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Study protocol

Natural corollaries and recovery after acute ACL injury – the NACOX cohort study protocol

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NACOX Cohort Study group

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ABSTRACT

Introduction

Anterior cruciate ligament (ACL) injury can result in joint instability, decreased functional performance, reduced physical activity and quality of life, and an increased risk for post-traumatic osteoarthritis. Despite the development of new treatment techniques and extensive research, the complex and multifaceted nature of ACL injury and its consequences are yet to be fully understood. The overall aim of the NACOX study is to evaluate the natural corollaries and recovery after an ACL injury.

Methods and analysis

The NACOX study is a multi-centre prospective prognostic cohort study of patients with acute ACL injury. At 7 sites in Sweden, we will include patients aged 15-40 years, within 6 weeks after primary ACL injury. Patients will complete questionnaires at multiple occasions over the 3 years following injury or the 3 years following ACL reconstruction (for participants who have surgical treatment). In addition, a subgroup of 130 patients will be followed with clinical examinations, several imaging modalities, and biological samples. Data analyses will be specific to each aim.

Ethics and dissemination

This study has been approved by the regional Ethical committee in Linköping, Sweden (Dnr 2016/44-31 and 2017/221-32). We plan to present the results at national and international conferences, and in peer-reviewed scientific journals. Participants will receive a short summary of the results following completion of the study.

Trial registration: NCT02931084

Strengths and limitations of this study

- The NACOX study will improve understanding of the biological, psychological and social consequences of an acute ACL injury.
- The combination of frequently-monitored biological, psychological and social data allows analysis of outcomes at key, clinically-relevant time points after injury.
- In collecting data from patients and clinicians (orthopaedic surgeons and physiotherapists), this study considers the perspectives of important stakeholders in acute ACL injury management.
- The utilisation of advanced imaging techniques and collection of biological samples for identification of proxies of early osteoarthritis that can be related to prospectively collected patient-reported outcome measures and clinical data.
- Loss to follow-up and missing data may be a risk due to the extensive collection of patient-reported outcomes. However, we have a dedicated study monitoring team, and a rigorous data analysis plan to appropriately deal with missing data.

INTRODUCTION

Anterior cruciate ligament (ACL) injuries are common in young athletes. In Sweden, there are approximately 7000 new injuries per year representing approximately 0.81/1000 inhabitants aged 10-64 years.[1] Despite the extensive research to identify the best treatment algorithms, there are still many patients who report unsatisfactory outcomes regarding knee stability, activity level and quality of life following ACL injury.[2 3] This may be because research has tended to focus on single factors, rather than accounting for the multifactorial nature of injury and recovery. There is also a clinical dogma that ACL reconstruction is necessary for a successful outcome after ACL injury and to resume sporting activities.[4 5] Although there is evidence that some patients have functional disability fulfilling the clinical indications for ACL reconstruction,[6] with high quality rehabilitation, many patients achieve satisfactory knee function and participation in sports without surgery.[7 8]

An ACL injury has biological, psychological and social corollaries,[9] that directly affect the patient (e.g. impaired quality of life and lower physical activity participation), and may affect the community (e.g. increased health utilisation costs, impaired productivity, potential for increased chronic disease burden through flow-on development of non-communicable diseases associated with physical inactivity such as cardiovascular disease and diabetes). Taking a biopsychosocial approach, factors including the extent of the initial injury (e.g. whether there were other knee structures involved), factors directly related to the treatment (e.g. which intervention and when), and patient preferences, expectations and past experiences may all be relevant when assessing outcomes.

The most serious long-term corollary after ACL injury is the increased risk for post-traumatic osteoarthritis, estimated to be up to 50% by 15 years after injury.[3] This risk of developing osteoarthritis is higher if the ACL injury is associated with a meniscus tear, and there are conflicting results regarding whether having ACL reconstruction reduces or increases the risk of osteoarthritis.[4 5 10-12] The underlying mechanisms behind the development of osteoarthritis are not well understood. Altered biological processes due to injury and joint bleeding, concomitant structural injuries to the cartilage and the subchondral bone, and joint instability and subsequent altered biomechanics, may be relevant for the development of osteoarthritis. Secondary joint trauma (e.g. with additional meniscal tears, or ACL reconstruction[13]) may also influence the risk for osteoarthritis.

Outcomes need to be evaluated from both the patient's and the clinician's perspective. Patient-reported outcomes provide important insights into aspects of injury and recovery that cannot otherwise be observed or measured with clinical tests or imaging.[14] Clinical outcomes provide the clinician (and by extension – in a well applied shared decision-making approach – the patient) with feedback regarding the effects of different clinical decisions on injury (i.e. which treatment and when), and any physical changes that occur following a clinical decision (e.g. change in effusion and muscle strength, incidence of new injuries, development of osteoarthritis).

The short-term aim of ACL injury management is to achieve satisfactory knee function and physical activity participation. In the long-term, treatment should aim to reduce the risk of developing osteoarthritis. Satisfaction is complex and short-term success (e.g. returning to pivoting sports) may facilitate longer term failure (e.g. developing osteoarthritis after sustaining a second or third meniscal injury). From the patient's perspective, satisfaction can relate to both the outcome of management of the injury (including knee function, confidence to participate in physical activity, fulfillment of expectations for recovery), and to the process of health care delivery (including being an active participant in the decision making process, communication with clinicians, information about the injury and treatment).[15-18] The clinician needs to monitor the resolution of impairments (knee stability, symmetrical lower limb muscle strength, absence of knee effusion), to ensure that treatment is tailored so that the patient has the physical capacity to reach his or her expectations (e.g. return to sport, return to occupation).[19] However, it is evident that these criteria cover only some of the spectrum of possible corollaries of ACL injury.

There is evidence that treatment after ACL injury needs to be individualised.[20] The clinician needs to be able to account for and (ideally) address the important biological, psychological and social factors for each patient. However, we still lack evidence regarding which factors, for which patient, at which time; and this poses challenges for clinical practice. Therefore, to enhance understanding of the consequences of ACL injury, and improve treatment, the overall aim of the NACOX study is to investigate the natural corollaries and recovery after ACL injury. Understanding the complexity of the consequences of ACL injury may improve clinical decision-making to ensure best health care for patients.

To achieve the overall aim, there are five main study objectives:

A. To assess biological, psychological and social factors and their relationships to the natural corollaries and recovery after acute ACL injury
B. To evaluate the choice of treatment after acute ACL-injury (i.e. ACL reconstruction, ACLR or non-ACL reconstruction, non-ACLR)
C. To evaluate the return to sport after acute ACL injury
D. To study knee problems in the short and long term after acute ACL-injury
E. To identify proxies (biomarkers and structural risk factors) for early detection of symptomatic and radiographic osteoarthritis

METHODS AND ANALYSIS

This study is a prospective multicenter prognostic cohort study. Patients will be consecutively recruited over approximately 12 months, from up to seven sites (mix of public and private health care clinics) in Sweden.

Inclusion and exclusion criteria

All patients with acute knee trauma presenting to the identified clinics are potentially eligible for participation.

Inclusion criteria: Patients with an ACL injury, sustained no more than 6 weeks prior to presentation, and aged between 15 and 40 years at time of ACL injury.

Exclusion criteria: previous ACL injury/ACL reconstruction on the same knee, serious concomitant knee injury, e.g. fracture that requires separate treatment, inability to understand written and spoken Swedish language, cognitive impairments, other illness or injury that impairs function (e.g. fibromyalgia, rheumatic diseases and other diagnoses associated with chronic pain).

Procedure

Recruitment of participants started in October 2016, and this study does not alter the usual course of treatment for patients with ACL injury at recruiting centres. This process is:

1. Patient receives a clinical diagnosis from an orthopedic surgeon, verified by MRI, within 2-6 weeks after their knee injury.

2. Initial treatment according to a supervised rehabilitation program of approximately three months duration.[21]
3. Scheduled follow-up visit after approximately 3 months, where further treatment is decided upon between patient and orthopaedic surgeon.

Consequently, patients of this cohort will follow one of the two following pathways: (1) ACLR plus post-operative supervised rehabilitation and (2) supervised rehabilitation alone (non-ACLR).

Patients are asked to participate in the study at their initial contact with the health care provider. Patients who accept participation will be sent questionnaires via smart phone or e-mail. Questionnaires will be sent weekly for the first 6 weeks, fortnightly from week 7 to week 24, monthly from month 7 to month 12, and bi-monthly from year 1 to year 3 after initial injury. Questionnaire length varies from very short (10 questions, approximately 2 completion time) to longer at specific critical time points (Figure 1 and Table 1).

A questionnaire about treatment choice (ACLR or non-ACLR) is completed by the patient, orthopedic surgeon and physiotherapist at the time the decision for ACLR or non-ACLR is made. A questionnaire about the decision to return to sport is completed by the patient and the physiotherapist when the patient reports that he/she is back to full participation in the goal sports/physical activity. For patients with ACLR, a new baseline questionnaire will be completed at the time of reconstruction. Subsequent data collection will continue according to the new baseline time point (Figure 1).

One subgroup (approximately 130 patients recruited from Linköping) will have extended follow-up data collection at baseline and 3, 6, 12 and 24 months after injury. At these time points, a clinical examination will be completed by a physiotherapist, physical activity will be registered over 5 consecutive days using a triaxial accelerometer (activPAL^{TM}, PALtechnologies, UK), knee MRI will be performed, and blood and urine samples will be collected. A joint fluid sample is acquired at baseline if indicated due to joint effusion, and at the time of any additional surgery including ACLR (if the patient has surgical treatment). Weight bearing radiographs are done at baseline and 5 years follow up. Patients who have ACLR are followed up with questionnaires and clinical examination with new baseline at the

time of reconstruction. MRIs and blood and urine samples are followed up with the injury according to the index baseline (Figure 1).

