Elisabeth Bandak[1,2], contributing to design and measurements, SAP revision. Marius Henriksen[2], statistics, contributing to design, SAP revision. Lauri Stenroth[3], contributing to design, measurements and outcome calculation, SAP revision.
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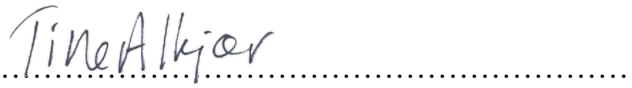
1.3 SIGNATURES

We the undersigned, certify that we read this SAP and approve it as adequate in scope of the main analyses of the MIRAKOS.

1.3.1 Author

Name: Tine Alkjær



Date:

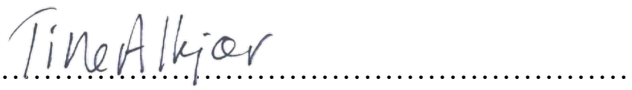
1.3.2 Statistician

Name: Marius Henriksen

Date: 23/02/2022

1.3.3 Principle investigator

Name: Tine Alkjær



Date:

2.0 INTRODUCTION

2.1 BACKGROUND AND RATIONALE

Knee osteoarthritis (OA) is the most common joint disease and a significant contributor to global disability [1]. The known knee OA risk factors include obesity, surgery, occupational load and injury [2,3]. Anterior cruciate ligament (ACL) rupture is a common knee injury [4,5] and the incidence is increasing, particularly among young people [6]. ACL injury affects the knee joint function and increases the risk of knee OA development [7-10] even at a young age, which prolongs the period of impaired function and pain [11]. Most research has focused on radiographic knee OA while fewer studies have investigated the prevalence of symptomatic knee OA after ACL injury [12]. It is important to discriminate between radiographic and symptomatic knee OA, as knee pain is a decisive criterion to diagnose knee OA [13], whereas radiographic changes serve more as a confirmatory measure. Indeed, the Framingham study showed that the prevalence of radiographic changes (indicative of OA) in the population older than 63 years was 33% whereas the prevalence of symptoms was only 9% [14]. A recent MRI study of 230 asymptomatic knees reported that 97% of these showed abnormalities in at least one knee structure [15]. This emphasizes that image-based signs of knee OA are not always accompanied by pain and OA symptoms.

Conventionally, mechanical joint loading is proposed as a key mechanism contributing to the development and progression of OA [16,17]. Thus, the knee joint loading during dynamic tasks in the ACL injured population has been studied extensively due to the supposed link between the knee joint compressive forces and the onset of post-traumatic knee OA [18-23]. However, the evidence for a causal link between knee joint loading and knee OA development and progression is weak [24,25]. Furthermore, a 15-year follow-up study, showed that ACL reconstructed persons returning to pivoting sport (presumably associated with high and multidirectional loads) had reduced odds of developing knee OA and had a better self-reported function in activities of daily living [26]. On the other hand, data suggest that ACL reconstructed individuals develop different adaptive neuromuscular functions [27,28], and it is possible that other mechanical factors than loading magnitude are implicated in the development of knee OA. Such other biomechanical factors may include force dissipation capacity of the musculoskeletal system [29], micro-incoordination [30], muscle strength and other aspects of muscle function. Low quadriceps muscle strength is associated with an increased risk of worsening symptoms and functional deterioration in people with and at risk of radiographic knee OA [31]. The quadriceps muscle strength and function are impaired after ACL injury and strength deficits persist even after ligament reconstruction [32-34]. Altogether, there are indices and a common agreement that poor musculoskeletal function is associated with increased risk of development of both symptomatic and radiographic knee OA, and that an ACL injury and reconstruction may lead to unfavourable changes in the musculoskeletal function accelerating the development of symptoms and/or degenerative OA changes. One study has compared individuals with definitive radiographic OA with and without symptoms and found that the symptomatic group had lower muscle strength and walking biomechanics indicative of a “stiffer” gait, possibly reflecting protective neuromuscular adaptations in the walking pattern [35]. As ACL injuries increase the risk of OA (symptomatic and radiographic) later in life, the musculoskeletal function may be changed alongside the early onset of symptoms but before definitive radiographic OA is present. Thus, the present study will compare the musculoskeletal function between ACL reconstructed individuals with and without knee pain. By this, we can deepen our understanding of the role of musculoskeletal function in relation to the development and progression of knee OA.

2.2 OBJECTIVES

2.2.1 Objectives and research questions

The objective of the present study is to compare the musculoskeletal function between ACL reconstructed individuals with and without knee pain to answer the research question: Are there differences in the musculoskeletal function in ACL reconstructed individuals with knee pain when compared to those without knee pain?

The musculoskeletal function will be assessed by

- Muscle strength of the knee extensor muscle (quadriceps)
- Biomechanics of the knee and quadriceps muscle during walking and a forward lunge movement

2.2.2 Hypotheses

1) ACL reconstructed individuals without knee pain have stronger quadriceps muscles compared to those with knee pain.
2) ACL reconstructed individuals without knee pain develop higher quadriceps muscle forces and knee joint loading during walking and forward lunging compared to those with knee pain.