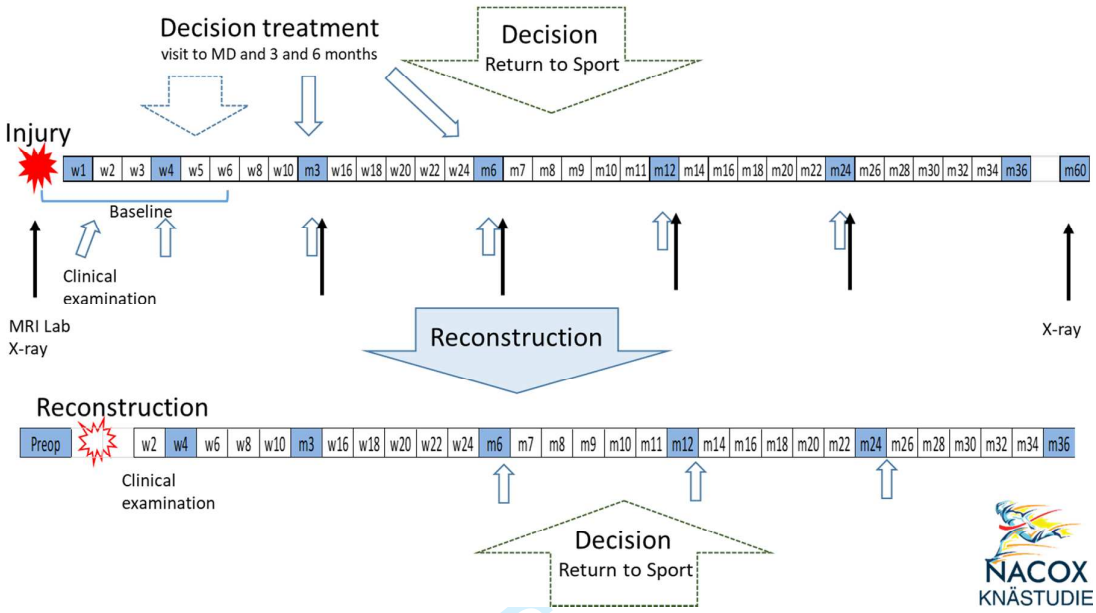

Figure 1. Flow chat of the NACOX study follow-up plan. w1, w2 and m3, m6 etc: weeks resp months after injury. Each box denotes when a questionnaire will be sent; blue marked boxes indicate extended questionnaires. Time points for clinical examination (blue arrows), Magnetic Resonance Imaging (MRI) and lab and x-rays (black arrows) are indicated Baseline questionnaire, clinical examination, MRI, lab and x-ray are within 6 weeks after the injury.

Outcomes

Reflecting a biopsychosocial approach, outcome measurement for this study will evaluate four main aspects: patient-reported outcomes, physical function, physical activity and physiological markers of joint injury (Table 1).

Patient-reported outcomes: all study participants

Demographic and baseline characteristics including age, sex, BMI, smoking habits, occupation, preinjury activity level, medical and injury history, sick leave, preferences regarding treatment will be collected with the baseline questionnaire.

Patient-reported *knee function and participation* will be assessed with the International Knee Documentation Committee Subjective Knee Form (IKDC-SKF),[22] a Single Assessment Numeric Evaluation (SANE) of global knee function,[23] and the four subscales of Knee injury and Osteoarthritis Outcome Score (Pain, other Symptoms, Function in sport and recreation (Sport/Rec) and knee related Quality of life (QOL); $KOOS_4$).[24] Subjective *knee stability* during ADL and sports will each be assessed with a single numeric rating scale (1-10 scale).

The frequency of self-reported participation in *physical activity* will be collected according to the recommendations from Swedish National Board of Welfare. Participants will report the type of physical activity they participated in (e.g. football, strength training), and the level of participation (e.g. recreational, elite), during the previous week. Participation in up to 3 activities can be recorded.[25]

Expectations for recovery (2 questions) and fulfillment of expectations (1 question) will be assessed using 6-item Likert scales. Participants will be asked to indicate if their goal was to return to sport and reasons for not returning. *Motivation* to return to the preinjury physical activity will be evaluated using a questionnaire we developed based on the transtheoretical model of behavior change.[18]

The General *Self Efficacy* scale (GSES) will be used at baseline to assess the individual's beliefs that his/her actions determine successful outcome.[26] Knee-specific self-efficacy will be assessed with the subscale of the Knee Self Efficacy Scale (K-SES) that evaluates patients' perception of future knee function (4 questions).[27] *Psychological readiness* for return to sport will be assessed with the ACL-Return to Sport after Injury (ACL-RSI) questionnaire that includes questions on confidence in performance, emotions and risk appraisal.[28] *Satisfaction* with present knee function will be evaluated with a 7-item Likert scale ranging from "delighted" to "terrible".[29] Knee-related quality of life (QoL) will be assessed with the ACL-QoL questionnaire.[30]

Participants will be asked to indicate the *number of rehabilitation sessions* they have completed. *Adherence* to rehabilitation will be assessed by the patient and physiotherapist with the Sports Injury Rehabilitation Adherence Scale.[31] The importance of rehabilitation for the current knee function will be assessed on a 5-response scale ranging from "necessary for

my current knee function" to "not necessary at all"[32]. Experience with health care will be assessed with a 5-item Likert scale ranging from "very good" to "very bad".

Information about *new knee injuries* will be collected using a direct question, which is followed up with phone call if the injury is severe, i.e. results in functional limitation during the following days or inability to participate in physical activity. *Knee problems during physical activity participation* will be assessed with the knee-specific part of the Oslo Sports Trauma Research Centre (OSTRC) questionnaire.[33]

Table 1. Reported outcomes at different time points after injury or reconstruction.

Blå färg enbart ejrec. Röd färg - enbart R																																				
																		once a month						every other month						every other month						
Injury	Baseline		w1	w2	w3	w4	w5	w6	w8	w10	m3	w16	w18	w20	w22	w24	m6	m7 - 11					m12	m14 - 22					m24	m26 - 36					m36	
																																				Extra
Reconstruction		Peop	-	w2	-	w4	-	w6	w8	w10	m3	w16	w18	w20	w22	w24	m6	m7 - 11					m12	m14 - 22					m24	m26 - 36					m36	
Demographics and baseline	x										(x)						(x)						(x)						(x)						(x)	
Knee function and participation																																				
IKDC-SKF		x				x					x						x						x						x						x	
SANE	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Subjective knee stability	x	x				x			x		x			x			x						x						x						x	
Giving way		x									x						x						x						x						x	
KOOS$_4$																													x						x	
Physical activity participation	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Expectations and fullfillment	x	x				x			x		x			x			x						x						x							
Motivation to RTS	x	x				x					x						x						x						x							
RTS (goal and actuall RTS)	x	x				x			x		x			x			x						x						x						x	
Reasons for not RTS									x		x			x			x						x						x						x	
Factors affecting RTS																																				x^2
Psychological factors																																				
General self efficacy	x																																			
Knee self efficacy	x	x				x					x						x						x						x							
ACL-RSI											x						x						x													
Satisfaction											x						x						x						x						x	
Quality of Life						x^4					x												x													
Rehabilitation and health care																																				
Frequency			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Adherence											x						x																			x^2
Importance		x									x						x						x						x							
Experiance with health care		x									x						x						x						x							
Decision of treatment*											x						x						x													x^1
New injuries			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Problems during physical activity																	x^3	x^3	x^3	x^3	x^3	x^3	x	x	x	x	x	x	x	x	x	x	x	x	x	

KOOS$_4$: Knee injury and Osteoarthritis Outcome Score, subscales for pain, symptoms, function in sport and recreation (sport/rec) and knee related Quality of life (QoL). RTS: return to sports. * answered by the patient, orthopedic surgeon and physical therapist. (x): only some questions are asked during these timepoints. x^1: Q answered when the decision fror ACLR is taken. x^2: Q is answered by the patient and physical therapist when the patient RTS to full sports participation. x^3: assessed only for non-ACLR. x^4 : Only the subscales "life style" and "social and emotional" of the ACL-QoL

Clinical examination: sub-group of study participants

The sub-group of participants recruited from one of the study sites (Linköping) will have clinical examinations of knee function, performed by either an orthopedic surgeon together with a physiotherapist (always for the baseline assessment), or physiotherapist alone, or physiotherapy student in the final year of education. All assessors will have standardised training in the clinical examination procedure.

Knee status will be assessed using knee joint effusion (circumference of the joint using a measurement tape, and the 'stroke test' [34]), knee joint laxity tests (Lachman test, Lever sign, anterior drawer and medial/lateral laxity), knee flexion and extension and ankle dorsiflexion range of motion, varus or valgus knee alignment. Instrumented knee laxity measurements will be assessed using the KT-1000 arthrometer at 133N and manual maximum (mean value of three repetitions).

Functional performance will be evaluated through qualitative assessment of gait (10m walking test), single leg squat, and four single-limb hop tests (single hop for distance, triple hop, crossover hop and 6-meter timed-hop).[35] *Postural control* will be assessed using a single-limb static balance task with eyes closed (SOLEC). Concentric quadriceps and hamstrings *strength* will be assessed using a Biodex dynamometer, at 60 degrees/second (5 repetitions) and 180 degrees/second (15 repetitions) angular velocities.

Activity registration: sub-group of study participants

At the conclusion of the clinical examination, participants will be asked to wear a triaxial accelerometer (activPAL™ micro, PAL Technologies Ltd.) for a minimum of 5 days (maximum 7 days) immediately following the examination. The accelerometer will be attached mid-way between the hip and the injured knee according to the manufacturer's recommendations.

Imaging: sub-group of study participants

Patients recruited in Linköping (approximately 130 patients) will undergo extensive imaging assessment and collection of biosamples.

Plain weight bearing *radiographs* – the current gold standard for assessment of radiographic osteoarthritis – will be obtained using a slightly modified method of the Lyon-Schuss view (participants stand, bearing equal weight through each limb [36]) and a standardised axial patellofemoral joint view (Table 2).[37]

Magnetic resonance (MR) images will be obtained from both knees at baseline for diagnostic purposes, to confirm an acute ACL tear in the index knee and to examine the status of the contralateral knee. At follow up, only the index knee will undergo MR image acquisition. MR images will be acquired using a Philips Ingenia 3T scanner with a 16-channel knee-coil and will be obtained at baseline, 3, 6, 12 and 24 months (Table 2).

Table 2. MR imaging, radiographic assessment and collection of biological samples over the study period for patients recruited at the Linköping site

	Baseline	3 months	6 months	12 months	24 months	60 months
WB radiographs	X	-	-	-	-	X
Full clinical protocol	X*	-	-	-		-
Compositional protocol	X	X	X	X	-	-
Explorative protocol	X	X	X	X	X	X
Blood	X	X	X	X	X	X
Urine	X	X	X	X	X	X
Joint fluid**	X					

** Bilateral assessment*

*** Collection will be performed under anesthesia at the time of any surgery during follow up*

For *clinical evaluation and diagnostics*, the normal clinical protocol at the Linköping site will be followed (total scantime 15 minutes, Table 3).

For *bone shape analyses*, a 3D PD sequence (scantime 6.5 minutes, Table 3) will be obtained.

For *compositional analysis* of cartilage and menisci, sagittal T2maps and T1Rho will be obtained (total scantime approx. 16 minutes, Table 3).

For exploratory purposes, a new in-house developed sequence (Qmap) with the potential of reducing clinical and compositional scantime and adding explorative measures of T1-maps T2-maps and PD-maps will be obtained (total scantime approximately 6 minutes, Table 3).[38]

Table 3. Detailed description of MR imaging sequences

Clinical package	Sagittal PD, 3 mm slice thickness with 0.3 mm gap. TE=20 ms; TR=1800 ms, ETL 10; FOV 160x145, ACQ matrix 516x384=0.31x0.38mm, recon matrix 528. Scantime 2:58 min. Axial PD FatSat, 3 mm slice thickness with 0.3 mm gap. TE=35 ms; TR=3981 ms, ETL 15; FOV 140x140, ACQ matrix 332x330=0.42x0.42mm, recon matrix 512. Scantime 4:15min Sagittal PD FatSat, 3 mm slice thickness with 0.3 mm gap. TE=30 ms; TR=3400 ms, ETL 15; FOV 160x145, ACQ matrix 468x399=0.31x0.40mm, recon matrix 528. Scantime 3:56min. Coronal PD FatSat, 3 mm slice thickness with 0.3 mm gap. TE=30 ms; TR=3572 ms, ETL 16; FOV 160x140, ACQ matrix 516x332=0.31x0.42mm, recon matrix 528. Scantime 3:56min
PD FS 3D	Sagittal PD FatSat 3D, 0.63 mm slice thickness, TE=185, TR=1300, ETL=63, FOV=144x162, AQC matrix 228x226=0.63x0.63, recon matrix 448. Scantime 6:31min
T2map	Sagittal T2-map (T2 relaxation), 3 mm slice thickness with 0.3 mm gap. TE=n*10 ms; TR=2371 ms, ETL 8; FOV 160x140, ACQ matrix 456x280=0.35x0.50mm, recon 560. Scantime 5:53min
T1Rho	3D sagittal SpinLock (T1Rho relaxation), 4 mm slice thickness. TE=3.3 ms; TR=6.4 ms, ETL 64; FOV 140x140, ACQ matrix 280x268=0.50x0.52mm, recon 352. Scantime 2:36min
Qmap	Sagittal Qmap (T1 relaxation, T2 relaxation, Proton Density), 3 mm slice thickness with 0.3 mm gap. TE=8.8/110 ms; TR=4217 ms, ETL 16; FOV 160x145, ACQ matrix 364x270=0.40x0.59mm, recon 576. Scantime 6:19min

Collection and storage of biological samples: sub-group of study participants

All samples will be stored in a dedicated biobank at -70°C.

Joint fluid (haemarthrosis) will be aspirated from the index knee at baseline to ascertain joint bleeding (highly indicative of severe knee injury), according to clinical routine. Due to the pain and discomfort associated with knee joint arthrocentesis (especially in knees without effusion), additional longitudinal collection of joint fluid will only be performed in case of

surgical procedure (collection will be during surgery). Collected samples will be centrifuged at 2500G for 10 minutes and aliquoted into 0.7ml tubes for storage.

Venous blood samples will be collected at the same visit as image acquisition. Collected samples will be centrifuged and aliquoted according to a specific protocol for storage.

Urine samples will be collected at first morning void (preferred). Collected samples will be centrifuged at 1800G for 10 minutes and aliquoted into 1.0mL tubes for storage at -70°C.

Analyses will include, but are not limited to, those presented in Table 4. In addition, several markers of inflammation, such as IL-6, IL-8, IL-10, IL-12, TNF-α, INF-ɣ, will be analysed.

Table 4. Planned analyses of biomarkers

BIOMARKER	FLUID	PROCESS	TISSUE
ARGS-AGGRECAN	Serum Synovial fluid	Cartilage turnover	Cartilage
CTX-II	Urine	Type II collagen degradation	Cartilage Bone
CTX-I	Serum Urine	Bone turnover	Bone
COMP	Serum Synovial fluid	Cartilage degradation	Cartilage
C2C	Serum Urine	Type II collagen degradation	Cartilage
NTX-I	Serum Urine	Bone resorption	Bone

Decision making for choice of treatment and return to sport

Factors affecting the *decision of choice of treatment* (ACLR or not) will be evaluated by the patient, orthopedic surgeon and physiotherapist with questionnaires. Respondents will answer questions about why the particular treatment was chosen, if they perceive it was the right treatment choice, the agreement for choice of treatment between the clinicians and patient, about the patient's involvement and understanding of information, and about the communication between clinicians. The physiotherapist and patient also answer questions about patient rehabilitation.

Factors affecting the *decision for return to sports* (RTS) is evaluated at the time the patient replies that she/he is back to full sports participation of the goal sport/physical activity (with or without knee problems) based on the response to a question from the OSTRC questionnaire [33] ("have you had any difficulties participating in your sport activity due to your knee problems" with the response "full participation without or with knee problems"). Other questions capture the areas on how the decision for RTS was taken, possible criteria used to approve RTS, activity and participation modification.

Primary outcomes and statistical analyses

A suite of analyses is planned for each of the 5 main study objectives.

Study objective A: assessment of the biological, psychological and social factors, and their relationships to the natural corollaries and recovery after acute ACL injury

Specific aims:

1. Assess whether there is a relationship between knee status and self-reported function early (up to 8 weeks) following ACL injury, and the IKDC-SKF at 3 and 12 months follow-up.
2. Assess whether there is a relationship between knee status in the first 8 weeks following injury and functional performance at 12 months follow-up
3. Evaluate how physical activity, self-reported activity participation or as measured by activPAL, in the first 8 weeks after ACL injury is related to self-reported function and functional performance at 3 and 12 months after injury.
4. Investigate the prognostic relationship between returning to physical activity after ACL injury, and key biological, psychological and social factors.

Primary outcome: Self-reported physical activity participation and IKDC subjective knee score at 12 months follow-up

Secondary outcomes: functional performance at 3, 6, 12 and 24 months follow-up, time to return to the goal physical activity

Statistical analysis: We will use generalised estimating equations (GEE) to assess longitudinal relationships between knee status and subjective knee function. The outcome variable will

be IKDC subjective knee form score. Predictor variables may include knee joint effusion, laxity and range of motion, and SANE.

We will use GEE to assess longitudinal relationships between knee status and functional performance. The outcome variables will be measures of hopping performance, strength and postural control. Predictor variables may include knee joint effusion, laxity and range of motion, and SANE.

We will use multilevel modelling to assess relationships between physical activity and knee status. The outcome variable will be IKDC subjective knee form score. Predictor variables may include physical activity (self-reported and objectively measured), knee status (knee joint effusion, laxity and range of motion), age and sex.

We will use multilevel modelling to assess the prognostic relationship between returning to the goal physical activity and biopsychosocial factors. The outcome variable will be time to return to the goal physical activity. The predictor variables will be different biopsychosocial factors (collected with questionnaires and clinical examination). We will use factor analysis to guide which independent variables are entered into the model.

Study objective B: evaluation of the choice of treatment after ACL injury

Specific aims:

1. Describe factors that are important for the choice of treatment after an ACL injury, i.e. ACLR or non-ACLR, from patients, orthopedic surgeons' and physical therapists' perspective
2. To confirm the factors identified as important for treatment choice, using demographic and patient-reported data
3. Assess the relationship between factors (biological, psychological, social factors and factors that affected the choice of treatment) and satisfactory knee function (IKDC subjective knee form) at 12 months
4. Describe the decision-making process for treatment and evaluate patient satisfaction with the decision that was made

Primary outcomes: satisfaction with the treatment choice and the relationship to patient-reported outcome (IKDC) at 12 months after injury or ACL-R

Secondary outcomes: factors affecting treatment decision

Statistical analysis: we will summarise the treatment decision factors reported by patients and clinicians descriptively using frequency tables. We will confirm whether specific treatment factors exist for individual patients, by matching the patient's own demographic and/or patient-reported data to the relevant factor.

We will use factor analysis to determine the common constructs underlying the factors that are important for the choice of treatment. The smaller number of related groups of factors will be used in a subsequent multivariable model. We will run separate analyses for the factors cited as important for the decision for ACLR and the factors cited as important for the decision for non-ACLR.

Finally, we will use a multilevel model to estimate the relationship between biopsychosocial factors and self-reported knee function at 12 months. The outcome variable will be IKDC subjective knee form score at 12 months. The predictor variables may include clusters of biopsychosocial factors (identified in A - Assessment of the biological, psychological and social factors, and their relationships to the natural corollaries and recovery after acute ACL injury) and treatment choice clusters (independent variables). The model will be adjusted for treatment received (i.e. ACLR or non-ACLR).

Study objective C: evaluation of return to sport after ACL injury

Specific aims:

1. Describe the decision-making process for return to sport following ACL injury
2. Describe the criteria physiotherapists use in clinical practice to clear patients to return to sport after ACL injury
3. Validate the criteria used to clear patients to return to sport after ACL injury

Primary outcome: return to sport rate at 24 months follow-up

Secondary outcomes: time to return to sport, sports participation rates over time, incidence of new knee injuries

Statistical analysis: we will summarise the return to sport decision factors reported by patients and clinicians descriptively using frequency tables. We will also summarise the criteria used by clinicians to decide when the patient was ready to return to sport descriptively using frequency tables. To assess the discriminant validity of the criteria used to clear patients to return to sport after ACL injury, we will use regression analyses to compare relevant outcomes (e.g. strength, effusion, range of motion) between participants who do and do not return to sport.