2.2.3 Scope

This SAP is structured as recommended for observational studies[36]. It will be the guiding document for the main analyses testing the two hypotheses and will exclusively include outcomes obtained from the ACL reconstructed leg (see section 6.1.1).

The data obtained from the participants' contralateral (i.e. non-operated) leg will be reported in subsequent sub-studies with separate SAP documents.

3.0 STUDY METHODS

3.1 GENERAL STUDY DESIGN AND PLAN

This is a cross-sectional observational study. Two groups of ACL reconstructed individuals identified as symptomatic (with knee pain) and asymptomatic (without knee pain) are invited to participate in the study that takes place at The Parker Institute/Bispebjerg-Frederiksberg Hospital, Copenhagen, Denmark. The study protocol (APPI2-PT-2020-02) was written and approved by the local ethics committee before study initiation. The final protocol (version 1.2) is published on the Parker Institute's website. This SAP was written after the initiation of the data collection (June 2021) and finalized before the inclusion of the last participant. The author of the SAP was not blinded to the database during writing the SAP. However, data were not summarized or analyzed before completing the SAP.

3.2 SAMPLE SIZE, POWER AND DETECTABLE DIFFERENCE

We will compare the quadriceps muscle strength between two groups of ACL reconstructed individuals: 1) symptomatic and 2) asymptomatic.

The variance in this population is unknown, while our sample size estimation will be pragmatic. To detect a group difference of 0.3 Nm/kg in the primary outcome with a common standard deviation of 0.5 Nm/kg, a sample size of 50 per group will have a power of 84%, ($\alpha = 0.05$). Thus, a total sample size of n=100 (50/50 per group) was originally intended to be applied. However, in November 2021 71 (17/54 symptomatic/asymptomatic) participants were recruited and included in the study, indicating a low prevalence of symptomatic participants. We estimated that it would be difficult to recruit

50 symptomatic participants within the time allotted for recruitment (scheduled to last until August 2022). In contrast, the recruitment of participants to the asymptomatic group has proven to be efficient. Therefore, we decided to re-calculate the sample size and change the group allocation ratio from 1:1 to 1:3. To detect that same group difference with the same common standard deviation of the primary outcome as stated above, a new total sample size was estimated to n=120 (n=30 symptomatic and n=90 asymptomatic participants) with a statistical power of 80.6%. This amendment was registered in the study protocol 4th November 2021. Currently, our goal is to continue the data collection until the inclusion of 120 eligible participants (symptomatic/asymptomatic; n=30/90) is reached. However, the inclusion of participants will end in August 2022, leaving some uncertainty about the final sample size.

3.3 TIMING OF FINAL ANALYSIS

The final analysis will be done after 31/8 2022 but no later than 31/12 2022 and presupposes that the inclusion of participants has ended, all data have been collected and the database has been closed. The SAP will be published on the Parker Institute's website along with the study protocol before any data analyses are conducted.

3.4 TIMING OF OUTCOME ASSESSMENTS

The processing of motion capture data and musculoskeletal modelling to provide biomechanical variables is time-consuming and will be done after the measurement visit of each participant. Thus, the outcomes to assess the knee and quadriceps muscle function during walking and forward lunge movement are generated continuously and registered in the database during the data collection period. This will continue for a short period after the inclusion of the last participant. All other outcomes and data are registered in the database at or immediately after the measurement visit. The participants fill out electronic-format questionnaires for assessment of self-reported knee function at the measurement visit and these data are registered directly in the database. While the research team will not be blinded to the data, there will not be any data extraction before the data collection has ended/the database closed and the SAP has been finalized.

4.0 STATISTICAL PRINCIPLES

4.1 MULTIPLICITY

We will not adjust for multiplicity. We are fully aware that the risk of type I error is present as we will make many comparisons and our outcomes are very likely correlated. Thus, we will explicitly state that the analyses are exploratory and hypothesis-generating and that the results may need replication in studies with a more causal design.

4.2 STATISTICAL SIGNIFICANCE AND CONFIDENCE INTERVALS

A P-value < 0.05 are considered statistically significant for our primary outcome. Results will be reported as mean values with standard deviation (SD), or group mean differences with a 95% confidence interval (CI).

4.3 ADHERENCE AND PROTOCOL DEVIATIONS

4.3.1 Definitions of protocol deviations

Protocol deviations are defined as study activities that diverge from the local institutional review board reviewed protocol but without significant consequences[37].