Study objective D: knee problems in the short- and long-term after acute ACL injury

Specific aim:

1. Describe the rate and nature of knee problems (new acute knee injury, gradual onset knee injury and osteoarthritis) after index ACL injury
2. Assess whether there is a relationship between biological, psychological and social factors, and new knee problems after acute ACL injury

Primary outcome: new acute knee injury

Secondary outcomes: gradual onset knee injury, osteoarthritis

Statistical analysis: we will use a time-to-event analysis to estimate the rate of new acute knee injuries (may include new ACL tears, new meniscus tears), the rate of radiographic osteoarthritis and the rate of symptomatic osteoarthritis. The predictor variables may include treatment (ACLR or non-ACLR), sex, age and clusters of biopsychosocial factors (identified in A - Assessment of the biological, psychological and social factors, and their relationships to the natural corollaries and recovery after acute ACL injury).

We will use a multi-level modeling approach to assess whether there is a relationship between biopsychosocial factors and gradual onset knee injuries. The independent variables may include clusters of biopsychosocial factors (identified in “Assessment of the biological, psychological and social factors, and their relationships to the natural corollaries and recovery after acute ACL injury”), treatment, sex and age.

Study objective E: identification of proxies for early detection of osteoarthritis

Specific aims

1. Identify imaging-based proxies of early radiographic and symptomatic osteoarthritis
2. Identify change in specific local and/or systemic molecular biomarkers (biological proxies) and investigate their relation to imaging based structural change and patient relevant outcomes
3. Investigate the temporal relation between symptoms, structure and biology after knee injury

Primary outcome: radiographic osteoarthritis at 5-years follow-up

Secondary outcomes: MR-defined at 2-years follow-up; symptoms as defined by IKDC subjective knee form and SANE at 2- and 5-years follow-up

Statistical analysis: We will use a multi-level modelling approach to relate predictor variables that may include imaging-based and biologically-based proxies, and possible risk factors (e.g. treatment (ACLR or non-ACLR), meniscus injury, activity participation) to the primary and secondary outcomes. We will adjust for potential confounders that may include sex, age, and body mass index.

Sample size calculation

For regression analysis in the different parts, using approximately 10 independent variables for each outcome, at least 130 participants will be included. For part B and C, evaluating decision for treatment and RTS, we need geographically spread collected data in order to be generalisable. Since there might be different routines and common praxis among different clinics even in the same geographical area, it is important to include different clinics when collecting data. We are collecting data from seven different counties, and several clinics within these counties, spreading from south to north of Sweden. We expect to collect data regarding decision making from at least about 25 orthopedic surgeons (about 10% of all surgeons performing ACL reconstructions over Sweden) and at least 45 physiotherapists (there is no registry for the number of physiotherapists treating ACL injured patients in Sweden).

Timeline

Patient recruitment started in October 2016 and will continue until June 2018.

Ethics and dissemination

Being included in this study will not influence which treatment the patient will receive. The study is approved by the regional Ethical committee in Linköping, Sweden (Dnr 2016/44-31 and 2017/221-32).

Results will be presented at national and international conferences and submitted for publication to peer-reviewed journals. Participants will receive short summary of the study.

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JK wrote the first draft and coordinated the preparation of the protocol together with CA. HG, HTG, MH, AS and RF were responsible for different parts of the protocol writing. All authors revised and accepted the final manuscript of the protocol.

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Competing interests statement

None declared

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Natural corollaries and recovery after acute ACL injury – the NACOX cohort study protocol

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Study protocol

Natural corollaries and recovery after acute ACL injury – the NACOX cohort study protocol

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ABSTRACT

Introduction

Anterior cruciate ligament (ACL) injury can result in joint instability, decreased functional performance, reduced physical activity and quality of life, and an increased risk for post-traumatic osteoarthritis. Despite the development of new treatment techniques and extensive research, the complex and multifaceted nature of ACL injury and its consequences are yet to be fully understood. The overall aim of the NACOX study is to evaluate the natural corollaries and recovery after an ACL injury.

Methods and analysis

The NACOX study is a multi-centre prospective prognostic cohort study of patients with acute ACL injury. At 7 sites in Sweden, we will include patients aged 15-40 years, within 6 weeks after primary ACL injury. Patients will complete questionnaires at multiple occasions over the 3 years following injury or the 3 years following ACL reconstruction (for participants who have surgical treatment). In addition, a subgroup of 130 patients will be followed with clinical examinations, several imaging modalities, and biological samples. Data analyses will be specific to each aim.

Ethics and dissemination

This study has been approved by the regional Ethical committee in Linköping, Sweden (Dnr 2016/44-31 and 2017/221-32). We plan to present the results at national and international conferences, and in peer-reviewed scientific journals. Participants will receive a short summary of the results following completion of the study.

Trial registration: NCT02931084

Strengths and limitations of this study

- The prospective observational design with frequently-monitored biological, psychological and social variables allows analysis of outcomes at key, clinically-relevant time points after injury.
- Quantitative methods are used to assess the perspectives of important stakeholders in acute ACL injury management (patients and clinicians (orthopaedic surgeons and physiotherapists)).
- The utilisation of advanced imaging techniques and collection of biological samples for identification of proxies of early osteoarthritis that can be related to prospectively collected patient-reported outcome measures and clinical data.
- Loss to follow-up and missing data may be a risk due to the extensive collection of patient-reported outcomes. However, we have a dedicated study monitoring team, and a rigorous data analysis plan to appropriately deal with missing data.

INTRODUCTION

Anterior cruciate ligament (ACL) injuries are common in young athletes. In Sweden, there are approximately 7000 new injuries per year representing approximately 0.81/1000 inhabitants aged 10-64 years.[1] Despite the extensive research to identify the best treatment algorithms, there are still many patients who report unsatisfactory outcomes regarding knee stability, activity level and quality of life following ACL injury.[2 3] This may be because research has tended to focus on single factors, rather than accounting for the multifactorial nature of injury and recovery. There is also a clinical dogma that ACL reconstruction is necessary for a successful outcome after ACL injury and to resume sporting activities.[4 5] Although there is evidence that some patients have functional disability fulfilling the clinical indications for ACL reconstruction,[6] with high quality rehabilitation, many patients achieve satisfactory knee function and participation in sports without surgery.[7 8]

An ACL injury has biological, psychological and social corollaries,[9] that directly affect the patient (e.g. impaired quality of life and lower physical activity participation), and may affect the community (e.g. increased health utilisation costs, impaired productivity, potential for increased chronic disease burden through flow-on development of non-communicable diseases associated with physical inactivity such as cardiovascular disease and diabetes). Taking a biopsychosocial approach, factors including the extent of the initial injury (e.g. whether there were other knee structures involved), factors directly related to the treatment (e.g. which intervention and when), and patient preferences, expectations and past experiences may all be relevant when assessing outcomes.

The most serious long-term corollary after ACL injury is the increased risk for post-traumatic osteoarthritis, estimated to be up to 50% by 15 years after injury.[3] This risk of developing osteoarthritis is higher if the ACL injury is associated with a meniscus tear, and there are conflicting results regarding whether having ACL reconstruction reduces or increases the risk of osteoarthritis.[4 5 10-12] The underlying mechanisms behind the development of osteoarthritis are not well understood. Altered biological processes due to injury and joint bleeding, concomitant structural injuries to the cartilage and the subchondral bone, and joint instability and subsequent altered biomechanics, may be relevant for the development of osteoarthritis. Secondary joint trauma (e.g. with additional meniscal tears, or ACL reconstruction[13]) may also influence the risk for osteoarthritis.

Outcomes need to be evaluated from both the patient's and the clinician's perspective. Patient-reported outcomes provide important insights into aspects of injury and recovery that cannot otherwise be observed or measured with clinical tests or imaging.[14] Clinical outcomes provide the clinician (and by extension – in a well applied shared decision-making approach – the patient) with feedback regarding the effects of different clinical decisions on injury (i.e. which treatment and when), and any physical changes that occur following a clinical decision (e.g. change in effusion and muscle strength, incidence of new injuries, development of osteoarthritis).

The short-term aim of ACL injury management is to achieve satisfactory knee function and physical activity participation. In the long-term, treatment should aim to reduce the risk of developing osteoarthritis. Satisfaction is complex and short-term success (e.g. returning to pivoting sports) may facilitate longer term failure (e.g. developing osteoarthritis after sustaining a second or third meniscal injury). From the patient's perspective, satisfaction can relate to both the outcome of management of the injury (including knee function, confidence to participate in physical activity, fulfillment of expectations for recovery), and to the process of health care delivery (including being an active participant in the decision making process, communication with clinicians, information about the injury and treatment).[15-18] The clinician needs to monitor the resolution of impairments (knee stability, symmetrical lower limb muscle strength, absence of knee effusion), to ensure that treatment is tailored so that the patient has the physical capacity to reach his or her expectations (e.g. return to sport, return to occupation).[19] However, it is evident that these criteria cover only some of the spectrum of possible corollaries of ACL injury.

There is evidence that treatment after ACL injury needs to be individualised.[20] The clinician needs to be able to account for and (ideally) address the important biological, psychological and social factors for each patient. However, we still lack evidence regarding which factors, for which patient, at which time; and this poses challenges for clinical practice. Therefore, to enhance understanding of the consequences of ACL injury, and improve treatment, the overall aim of the NACOX study is to investigate the natural corollaries and recovery after ACL injury. Understanding the complexity of the consequences of ACL injury may improve clinical decision-making to ensure best health care for patients.

To achieve the overall aim, there are five main study objectives:

A. To assess biological, psychological and social factors and their relationships to the natural corollaries and recovery after acute ACL injury
B. To evaluate the choice of treatment after acute ACL-injury (i.e. ACL reconstruction, ACLR or non-ACL reconstruction, non-ACLR)
C. To evaluate the return to sport after acute ACL injury
D. To study knee problems in the short and long term after acute ACL-injury
E. To identify proxies (biomarkers and structural risk factors) for early detection of symptomatic and radiographic osteoarthritis

METHODS AND ANALYSIS

This study is a prospective multicenter prognostic cohort study. Patients will be consecutively recruited over approximately 20 months, from up to seven sites (mix of public and private health care clinics) in Sweden.

Inclusion and exclusion criteria

All patients with acute knee trauma presenting to the identified clinics are potentially eligible for participation.

Inclusion criteria: Patients with an ACL injury, sustained no more than 6 weeks prior to presentation, and aged between 15 and 40 years at time of ACL injury.

Exclusion criteria: previous ACL injury/ACL reconstruction on the same knee, serious concomitant knee injury (e.g. posterior cruciate ligament rupture, fracture that requires separate treatment), inability to understand written and spoken Swedish language, cognitive impairments, other illness or injury that impairs function (e.g. fibromyalgia, rheumatic diseases and other diagnoses associated with chronic pain).