4.3.2 Protocol deviations to be summarised

The following deviations from the protocol have been identified:

- The pressure pain sensitivity was assessed by *two* pressure pain thresholds: 1) the pressure pain detection threshold (PDT) and 2) the pressure pain tolerance threshold (PTT). The PTT defines the pressure, at which the pain becomes intolerable (section 6.2.7).
- The current knee pain was assessed by a verbal rating scale (VRS) 0-10 *both* during walking/forward lunging *and* during the muscle strength testing, meaning that after each walking/lunging/contraction trial the participant verbally rated the current knee pain on a 0-10 VRS (section 6.2.9).
- The knee joint laxity testing was part of the clinical examination *but* an instrumented knee joint laxity test was also done during the experiments to quantify the laxity and report it as a participant characteristic (section 6.2.10).
- The second hypothesis was originally formulated: "ACL reconstructed individuals without knee pain develop higher quadriceps muscle forces and knee joint compressive forces during walking and forward lunging compared to those with knee pain". This has been refined: "ACL reconstructed individuals without knee pain develop higher quadriceps muscle forces and knee joint loading during walking and forward lunging compared to those with knee pain". The reason for this change is that we assess *both* the knee joint compressive force *and* the knee extensor moment and these two parameters are covered under "knee joint loading".

The above was either not explicitly stated in the study protocol or reflects refinements to its content.

This SAP focus on measurements from the ACL reconstructed leg while observations from the contralateral leg will be reported in subsequent sub-studies (section 2.2.3).

5.0 STUDY POPULATION

5.1 SCREENING DATA

Screening data were collected with the purpose to describe the eligibility of all potential participants responding positively to the study invitation letter (see section 5.3). Thus, reasons for non-eligibility will be documented, and includes (but are not limited to):

- Major surgery to the other knee, e.g., ACL reconstruction.
- Other musculoskeletal pain in the lower extremities.
- BMI > 30.
- Neuromuscular diseases.

5.2 ELIGIBILITY

The aim was to compare participants with and without knee pain, the eligibility criteria were as follows:

Participants with knee pain ("Symptomatic group"):

Inclusion criteria:

- Age between 18 and 40 years at the time of ACL reconstruction.
- Primary ACL reconstruction using the semitendinosus-gracilis tendon graft.
- Post-surgery time of at least 3 years.

- Current body mass index (BMI) of ≤30.
- Pain score of at least 3 (VRS 0-10) in the reconstructed knee during activities of daily living (ADL) within the last week.

Participants without knee pain ("Asymptomatic group"):

Inclusion criteria:

- Age between 18 and 40 years at the time of ACL reconstruction.
- Primary ACL reconstruction using the semitendinosus-gracilis tendon graft.
- Post-surgery time of at least 3 years.
- Current body mass index (BMI) of ≤30.
- Pain score of 0 (VRS 0-10) in the reconstructed knee during activities of daily living (ADL) within the last week.

For both groups, the exclusion criteria are the same:

Exclusion criteria:

- Known neuromuscular diseases.
- Evidence of cartilage lesions ICRS grade 4 (full thickness) from MRI at time of ACL reconstruction or documented peri-surgically.
- ACL reconstruction or other major surgery to the other knee.
- Congenital deformities in the lower extremities preventing full participation in the tests.
- Current musculoskeletal pain in other regions of the lower extremity other than the injured knee.
- Any other condition that in the opinion of the investigator makes a potential participant unfit for participation or conditions that puts a potential participant at risk by participation.

5.3 RECRUITMENT

ACL reconstructed persons were identified in the Danish Ligament Reconstruction (DLR) Register and invited to participate in the study by sending them an invitation letter via digital mail (e-Boks) stating the main criteria for participation. A flow diagram will be used to visualize the flow of participants. Here we will report the population identified in the DLR register and from where eligible participants were selected, reasons for exclusions and how many were included and allocated to the symptomatic and asymptomatic group and any withdrawals. See figure 1 for an example.

5.4 WITHDRAWAL/FOLLOW-UP

This study is a cross-sectional observational study with no interventions applied. Thus, we expect the withdrawal rate to be negligible. Withdrawal can occur when the eligible participants refuse to participate or if other issues emerge preventing the participant from participating in the experiments after written informed consent has been obtained.

5.5 BASELINE PARTICIPANT CHARACTERISTICS

5.5.1 Collected baseline participant characteristics

Most of the data are collected at the measurement visit (one day) while only a few clinical data are extracted from electronic registry databases (e.g. DLR Register or patient record via the electronic medical journal "Sundhedsplatformen" (SP)) and few participants characteristics are registered during screening. Table 1 displays an overview of all the collected variables.

5.6 ASSUMED CONFOUNDING COVARIATES

Although, we cannot exclude that our measured variables may be influenced by measured and unmeasured variables (e.g. genetic, environmental, psychological) that potentially confound the interpretation of the results leading to wrong conclusions, we have not been able to identify any covariates that clearly would influence *both* the exposure (presence of knee pain) *and* outcome (musculoskeletal function). Thus, no adjustments for confounding covariates will be applied in our statistical analyses.

6.0 ANALYSIS

6.1 OUTCOME DEFINITIONS

The analysis of our primary and secondary outcomes shall answer the research question and test the two hypotheses (see section 2.2).

6.1.1 Study knee

The study knee is defined as the knee at which the ACL was reconstructed. The outcomes are obtained from the participants' study knee and used as input parameters to the statistical analyses (section 6.3).

6.1.2 Primary outcome

The primary outcome is the maximal isometric quadriceps muscle strength defined as the highest torque value measured among three separate maximal voluntary isometric contraction (MVIC) repetitions. The unit for the primary outcome is Nm/kg (see section 6.2.1).