Procedure

Recruitment of participants started in October 2016, and this study does not alter the usual course of treatment for patients with ACL injury at recruiting centres. This process is:

1. Patient receives a clinical diagnosis from an orthopedic surgeon, verified by MRI, within 2-6 weeks after their knee injury.

2. Initial treatment according to a supervised rehabilitation program of approximately three months duration.[21]
3. Scheduled follow-up after approximately 3 months, where further treatment is decided upon between patient and orthopaedic surgeon.

Consequently, patients of this cohort will follow one of the two following pathways: (1) ACLR plus post-operative supervised rehabilitation and (2) supervised rehabilitation alone (non-ACLR).

Patients are asked to participate in the study at their initial contact with the health care provider. Patients who accept participation will be sent questionnaires via smart phone or e-mail. Questionnaires will be sent weekly for the first 6 weeks, fortnightly from week 7 to week 24, monthly from month 7 to month 12, and bi-monthly from year 1 to year 3 after initial injury. Questionnaire length varies from very short (10 questions, approximately 2 min completion time) to longer at specific critical time points (Figure 1 and Table 1).

A questionnaire about treatment choice (ACLR or non-ACLR) is completed by the patient, orthopedic surgeon and physiotherapist at the time the decision for ACLR or non-ACLR is made. A questionnaire about the decision to return to sport is completed by the patient and the physiotherapist when the patient reports that he/she is back to full participation in the goal sports/physical activity. For patients with ACLR, a new baseline questionnaire will be completed at the time of reconstruction. Subsequent data collection will continue according to the new baseline time point (Figure 1).

One subgroup (approximately 130 patients recruited from Linköping) will have extended follow-up data collection at baseline and 3, 6, 12 and 24 months after injury. At these time points, a clinical examination will be completed by a physiotherapist, physical activity will be registered over 5 consecutive days using a triaxial accelerometer (activPAL™, PALtechnologies, UK), knee MRI will be performed, and blood and urine samples will be collected. A joint fluid sample is acquired at baseline if indicated due to joint effusion, and at the time of any additional surgery including ACLR (if the patient has surgical treatment). Weight bearing radiographs are done at baseline and 5 years follow up. Patients who have ACLR are followed up with questionnaires and clinical examination with new baseline at the

time of reconstruction. MRIs and blood and urine samples are followed up with the injury according to the index baseline (Figure 1).

Figure 1 about here

Outcomes

Reflecting a biopsychosocial approach, outcome measurement for this study will evaluate four main aspects: patient-reported outcomes, physical function, physical activity and physiological markers of joint injury (Figure 1).

Patient-reported outcomes: all study participants

Demographic and baseline characteristics including age, sex, BMI, smoking habits, occupation, preinjury activity level, medical and injury history, sick leave, preferences regarding treatment will be collected with the baseline questionnaire (Figure 2).

Patient-reported *knee function and participation* will be assessed with the International Knee Documentation Committee Subjective Knee Form (IKDC-SKF),[22] a Single Assessment Numeric Evaluation (SANE) of global knee function,[23] and the four subscales of Knee injury and Osteoarthritis Outcome Score (Pain, other Symptoms, Function in sport and recreation (sport/rec) and knee-related quality of life (QOL); $KOOS_4$).[24] Subjective *knee stability* during ADL and sports will each be assessed with a single numeric rating scale (1-10 scale) (Figure 2).

The frequency of self-reported participation in *physical activity* will be collected according to the recommendations from Swedish National Board of Welfare. Participants will report the type of physical activity they participated in (e.g. football, strength training), and the level of participation (e.g. recreational, elite), during the previous week. Participation in up to 3 activities can be recorded (Figure 2).[25]

Expectations for recovery (2 questions) and fulfillment of expectations (1 question) will be assessed using 6-item Likert scales. Participants will be asked to indicate if their goal was to return to sport and reasons for not returning. *Motivation* to return to the preinjury physical

activity will be evaluated using a questionnaire we developed based on the transtheoretical model of behavior change (Figure 2).[18]

The General *Self Efficacy* scale (GSES) will be used at baseline to assess the individual's beliefs that his/her actions determine successful outcome.[26] Knee-specific self-efficacy will be assessed with the subscale of the Knee Self Efficacy Scale (K-SES) that evaluates patients' perception of future knee function (4 questions).[27] *Psychological readiness* for return to sport will be assessed with the ACL-Return to Sport after Injury (ACL-RSI) questionnaire that includes questions on confidence in performance, emotions and risk appraisal.[28] *Satisfaction* with present knee function will be evaluated with a 7-item Likert scale ranging from "delighted" to "terrible".[29] Knee-related quality of life (QoL) will be assessed with the ACL-QoL questionnaire and KOOS QOL (Figure 2).[30]

Participants will be asked to indicate the *number of rehabilitation sessions* they have completed. *Adherence* to rehabilitation will be assessed by the patient and physiotherapist with the Sports Injury Rehabilitation Adherence Scale.[31] The importance of rehabilitation for the current knee function will be assessed on a 5-response scale ranging from "necessary for my current knee function" to "not necessary at all"[32]. Experience with health care will be assessed with a 5-item Likert scale ranging from "very good" to "very bad" (Figure 2).

Information about *new knee injuries* will be collected using a direct question, which is followed up with phone call if the injury is severe, i.e. results in functional limitation during the following days or inability to participate in physical activity. *Knee problems during physical activity participation* will be assessed with the knee-specific part of the Oslo Sports Trauma Research Centre (OSTRC) questionnaire (Figure 2).[33]

Figure 2 about here.

Clinical examination: sub-group of study participants

The sub-group of participants recruited from one of the study sites (Linköping) will have clinical examinations of knee function, performed by either an orthopedic surgeon together with a physiotherapist (always for the baseline assessment), or physiotherapist alone, or

physiotherapy student in the final year of education. All assessors will have standardised training in the clinical examination procedure.

Knee status will be assessed using knee joint effusion (circumference of the joint using a measurement tape, and the 'stroke test' [34]), knee joint laxity tests (Lachman test, Lever sign, anterior drawer and medial/lateral laxity), knee flexion and extension and ankle dorsiflexion range of motion, varus or valgus knee alignment. Instrumented knee laxity measurements will be assessed using the KT-1000 arthrometer at 133N and manual maximum (mean value of three repetitions).

Functional performance will be evaluated through qualitative assessment of gait (10m walking test), single leg squat, and four single-limb hop tests (single hop for distance, triple hop, crossover hop and 6-meter timed-hop).[35] *Postural control* will be assessed using a single-limb static balance task with eyes closed (SOLEC). Concentric quadriceps and hamstrings *strength* will be assessed using a Biodex dynamometer, at 60 degrees/second (5 repetitions) and 180 degrees/second (15 repetitions) angular velocities.

Activity registration: sub-group of study participants

At the conclusion of the clinical examination, participants will be asked to wear a triaxial accelerometer (activPAL™ micro, PAL Technologies Ltd.) for a minimum of 5 days (maximum 7 days) immediately following the examination. The accelerometer will be attached mid-way between the hip and the injured knee according to the manufacturer's recommendations.

Imaging: sub-group of study participants

Patients recruited in Linköping (approximately 130 patients) will undergo extensive imaging assessment and collection of biosamples.

Plain weight bearing *radiographs* – the current gold standard for assessment of radiographic osteoarthritis – will be obtained using a slightly modified method of the Lyon-Schuss view (participants stand, bearing equal weight through each limb [36]) and a standardised axial patellofemoral joint view (Table 1).[37]

Magnetic resonance (MR) images will be obtained from both knees at baseline for diagnostic purposes, to confirm an acute ACL tear in the index knee and to examine the status of the contralateral knee. At follow up, only the index knee will undergo MR image acquisition. MR images will be acquired using a Philips Ingenia 3T scanner with a 16-channel knee-coil and will be obtained at baseline, 3, 6, 12 and 24 months (Table 1).

Table 1. MR imaging, radiographic assessment and collection of biological samples over the study period for patients recruited at the Linköping site

	Baseline	3 months	6 months	12 months	24 months	60 months
WB radiographs	X	-	-	-	-	X
Full clinical protocol	X*	-	-	-		-
Compositional protocol	X	X	X	X	-	-
Explorative protocol	X	X	X	X	X	X
Blood	X	X	X	X	X	X
Urine	X	X	X	X	X	X
Joint fluid**	X					

** Bilateral assessment*

*** Collection will be performed under anesthesia at the time of any surgery during follow up*

For *clinical evaluation and diagnostics*, the normal clinical protocol at the Linköping site will be followed (scan time 15 minutes, Table 2).

For *bone shape analyses*, a 3D PD sequence (scan time 6.5 minutes, Table 2) will be obtained.

For *compositional analysis* of cartilage and menisci, sagittal T2maps and T1Rho will be obtained (scan time approx. 16 minutes, Table 2).

For exploratory purposes, a new in-house developed sequence (Qmap) with the potential of reducing clinical and compositional scan time and adding explorative measures of T1-maps T2-maps and PD-maps will be obtained (scan time approximately 6 minutes, Table 2).[38]

Table 2. Detailed description of magnetic resonance imaging sequences

Clinical package	Sagittal PD, 3 mm slice thickness with 0.3 mm gap. TE=20 ms; TR=1800 ms, ETL 10; FOV 160x145, ACQ matrix 516x384=0.31x0.38mm, recon matrix 528. Scan time 2:58 min. Axial PD FatSat, 3 mm slice thickness with 0.3 mm gap. TE=35 ms; TR=3981 ms, ETL 15; FOV 140x140, ACQ matrix 332x330=0.42x0.42mm, recon matrix 512. Scan time 4:15min Sagittal PD FatSat, 3 mm slice thickness with 0.3 mm gap. TE=30 ms; TR=3400 ms, ETL 15; FOV 160x145, ACQ matrix 468x399=0.31x0.40mm, recon matrix 528. Scan time 3:56min. Coronal PD FatSat, 3 mm slice thickness with 0.3 mm gap. TE=30 ms; TR=3572 ms, ETL 16; FOV 160x140, ACQ matrix 516x332=0.31x0.42mm, recon matrix 528. Scan time 3:56min
PD FS 3D	Sagittal PD FatSat 3D, 0.63 mm slice thickness, TE=185, TR=1300, ETL=63, FOV=144x162, AQC matrix 228x226=0.63x0.63, recon matrix 448. Scan time 6:31min
T2map	Sagittal T2-map (T2 relaxation), 3 mm slice thickness with 0.3 mm gap. TE=n*10 ms; TR=2371 ms, ETL 8; FOV 160x140, ACQ matrix 456x280=0.35x0.50mm, recon 560. Scan time 5:53min
T1Rho	3D sagittal spin lock (T1Rho relaxation), 4 mm slice thickness. Spin lock time (1, 10, 20 and 40ms), (TE=3.3 ms; TR=6.4 ms, ETL 64; FOV 140x140, ACQ matrix 280x268=0.50x0.52mm, recon 352. Scan time 2:36min
Qmap	Sagittal Qmap (T1 relaxation, T2 relaxation, Proton Density), 3 mm slice thickness with 0.3 mm gap. TE=8.8/110 ms; TR=4217 ms, ETL 16; FOV 160x145, ACQ matrix 364x270=0.40x0.59mm, recon 576. Scan time 6:19min

Collection and storage of biological samples: sub-group of study participants

All samples will be stored in a dedicated biobank at -70°C.

Joint fluid (haemarthrosis) will be aspirated from the index knee at baseline to ascertain joint bleeding (highly indicative of severe knee injury), according to clinical routine. Due to the pain and discomfort associated with knee joint arthrocentesis (especially in knees without effusion), additional longitudinal collection of joint fluid will only be performed in case of

surgical procedure (collection will be during surgery). Collected samples will be centrifuged at 2500G for 10 minutes and aliquoted into 0.7ml tubes for storage.

Venous blood samples will be collected at the same visit as image acquisition. Collected samples will be centrifuged and aliquoted according to a specific protocol for storage.

Urine samples will be collected at first morning void (preferred). Collected samples will be centrifuged at 1800G for 10 minutes and aliquoted into 1.0mL tubes for storage at -70°C.

Analyses will include, but are not limited to, those presented in Table 3. In addition, several markers of inflammation, such as IL-6, IL-8, IL-10, IL-12, TNF-α, INF-ɣ, will be analysed.

Table 3. Planned analyses of biomarkers

BIOMARKER	FLUID	PROCESS	TISSUE
ARGS-AGGRECAN	Serum Synovial fluid	Cartilage turnover	Cartilage
CTX-II	Urine	Type II collagen degradation	Cartilage Bone
CTX-I	Serum Urine	Bone turnover	Bone
COMP	Serum Synovial fluid	Cartilage degradation	Cartilage
C2C	Serum Urine	Type II collagen degradation	Cartilage
NTX-I	Serum Urine	Bone resorption	Bone

Decision making for choice of treatment and return to sport

Factors affecting the *decision of choice of treatment* (ACLR or not) will be evaluated by the patient, orthopedic surgeon and physiotherapist with questionnaires. Respondents will answer questions about why the particular treatment was chosen, if they perceive it was the right treatment choice, the agreement for choice of treatment between the clinicians and patient, about the patient's involvement and understanding of information, and about the communication between clinicians. The physiotherapist and patient also answer questions about patient rehabilitation.

Factors affecting the *decision for return to sports* (RTS) is evaluated at the time the patient replies that she/he is back to full sports participation of the goal sport/physical activity (with or without knee problems) based on the response to a question from the OSTRC questionnaire [33] ("have you had any difficulties participating in your sport activity due to your knee problems" with the response "full participation without or with knee problems"). Other questions capture the areas on how the decision for RTS was taken, possible criteria used to approve RTS, activity and participation modification.

Primary outcomes and statistical analyses

A suite of analyses is planned for each of the 5 main study objectives.

Study objective A: assessment of the biological, psychological and social factors, and their relationships to the natural corollaries and recovery after acute ACL injury

Specific aims:

1. Assess whether there is a relationship between knee status and self-reported function early (up to 8 weeks) following ACL injury, and the IKDC-SKF at 3 and 12 months follow-up.
2. Assess whether there is a relationship between knee status in the first 8 weeks following injury and functional performance at 12 months follow-up
3. Evaluate how physical activity, self-reported activity participation or as measured by activPAL, in the first 8 weeks after ACL injury is related to self-reported function and functional performance at 3 and 12 months after injury.
4. Investigate the prognostic relationship between returning to physical activity after ACL injury, and key biological, psychological and social factors.

Primary outcome: Self-reported physical activity participation and IKDC subjective knee score at 12 months follow-up

Secondary outcomes: functional performance at 3, 6, 12 and 24 months follow-up, time to return to the goal physical activity

Statistical analysis: We will use generalised estimating equations (GEE) to assess longitudinal relationships between knee status and subjective knee function. The outcome variable will

be IKDC subjective knee form score. Predictor variables may include knee joint effusion, laxity and range of motion, and SANE.

We will use GEE to assess longitudinal relationships between knee status and functional performance. The outcome variables will be measures of hopping performance, strength and postural control. Predictor variables may include knee joint effusion, laxity and range of motion, and SANE.

We will use multilevel modelling to assess relationships between physical activity and knee status. The outcome variable will be IKDC subjective knee form score. Predictor variables may include physical activity (self-reported and objectively measured), knee status (extent of index injury (i.e. concomitant injuries), knee joint effusion, laxity and range of motion), age and sex.

We will use multilevel modelling to assess the prognostic relationship between returning to the goal physical activity and biopsychosocial factors. The outcome variable will be time to return to the goal physical activity. The predictor variables will be different biopsychosocial factors (collected with questionnaires and clinical examination). We will use factor analysis to guide which independent variables are entered into the model.

Study objective B: evaluation of the choice of treatment after ACL injury

Specific aims:

1. Describe factors that are important for the choice of treatment after an ACL injury, i.e. ACLR or non-ACLR, from patients, orthopedic surgeons' and physical therapists' perspective
2. To confirm the factors identified as important for treatment choice, using demographic and patient-reported data
3. Assess the relationship between factors (biological, psychological, social factors and factors that affected the choice of treatment) and satisfactory knee function (IKDC subjective knee form) at 12 months
4. Describe the decision-making process for treatment and evaluate patient satisfaction with the decision that was made

Primary outcomes: satisfaction with the treatment choice and the relationship to patient-reported outcome (IKDC) at 12 months after injury or ACL-R

Secondary outcomes: factors affecting treatment decision

Statistical analysis: we will summarise the treatment decision factors reported by patients and clinicians descriptively using frequency tables. We will confirm whether specific treatment factors exist for individual patients, by matching the patient's own demographic and/or patient-reported data to the relevant factor.

We will use factor analysis to determine the common constructs underlying the factors that are important for the choice of treatment. The smaller number of related groups of factors will be used in a subsequent multivariable model. We will run separate analyses for the factors cited as important for the decision for ACLR and the factors cited as important for the decision for non-ACLR.

Finally, we will use a multilevel model to estimate the relationship between biopsychosocial factors and self-reported knee function at 12 months. The outcome variable will be IKDC subjective knee form score at 12 months. The predictor variables may include clusters of biopsychosocial factors (identified in A - Assessment of the biological, psychological and social factors, and their relationships to the natural corollaries and recovery after acute ACL injury) and treatment choice clusters (independent variables). The model will be adjusted for treatment received (i.e. ACLR or non-ACLR).

Study objective C: evaluation of return to sport after ACL injury

Specific aims:

1. Describe the decision-making process for return to sport following ACL injury
2. Describe the criteria physiotherapists use in clinical practice to clear patients to return to sport after ACL injury
3. Validate the criteria used to clear patients to return to sport after ACL injury

Primary outcome: return to sport rate at 24 months follow-up

Secondary outcomes: time to return to sport, sports participation rates over time, incidence of new knee injuries

Statistical analysis: we will summarise the return to sport decision factors reported by patients and clinicians descriptively using frequency tables. We will also summarise the criteria used by clinicians to decide when the patient was ready to return to sport descriptively using frequency tables. To assess the discriminant validity of the criteria used to clear patients to return to sport after ACL injury, we will use logistic regression analyses to compare relevant outcomes (e.g. strength, effusion, range of motion) between participants who do and do not return to sport.

Study objective D: knee problems in the short- and long-term after acute ACL injury

Specific aim:

1. Describe the rate and nature of knee problems (new acute knee injury, gradual onset knee injury and osteoarthritis) after index ACL injury
2. Assess whether there is a relationship between biological, psychological and social factors, and new knee problems after acute ACL injury

Primary outcome: new acute knee injury

Secondary outcomes: gradual onset knee injury, osteoarthritis

Statistical analysis: we will use a time-to-event analysis to estimate the rate of new acute knee injuries (may include new ACL tears, new meniscus tears), the rate of radiographic osteoarthritis and the rate of symptomatic osteoarthritis. The predictor variables may include concomitant injury to other knee structures at index ACL injury, treatment (ACLR or non-ACLR), sex, age and clusters of biopsychosocial factors (identified in A - Assessment of the biological, psychological and social factors, and their relationships to the natural corollaries and recovery after acute ACL injury).

We will use a multi-level modeling approach to assess whether there is a relationship between biopsychosocial factors and gradual onset knee injuries. The independent variables may include clusters of biopsychosocial factors (identified in “Assessment of the biological, psychological and social factors, and their relationships to the natural corollaries and recovery after acute ACL injury”), treatment, sex and age.

Study objective E: identification of proxies for early detection of osteoarthritis

Specific aims

1. Identify imaging-based proxies of early radiographic and symptomatic osteoarthritis
2. Identify change in specific local and/or systemic molecular biomarkers (biological proxies) and investigate their relation to imaging based structural change and patient relevant outcomes
3. Investigate the temporal relation between symptoms, structure and biology after knee injury

Primary outcome: radiographic osteoarthritis at 5-years follow-up

Secondary outcomes: MR-defined at 2-years follow-up; symptoms as defined by IKDC subjective knee form and SANE at 2- and 5-years follow-up

Statistical analysis: We will use a multi-level modelling approach to relate predictor variables that may include imaging-based and biologically-based proxies, and possible risk factors (e.g. concomitant injury to other knee structures at index ACL injury, treatment (ACLR or non-ACLR), new meniscus injury, activity participation) to the primary and secondary outcomes. We will adjust for potential confounders that may include sex, age, and body mass index.