6.1.3 Key secondary outcomes

The following outcomes are assessed as key secondary outcomes:

- The peak knee extensor moment during walking defined as the mean of the individual peak knee extensor moment values across six walking trials. The unit for this outcome is Nm/kg (section 6.2.2).
- The peak knee extensor moment during the forward lunge defined as the mean of the individual peak knee extensor moment values across three forward lunge trials. The unit for this outcome is Nm/kg (section 6.2.2).
- The peak quadriceps muscle force during walking defined as the mean of the individual peak quadriceps muscle force values across six walking trials. The unit for this outcome is N/kg (section 6.2.2).
- The peak quadriceps muscle force during the forward lunge defined as the mean of the individual peak quadriceps muscle force values across three forward lunge trials. The unit for this outcome is N/kg (section 6.2.2).
- The peak knee joint contact force during walking defined as the mean of the individual peak knee joint contact force values across six walking trials. The unit for this outcome is N/kg (section 6.2.2).
- The peak knee joint contact force during the forward lunge defined as the mean of the individual peak knee joint contact force values across three forward lunge trials. The unit for this outcome is N/kg (section 6.2.2).

6.1.4 Other secondary outcomes

The following outcomes are assessed as other secondary outcomes:

- The maximal isometric hamstring muscle strength defined as the highest torque value measured among three separate MVIC repetitions. The unit for the primary outcome is Nm/kg (section 6.2.1).
- The five Knee Injury and Osteoarthritis Outcome Score (KOOS) subscales (pain; symptoms; function in activities of daily living; function in sports and recreational activity; knee-related quality of life (QoL) (section 6.2.3).
- The International Knee Documentation Committee (IKDC) score (section 6.2.4).
- The intermittent and constant osteoarthritis pain (ICOAP) total score and two subscales: the constant pain subscale and intermittent pain subscale (section 6.2.5).
- The change in Tegner scores from the pre-injury activity level to the current activity level (section 6.2.6).
- The pressure pain detection threshold (PDT) defined as mean of the three measurements. The unit for this outcome is kPa, (section 6.2.7).
- The pressure pain tolerance threshold (PTT) defined as mean of the three measurements. The unit for this outcome is kPa, (section 6.2.7).
- The Kellgren and Lawrence grading scale (the radiographic knee OA level) (section 6.2.8).
- The peak knee flexion angle in the first half of the stance phase during walking defined as the mean of the individual peak knee flexion angle values across six walking trials. The unit for this outcome is ° (section 6.2.2).
- The peak knee flexion angle during the forward lunge defined as the mean of the individual peak knee flexion angle values across three forward lunge trials. The unit for this outcome is ° (section 6.2.2).
- The walking speed defined as the mean of the individual speeds across six walking trials. The unit for this outcome is m/s (section 6.2.2).
- The forward lunge foot-ground contact time defined as the mean of the individual time duration of foot-ground contact across three forward lunge trials. The unit of this outcome is s (section 6.2.2).
- The current knee pain during walking defined as the mean of the individual VRS scores reported during six walking trials (section 6.2.9).
- The current knee pain during the forward lunge defined as the mean of the individual VRS scores reported during three forward lunge trials (section 6.2.9).
- The current knee pain during the quadriceps muscle strength test defined as the mean of the individual VRS scores reported after three MVIC trials (section 6.2.9).
- The current knee pain during the hamstring muscle strength test defined as the mean of the individual VRS scores reported after three MVIC trials (section 6.2.9).

6.2 MEASUREMENTS AND CALCULATION OF OUTCOMES

6.2.1 Muscle strength

MVICs of the quadriceps and hamstring muscle strength will be assessed using an isokinetic dynamometer (Biodex System4 Pro, Biodex Medical System, NY, USA) at 60° knee flexion. The participants are seated in a rigid chair firmly strapped to the seat across the chest, at the hip and distal thigh. The rotation axis of the dynamometer is visually aligned to the lateral femoral epicondyle and the lower leg attached to the lever arm of the dynamometer. The lever arm is placed just above the lateral malleolus and firmly fixed with a cuff. The participants are asked to perform the MVICs with maximal effort and verbal encouragement will be provided during testing that comprises three repetitions

of which the highest peak torque value defines the maximal quadriceps/hamstring muscle strength and will be reported as body mass normalized values (Nm/kg)[38].

6.2.2 Biomechanics modelling and simulation

Experimental data

Anthropometric parameters required for scaling the biomechanical model are obtained from the participants. Participants are fitted with 39 reflective markers. A static standing calibration and functional calibration movements for the hip joint centre (star-arc) and knee joint axis (half squat) are first performed. Then the participants perform walking with self-select walking speed and forward lunges in the motion capture laboratory. The instruction in the forward lunge is to take a long step forward, go down to 90 degrees of knee flexion and return to the standing posture as fast as possible. During the movements marker trajectories (100 Hz, Vicon Motion Systems Ltd, UK) and ground reaction forces (1000 Hz, OR-6, Advanced Mechanical Technology Inc., USA) will be recorded. Gaps in the marker trajectories are filled and marker trajectories and ground reaction forces data are low-pass filtered (recursive 4th order low-pass Butterworth filter with 6Hz cut-off frequency[39]). Six walking trials all within ±0.1 km/h and three forward lunges are selected for further analysis using musculoskeletal modelling and simulation.

Musculoskeletal modelling and simulation

Musculoskeletal modelling simulation is performed in OpenSim software[40]. A musculoskeletal model designed for analysis of movement with large hip and knee joint excursions is used[41]. The model is further modified to improve knee extensor muscle moment arm at large knee flexion angles and to account for autograft donor muscle impairment by adjusting semitendinosus and gracilis muscle optimal fibre length and maximal isometric muscle force as per Saxby et al. (2016)[42]. Then, the model is scaled to match the mass and dimensions of the participant based on the measured body mass and marker locations from a standing calibration trial. The estimated hip joint centre[43] and knee joint axis[44] are used to assist scaling of the pelvis, femur, and tibia segments and subsequently to locate joint centres. The maximum isometric force of the knee extensor and flexor muscles are scaled to match experimentally measured knee extension and flexion strength, respectively. In all other muscles, it is assumed that the muscle strength scales relative to the body mass of the participants. Joint kinematics are calculated using inverse kinematics algorithm in OpenSim followed by inverse dynamics to calculate intersegmental resultant forces and moments. Static optimization is used to estimate muscle forces while accounting for muscle force-length properties with a cost function minimizing the sum of squared muscle activations[45]. Finally, a joint reaction analysis tool is used to estimate knee joint contact forces. The simulations are performed for the ground contact phase of walking and forward lunge.

The biomechanical outcomes are means of six successful walking trials/three successful forward lunge trials. The outcomes of the walking trials (peak knee extensor moment, peak quadriceps muscle force, peak knee joint contact force, peak knee flexion angle) are extracted from the 1st half of the stance phase. We focus on the 1st half of the stance phase since in this phase it is the knee extensor muscles that are controlling the knee flexion and are the main contributors of the compressive forces at the tibiofemoral joint. Later in the stance, the main contributor to the compressive force is gastrocnemius muscles [46]. Regarding the forward lunge trials these outcomes are extracted across the whole foot-ground contact phase. The peak knee extensor moment, peak quadriceps muscle force, peak knee joint contact force outcomes will be normalized to body mass; moments are expressed as Nm/kg and forces as N/kg.

The walking speed is assessed by photocells during each individual walking trial and calculated as the mean walking speed (m/s) of the six walking trials selected for further biomechanical analysis.

The forward lunge foot-ground contact time is assessed as the time in s where the foot is in contact with the force plate during the lunge movement. The vertical ground reaction force signal is used to detect this period. The foot is considered to be in contact with the ground when the ground reaction force signal is above 10 N. The forward lunge foot-ground contact time outcome is calculated as the mean of the three forward lunge trials selected for the biomechanical analyses.

6.2.3 KOOS

The Knee injury and Osteoarthritis Outcome Score (KOOS), a disease-specific instrument, is an extension of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) [47]. The KOOS consists of 42 items covering five domains, namely, Pain (9 items), Symptoms (7 items), Activities of Daily Living (ADL) (17 items), Sports and Recreation (5 items), and knee-related QoL (4 items). The KOOS adopts a five-point Likert scale scoring system (ranging from 0 (least severe) to 4 (most severe). A normalized score is calculated for each domain with 100 indicating no symptoms and functional impairment and 0 indicating extreme symptoms and functional impairment. In accordance with the user guide (http://www.koos.nu), if the number of missing items is less than or equal to 2 in a subscale, they will be substituted by the average item value for that subscale. If more than two items of the subscale are omitted, the response will be considered invalid, and no subscale score calculated.

6.2.4 The International Knee Documentation Committee (IKDC questionnaire)

The IKDC questionnaire is an instrument to assess patients with a variety of knee disorders including ligamentous and meniscal injuries as well as patellofemoral pain and osteoarthritis[48]. The questionnaire consists of three subscales: symptoms (7 items), sports activity (2 items), and knee function (2 items) and provides an overall function score. The scores are obtained by summing the individual items and then converting the crude total to a scaled number that ranges from 0 to 100. This final number represents a measure of function with higher scores representing higher levels of function. Thus, a score of 100 reflects no functional limitations. The IKDC score may be calculated if there are missing data, providing that responses have been given for at least 90% of the items. To calculate the IKDC score in case data are missing, the average score of the items that have been answered will be used to substitute for the missing item score(s). In case responses are missing for more than 90% of the items the IKDC response will be considered invalid.

6.2.5 Intermittent and Constant Osteoarthritis Pain questionnaire (ICOAP)

The ICOAP is a diagnosis-specific 11-item questionnaire designed to assess the pain experienced within the last week among people suffering from knee and hip OA[49]. The questionnaire is divided into two domains, a 5-item scale for constant pain and a 6-item scale for intermittent pain (so-called ‘‘pain that comes and goes’’). Each domain captures pain intensity as well as related distress and the impact of OA pain on quality of life. All items are scored on anchored rating scales with five levels of response (0–4). The ICOAP outcomes comprise the two subscales 1) the constant pain subscale (0–20) and 2) the intermittent pain subscale (0–24), and the total pain score (0–44). Normalized scores for the two subscales and the total pain score, from 0 (best) to 100 (worst), are calculated. If there are three or more items missing, the response is considered invalid. If there are less than 3 items missing,

the missing item can be replaced with the mean of the responses to other items within the same subscale[1].