Sample size calculation

For regression analysis in the different parts, using approximately 10 independent variables for each outcome, at least 130 participants will be included.[39] For part B and C, evaluating decision for treatment and RTS, we need geographically spread collected data in order to be generalisable. Since there might be different routines and common praxis among different clinics even in the same geographical area, it is important to include different clinics when collecting data. We are collecting data from seven different counties, and several clinics within these counties, spreading from south to north of Sweden. We expect to collect data regarding decision making from at least about 25 orthopedic surgeons (about 10% of all surgeons performing ACL reconstructions over Sweden) and at least 45 physiotherapists (there is no registry for the number of physiotherapists treating ACL injured patients in Sweden).

Patient and Public Involvement

Participants will receive a short summary of the results following completion of the study.

Timeline

Patient recruitment started in October 2016 and will continue until June 2018.

Ethics and dissemination

Being included in this study will not influence which treatment the patient will receive. The study is approved by the regional Ethical committee in Linköping, Sweden (Dnr 2016/44-31 and 2017/221-32).

Results will be presented at national and international conferences and submitted for publication to peer-reviewed journals. Participants will receive short summary of the study.

Figure legends

Figure 1. Flow chart of the NACOX study follow-up plan. w1, w2 and m3, m6 etc: denote weeks or months after injury. Each box denotes when a questionnaire will be sent; shaded boxes indicate extended questionnaires. Time points for clinical examination (blue arrows), Magnetic Resonance Imaging (MRI) and lab and x-rays (black arrows) are indicated. Baseline questionnaire, clinical examination, MRI, lab and x-ray are within 6 weeks after the injury.

Figure 2. Reported outcomes at different time points after injury or reconstruction. $KOOS_4$, Knee Injury and Osteoarthritis Outcome Score, subscales for pain, symptoms, function in sport and recreation and knee-related quality of life; RTS, return to sport; *, answered by the patient, orthopedic surgeon and physiotherapist; (x), only some questions are asked during these timepoints; [1], question answered when the decision for ACLR is made; [2],question answered by the patient and physiotherapist when the patient has returned to full sports participation; [3],assessed only for non-ACLR; [4],only the subscales "life style" and "social and emotional" of the ACL-QoL; QoL, quality of life.

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Authors' contributions:

JK wrote the first draft and coordinated the preparation of the protocol together with CA. HG, HTG, MH, AS and RF were responsible for different parts of the protocol writing. All authors revised and accepted the final manuscript of the protocol.

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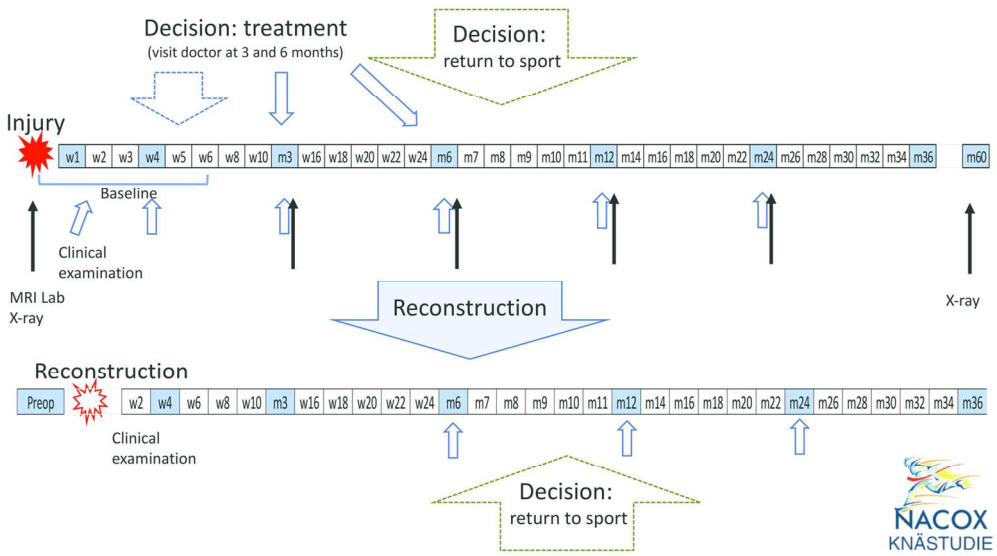
Competing interests statement

None declared



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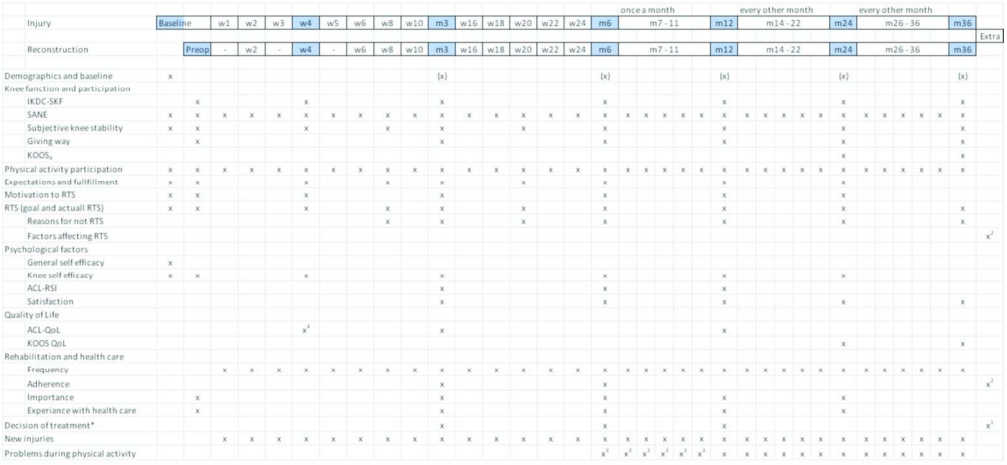

Figure 2. Reported outcomes at different time points after injury or reconstruction. KOOS4, Knee Injury and Osteoarthritis Outcome Score, subscales for pain, symptoms, function in sport and recreation and knee-related quality of life; RTS, return to sport; *, answered by the patient, orthopedic surgeon and physiotherapist; (x), only some questions are asked during these timepoints; 1, question answered when the decision for ACLR is made; 2,question answered by the patient and physiotherapist when the patient has returned to full sports participation; 3,assessed only for non-ACLR; 4,only the subscales "life style" and "social and emotional" of the ACL-QoL; QoL, quality of life.

126x59mm (300 x 300 DPI)

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Natural corollaries and recovery after acute ACL injury – the NACOX cohort study protocol

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Study protocol

Natural corollaries and recovery after acute ACL injury – the NACOX cohort study protocol

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ABSTRACT

Introduction

Anterior cruciate ligament (ACL) injury can result in joint instability, decreased functional performance, reduced physical activity and quality of life, and an increased risk for post-traumatic osteoarthritis. Despite the development of new treatment techniques and extensive research, the complex and multifaceted nature of ACL injury and its consequences are yet to be fully understood. The overall aim of the NACOX study is to evaluate the natural corollaries and recovery after an ACL injury.

Methods and analysis

The NACOX study is a multi-centre prospective prognostic cohort study of patients with acute ACL injury. At 7 sites in Sweden, we will include patients aged 15-40 years, within 6 weeks after primary ACL injury. Patients will complete questionnaires at multiple occasions over the 3 years following injury or the 3 years following ACL reconstruction (for participants who have surgical treatment). In addition, a subgroup of 130 patients will be followed with clinical examinations, several imaging modalities, and biological samples. Data analyses will be specific to each aim.

Ethics and dissemination

This study has been approved by the regional Ethical committee in Linköping, Sweden (Dnr 2016/44-31 and 2017/221-32). We plan to present the results at national and international conferences, and in peer-reviewed scientific journals. Participants will receive a short summary of the results following completion of the study.

Trial registration: NCT02931084

Strengths and limitations of this study

- The prospective observational design with frequently-monitored biological, psychological and social variables allows analysis of outcomes at key, clinically-relevant time points after injury.
- Quantitative methods are used to assess the perspectives of important stakeholders in acute ACL injury management (patients and clinicians (orthopaedic surgeons and physiotherapists)).
- The utilisation of advanced imaging techniques and collection of biological samples for identification of proxies of early osteoarthritis that can be related to prospectively collected patient-reported outcome measures and clinical data.
- Loss to follow-up and missing data may be a risk due to the extensive collection of patient-reported outcomes. However, we have a dedicated study monitoring team, and a rigorous data analysis plan to appropriately deal with missing data.

INTRODUCTION

Anterior cruciate ligament (ACL) injuries are common in young athletes. In Sweden, there are approximately 7000 new injuries per year representing approximately 0.81/1000 inhabitants aged 10-64 years.[1] Despite the extensive research to identify the best treatment algorithms, there are still many patients who report unsatisfactory outcomes regarding knee stability, activity level and quality of life following ACL injury.[2 3] This may be because research has tended to focus on single factors, rather than accounting for the multifactorial nature of injury and recovery. There is also a clinical dogma that ACL reconstruction is necessary for a successful outcome after ACL injury and to resume sporting activities.[4 5] Although there is evidence that some patients have functional disability fulfilling the clinical indications for ACL reconstruction,[6] with high quality rehabilitation, many patients achieve satisfactory knee function and participation in sports without surgery.[7 8]

An ACL injury has biological, psychological and social corollaries,[9] that directly affect the patient (e.g. impaired quality of life and lower physical activity participation), and may affect the community (e.g. increased health utilisation costs, impaired productivity, potential for increased chronic disease burden through flow-on development of non-communicable diseases associated with physical inactivity such as cardiovascular disease and diabetes). Taking a biopsychosocial approach, factors including the extent of the initial injury (e.g. whether there were other knee structures involved), factors directly related to the treatment (e.g. which intervention and when), and patient preferences, expectations and past experiences may all be relevant when assessing outcomes.

The most serious long-term corollary after ACL injury is the increased risk for post-traumatic osteoarthritis, estimated to be up to 50% by 15 years after injury.[3] This risk of developing osteoarthritis is higher if the ACL injury is associated with a meniscus tear, and there are conflicting results regarding whether having ACL reconstruction reduces or increases the risk of osteoarthritis.[4 5 10-12] The underlying mechanisms behind the development of osteoarthritis are not well understood. Altered biological processes due to injury and joint bleeding, concomitant structural injuries to the cartilage and the subchondral bone, and joint instability and subsequent altered biomechanics, may be relevant for the development of osteoarthritis. Secondary joint trauma (e.g. with additional meniscal tears, or ACL reconstruction[13]) may also influence the risk for osteoarthritis.