6.2.6 Tegner score

The Tegner activity scale is an instrument to measure activity following knee injuries[50]. It grades activity based on work and sports activities on a scale of 0 to 10 one-item scores. Zero represents disability due to knee problems and 10 represents competitive sports (e.g., soccer - national and international elite level). The subjects report the level of participation that best describes their current level of activity and that before the injury. The change in Tegner score is calculated as the difference between the current activity level Tegner score and pre-injury activity level Tegner score. Negative values will indicate a decline in the activity level.

6.2.7 Pressure pain sensitivity

The pain sensitivity will be assessed by computerised cuff pressure algometry (CPA)[51]. A double-chambered Tourniquet cuff is wrapped around the calf by the gastrocnemius muscles of the lower extremity of the ACL reconstructed leg. A computer-controlled compressor inflates the cuff with air at 1 kPa/s[52]. The participant is asked to indicate the first sensation of pain on the handheld device with a slider by moving the slider upwards (lowest level indicate no pain, highest level indicate worst imaginable pain). The inflation continues until the participant presses the stop button on the handheld device. The pressure pain sensitivity is assessed by two pressure pain thresholds: the pressure pain detection threshold (PDT) and the pressure pain tolerance threshold (PTT). The PDT defines the pressure where the pain is detected (i.e., the first time the participant moves the slider away from zero) and PTT defines the pressure, at which the pain becomes intolerable (i.e., where the participant presses the stop button). The recorded pressures are measured in kPa. The test is repeated four times separated by resting periods of 3 minutes. The first measurement is used for familiarization and the following three are used for the analysis. The PDT and PTT outcomes are calculated as means of the three measurements.

6.2.8 The radiographic knee OA level

The evaluation of radiographic signs of knee OA is done according to Kellgren-Lawrence grading[53] at Frederiksberg Hospital by the same highly experienced rheumatologist. Scores on the Kellgren–Lawrence scale range from 0 to 4, with a score of 2, 3, or 4 indicating definite osteoarthritis and higher scores indicating more severe disease.

6.2.9 Knee pain during movement/muscle strength tests

The current knee pain during the muscle strength, walking and forward lunge tests will be assessed by a VRS 0-10 immediately after each trial, where 0 indicates 'no pain at all' and 10 indicates 'worst imaginable pain'. The knee pain will be calculated as the mean VRS reported during the trials selected for further analysis (for walking this is six and for forward lunge/muscle strength, it is three trials).

6.2.10 Knee joint laxity

Instrumented knee joint laxity testing will be measured using a digital arthrometer (Lachmeter, Lachmeter Company Equipamentos Ortopedicos LTDA, Ribeirao Preto, Brazil). This quantifies the anterior translation of the tibia relative to the femur. The participant is lying supine on an examination table with a wedge cushion behind the thigh ensuring the same degree of knee flexion for all measurements. The participant is asked to relax the thigh muscles and especially the hamstrings during the

[1] https://oarsi.org/sites/default/files/docs/2013/icoap_users_guide_07072010.pdf

test. The knee joint laxity will be assessed three times for each knee. The mean of the three assessments for each leg are calculated and the knee joint laxity side-to-side difference will be calculated by subtracting the mean knee joint laxity of the contralateral knee from the mean knee joint laxity of the ACL-reconstructed knee. The unit of this variable is mm. The knee joint laxity is included in the participant characteristics (Table 2).

6.3 STATISTICAL ANALYSIS METHODS

6.3.1 Primary analysis

The primary analysis applied for estimation of between-group differences of all selected outcomes (section 6.1) will be an analysis of covariance (ANCOVA) for continuous data. The results will be reported as mean ± SD, mean differences with 95% confidence interval (CI) and the level of significance is set to 0.05.

Categorical data and counts (percentages) will be analysed using Chi-square statistics comparing distributions between groups.

Binary data will be presented as risk differences with 95% CI.

The results of the analyses will be presented in tables that resemble the Tables 2-4 shown in section 9.0.

6.4 MISSING DATA

6.4.1 Reasons for missing data

Missing data may potentially occur due to technical issues, electrical power supply breakdown, or other unforeseen issues related to the test equipment. If this should happen the missing data will be considered as missing completely at random.

6.4.2 Imputation method

In case, a given variable has more than 10% missing data, multiple imputation will be applied. All the existing data of that variable will be used to predict the missing values.

6.5 STATISTICAL SOFTWARE

The analyses are done using the statistical software SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

7.0 DISCUSSION

The overall aim of the MIRAKOS study is to compare the musculoskeletal function between ACL reconstructed individuals with and without knee pain to answer the research question: Are there differences in the musculoskeletal function in ACL reconstructed individuals with knee pain when compared to those without knee pain? Specifically, we will test two hypotheses:

1) ACL reconstructed individuals without knee pain have stronger quadriceps muscles compared to those with knee pain.
2) ACL reconstructed individuals without knee pain develop higher quadriceps muscle forces and knee joint loading during walking and forward lunging compared to those with knee pain.

The rationale for the hypotheses is based on research documenting that quadriceps muscle weakness is associated with an increased risk of worsening symptoms and functional deterioration in people

with and at risk for radiographic knee OA[31,35], and that knee joint pain has a negative impact on quadriceps muscle activation and force production[54].

Although, the aim is to test these hypotheses we are fully aware of the fact that the cross-sectional study design will render our analyses exploratory. We will be able to describe and quantify possible differences in the musculoskeletal function between the two study groups; and then likely generate new hypotheses that may be investigated in future studies designed to determine causations.

8.0 CONCLUSION

This SAP presents the basis for the analyses and outcome selection in the MIRAKOS cross-sectional study and discusses the methodological and statistical concerns associated with it. We aim to report the results of the MIRAKOS study as transparently and clearly as possible in order to mitigate outcome reporting bias and data-driven results.

9.0 TABLES AND FIGURES

Figure 1. Flow diagram (example)

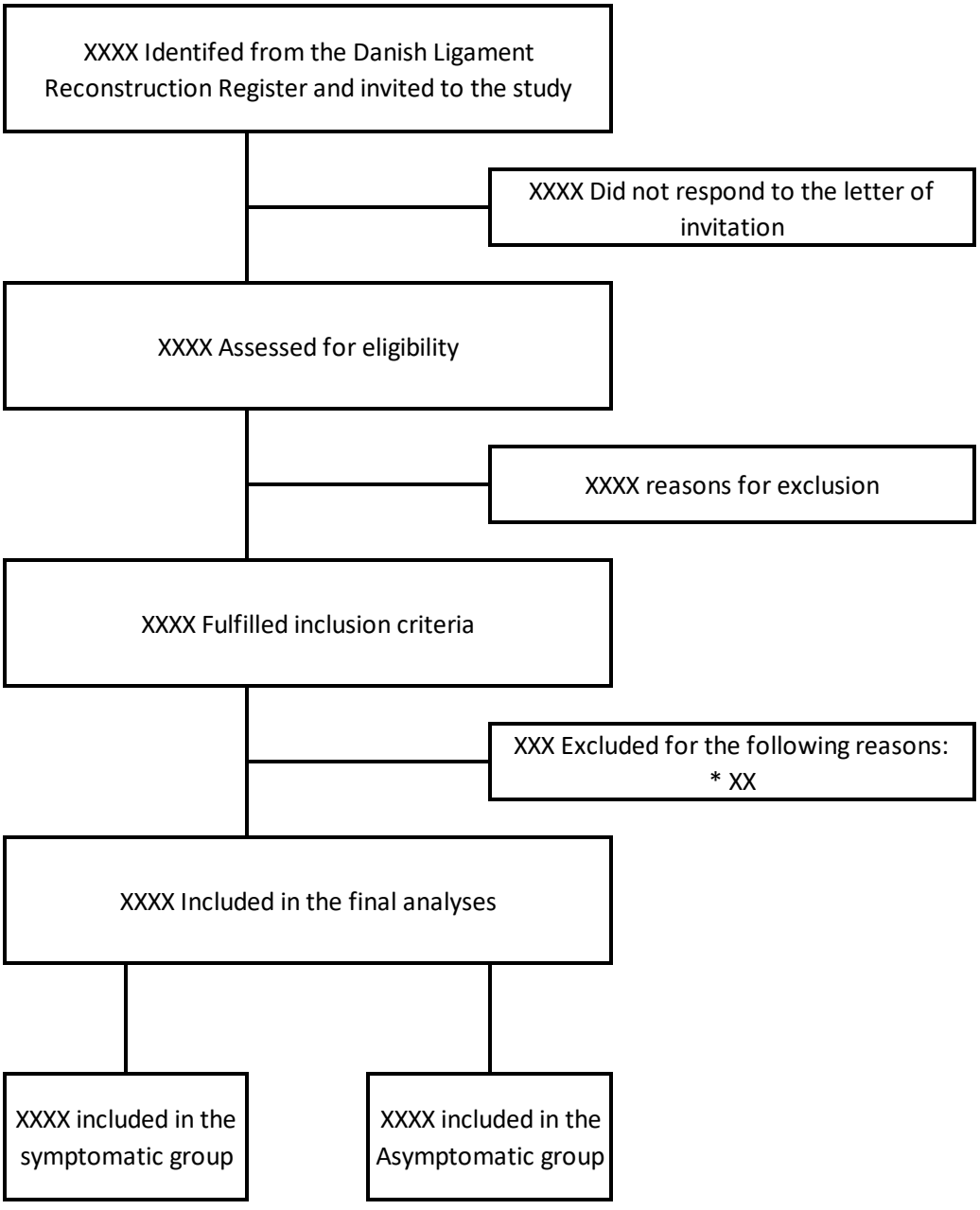


Table 1. Overview of all measured variables.

Variable	At study visit	Not at study visit (tool)
Demographic/Clinical		
Age	X	
Sex	X	
Height	X	
Weight	X	
Body mass index	X	
Injury situation	X	
Injured knee (side)	X	
Reconstruction graft type		X (DLR Register)
Time since surgery (month)		X (DLR Register)
Knee joint laxity	X	
Radiographic knee OA level (K-L score)	X	
Questionnaires		
Pre-injury activity level (Tegner score)	X	
Current activity level (Tegner score)	X	
Knee function (IKDC)	X	
Knee function (KOOS)	X	
Knee pain experience (ICOAP)	X	
Muscle strength		
Maximal isometric quadriceps strength	X	
Maximal isometric hamstring strength	X	
Movement biomechanics		
Walking speed	X	
Peak knee extensor moment (walking/forward lunge)		X (musculoskeletal modelling)
Peak knee flexion angle (walking/ forward lunge)		X (musculoskeletal modelling)
Forward lunge movement time		X (musculoskeletal modelling)
Peak quadriceps muscle force (walking/ forward lunge)		X (musculoskeletal modelling)
Peak knee joint contact force (walking/ forward lunge)		X (musculoskeletal modelling)
Knee pain		
VRS during muscle strength tests	X	
VRS during walking/forward lunge tests	X	
Pressure pain sensitivity		
Pressure pain detection threshold (PDT)	X	
Pressure pain tolerance threshold (PTT)	X	

Table 2. Participant characteristics

Variable	Asymptomatic (N=xx)	Symptomatic (N=xx)	Estimated difference	P-value
Demographic/Clinical				
Age, years				
Male sex, no. (%)				
Height, m				
Body mass, kg				
Body mass index, kg/m^2				
Injured knee (right), no. (%)				
Reconstruction graft type				
Time since surgery, month				
Knee joint laxity, mm				
Injury situation[*], no. (%):				
Traffic accident				
Sport injury				
Other				
Type of sport[*], no. (%)				
Team ball sports				
Racket sports				
Martial arts				
Other				
Return to sport[*], no. (%)				
No				
Yes				
Partly[#]				
Radiographic knee OA level (K-L score)[$], no. (%)				
0				
1				
2				
3				
4				
Activity level				
Pre-injury activity level (Tegner score)				
Current activity level (Tegner score)				

[*]Obtained during screening interview.
[#]Meaning "yes but not at the same pre-injury level".
[$]Scores on the Kellgren–Lawrence scale range from 0 to 4, with a score of 2, 3, or 4 indicating definite osteoarthritis and higher scores indicating more severe disease.

Table 3. Group means (SD) and mean differences (95% CI) of muscle strength, walking and forward lunge knee biomechanics, knee pain during movement/muscle strength tests and pressure pain sensitivity variables including statistical probability.

	Asymptomatic (N=xx)	Symptomatic (N=xx)	Estimated difference	P-value
	Mean (SD)	Mean (SD)	Group Mean Difference (95% CI)	
Muscle strength				
Maximal isometric quadriceps muscle strength (Nm/kg)*				
Maximal isometric hamstring muscle strength (Nm/kg)ǂ				
Walking biomechanics				
Peak knee extensor moment during walking (Nm/kg)§				
Peak quadriceps muscle force during walking (N/kg)§				
Peak knee joint contact force during walking (N/kg)§				
Peak knee flexion, walking (°)ǂ				
Walking speed, (m/s)ǂ				
Forward lunge biomechanics				
Peak knee extensor moment during forward lunge (Nm/kg)§				
Peak quadriceps muscle force during forward lunge (N/kg)§				
Peak knee joint contact force during forward lunge (N/kg)§				
Peak knee flexion, forward lunging (°)ǂ				
Forward lunge foot-ground contact time (s)ǂ				
Pain during movement/muscle strength tests				
Current knee pain during walkingǂ				
Current knee pain during forward lungeǂ				
Current knee pain during quadriceps muscle strength testǂ				
Current knee pain during hamstring muscle strength testǂ				
Pressure pain sensitivity				
Pressure pain detection threshold (kPa)ǂ				
Pressure pain tolerance threshold (kPa)ǂ				
*Primary outcome measure; §Key secondary outcome measures; ǂOther secondary outcome measures				

Table 4. Group means (SD) and mean differences (95% CI) of questionnaires (patient reported outcomes) and activity level variables including statistical probability.

	Asymptomatic (N=xx)	Symptomatic (N=xx)	Estimated difference	P-value
	Mean (SD)	Mean (SD)	Group Mean Difference (95% CI)	
Questionnairesǂ				
KOOS Pain score				
KOOS Symptoms score				
KOOS Quality of life score				
KOOS Sports and recreation score				
KOOS Quality of life score				
International Knee Documentation Committee score				
ICOAP Total score				
ICOAP Constant Pain subscore				
ICOAP Intermittent Pain subscore				
Activity levelǂ				
Change in Tegner score activity level (current - preinjury)				
ǂOther secondary outcome measures				

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