Outcomes need to be evaluated from both the patient's and the clinician's perspective. Patient-reported outcomes provide important insights into aspects of injury and recovery that cannot otherwise be observed or measured with clinical tests or imaging.[14] Clinical outcomes provide the clinician (and by extension – in a well applied shared decision-making approach – the patient) with feedback regarding the effects of different clinical decisions on injury (i.e. which treatment and when), and any physical changes that occur following a clinical decision (e.g. change in effusion and muscle strength, incidence of new injuries, development of osteoarthritis).

The short-term aim of ACL injury management is to achieve satisfactory knee function and physical activity participation. In the long-term, treatment should aim to reduce the risk of developing osteoarthritis. Satisfaction is complex and short-term success (e.g. returning to pivoting sports) may facilitate longer term failure (e.g. developing osteoarthritis after sustaining a second or third meniscal injury). From the patient's perspective, satisfaction can relate to both the outcome of management of the injury (including knee function, confidence to participate in physical activity, fulfillment of expectations for recovery), and to the process of health care delivery (including being an active participant in the decision making process, communication with clinicians, information about the injury and treatment).[15-18] The clinician needs to monitor the resolution of impairments (knee stability, symmetrical lower limb muscle strength, absence of knee effusion), to ensure that treatment is tailored so that the patient has the physical capacity to reach his or her expectations (e.g. return to sport, return to occupation).[19] However, it is evident that these criteria cover only some of the spectrum of possible corollaries of ACL injury.

There is evidence that treatment after ACL injury needs to be individualised.[20] The clinician needs to be able to account for and (ideally) address the important biological, psychological and social factors for each patient. However, we still lack evidence regarding which factors, for which patient, at which time; and this poses challenges for clinical practice. Therefore, to enhance understanding of the consequences of ACL injury, and improve treatment, the overall aim of the NACOX study is to investigate the natural corollaries and recovery after ACL injury. Understanding the complexity of the consequences of ACL injury may improve clinical decision-making to ensure best health care for patients.

To achieve the overall aim, there are five main study objectives:

A. To assess biological, psychological and social factors and their relationships to the natural corollaries and recovery after acute ACL injury
B. To evaluate the choice of treatment after acute ACL-injury (i.e. ACL reconstruction, ACLR or non-ACL reconstruction, non-ACLR)
C. To evaluate the return to sport after acute ACL injury
D. To study knee problems in the short and long term after acute ACL-injury
E. To identify proxies (biomarkers and structural risk factors) for early detection of symptomatic and radiographic osteoarthritis

METHODS AND ANALYSIS

This study is a prospective multicenter prognostic cohort study. Patients will be consecutively recruited over approximately 20 months, from up to seven sites (mix of public and private health care clinics) in Sweden.

Inclusion and exclusion criteria

All patients with acute knee trauma presenting to the identified clinics are potentially eligible for participation.

Inclusion criteria: Patients with an ACL injury, sustained no more than 6 weeks prior to presentation, and aged between 15 and 40 years at time of ACL injury.

Exclusion criteria: previous ACL injury/ACL reconstruction on the same knee, serious concomitant knee injury (e.g. posterior cruciate ligament rupture, fracture that requires separate treatment), inability to understand written and spoken Swedish language, cognitive impairments, other illness or injury that impairs function (e.g. fibromyalgia, rheumatic diseases and other diagnoses associated with chronic pain).

Procedure

Recruitment of participants started in October 2016, and this study does not alter the usual course of treatment for patients with ACL injury at recruiting centres. This process is:

1. Patient receives a clinical diagnosis from an orthopedic surgeon, verified by MRI, within 2-6 weeks after their knee injury.

2. Initial treatment according to a supervised rehabilitation program of approximately three months duration.[21]
3. Scheduled follow-up after approximately 3 months, where further treatment is decided upon between patient and orthopaedic surgeon.

Consequently, patients of this cohort will follow one of the two following pathways: (1) ACLR plus post-operative supervised rehabilitation and (2) supervised rehabilitation alone (non-ACLR).

Patients will receive information about the study at their initial contact with the health care provider. Subsequently, a member of the research team will contact the patient by phone to provide additional verbal information and obtain verbal consent. Patients who accept participation will be asked to sign a written informed consent form before questionnaires are sent via smartphone or e-mail. Questionnaires will be sent weekly for the first 6 weeks, fortnightly from week 7 to week 24, monthly from month 7 to month 12, and bi-monthly from year 1 to year 3 after initial injury. Questionnaire length varies from very short (10 questions, approximately 2 min completion time) to longer at specific critical time points (Figure 1 and Table 1).

A questionnaire about treatment choice (ACLR or non-ACLR) is completed by the patient, orthopedic surgeon and physiotherapist at the time the decision for ACLR or non-ACLR is made. A questionnaire about the decision to return to sport is completed by the patient and the physiotherapist when the patient reports that he/she is back to full participation in the goal sports/physical activity. For patients with ACLR, a new baseline questionnaire will be completed at the time of reconstruction. Subsequent data collection will continue according to the new baseline time point (Figure 1).

One subgroup (approximately 130 patients recruited from Linköping) will have extended follow-up data collection at baseline and 3, 6, 12 and 24 months after injury. At these time points, a clinical examination will be completed by a physiotherapist, physical activity will be registered over 5 consecutive days using a triaxial accelerometer (activPAL™, PALtechnologies, UK), knee MRI will be performed, and blood and urine samples will be collected. A joint fluid sample is acquired at baseline if indicated due to joint effusion, and at the time of any additional surgery including ACLR (if the patient has surgical treatment).

Weight bearing radiographs are done at baseline and 5 years follow up. Patients who have ACLR are followed up with questionnaires and clinical examination with new baseline at the time of reconstruction. MRIs and blood and urine samples are followed up with the injury according to the index baseline (Figure 1). Additional verbal and written consent for collection of biological samples and imaging is obtained prior to any data collection.

Figure 1 about here

Outcomes

Reflecting a biopsychosocial approach, outcome measurement for this study will evaluate four main aspects: patient-reported outcomes, physical function, physical activity and physiological markers of joint injury (Figure 1).

Patient-reported outcomes: all study participants

Demographic and baseline characteristics including age, sex, BMI, smoking habits, occupation, preinjury activity level, medical and injury history, sick leave, preferences regarding treatment will be collected with the baseline questionnaire (Figure 2).

Patient-reported *knee function and participation* will be assessed with the International Knee Documentation Committee Subjective Knee Form (IKDC-SKF),[22] a Single Assessment Numeric Evaluation (SANE) of global knee function,[23] and the four subscales of Knee injury and Osteoarthritis Outcome Score (Pain, other Symptoms, Function in sport and recreation (sport/rec) and knee-related quality of life (QOL); $KOOS_4$).[24] Subjective *knee stability* during ADL and sports will each be assessed with a single numeric rating scale (1-10 scale) (Figure 2).

The frequency of self-reported participation in *physical activity* will be collected according to the recommendations from Swedish National Board of Welfare. Participants will report the type of physical activity they participated in (e.g. football, strength training), and the level of participation (e.g. recreational, elite), during the previous week. Participation in up to 3 activities can be recorded (Figure 2).[25]

Expectations for recovery (2 questions) and fulfillment of expectations (1 question) will be assessed using 6-item Likert scales. Participants will be asked to indicate if their goal was to return to sport and reasons for not returning. *Motivation* to return to the preinjury physical activity will be evaluated using a questionnaire we developed based on the transtheoretical model of behavior change (Figure 2).[18]

The General *Self Efficacy* scale (GSES) will be used at baseline to assess the individual's beliefs that his/her actions determine successful outcome.[26] Knee-specific self-efficacy will be assessed with the subscale of the Knee Self Efficacy Scale (K-SES) that evaluates patients' perception of future knee function (4 questions).[27] *Psychological readiness* for return to sport will be assessed with the ACL-Return to Sport after Injury (ACL-RSI) questionnaire that includes questions on confidence in performance, emotions and risk appraisal.[28] *Satisfaction* with present knee function will be evaluated with a 7-item Likert scale ranging from "delighted" to "terrible".[29] Knee-related quality of life (QoL) will be assessed with the ACL-QoL questionnaire and KOOS QOL (Figure 2).[30]

Participants will be asked to indicate the *number of rehabilitation sessions* they have completed. *Adherence* to rehabilitation will be assessed by the patient and physiotherapist with the Sports Injury Rehabilitation Adherence Scale.[31] The importance of rehabilitation for the current knee function will be assessed on a 5-response scale ranging from "necessary for my current knee function" to "not necessary at all"[32]. Experience with health care will be assessed with a 5-item Likert scale ranging from "very good" to "very bad" (Figure 2).

Information about *new knee injuries* will be collected using a direct question, which is followed up with phone call if the injury is severe, i.e. results in functional limitation during the following days or inability to participate in physical activity. *Knee problems during physical activity participation* will be assessed with the knee-specific part of the Oslo Sports Trauma Research Centre (OSTRC) questionnaire (Figure 2).[33]

Figure 2 about here.

Clinical examination: sub-group of study participants

The sub-group of participants recruited from one of the study sites (Linköping) will have clinical examinations of knee function, performed by either an orthopedic surgeon together with a physiotherapist (always for the baseline assessment), or physiotherapist alone, or physiotherapy student in the final year of education. All assessors will have standardised training in the clinical examination procedure.

Knee status will be assessed using knee joint effusion (circumference of the joint using a measurement tape, and the 'stroke test' [34]), knee joint laxity tests (Lachman test, Lever sign, anterior drawer and medial/lateral laxity), knee flexion and extension and ankle dorsiflexion range of motion, varus or valgus knee alignment. Instrumented knee laxity measurements will be assessed using the KT-1000 arthrometer at 133N and manual maximum (mean value of three repetitions).

Functional performance will be evaluated through qualitative assessment of gait (10m walking test), single leg squat, and four single-limb hop tests (single hop for distance, triple hop, crossover hop and 6-meter timed-hop).[35] *Postural control* will be assessed using a single-limb static balance task with eyes closed (SOLEC). Concentric quadriceps and hamstrings *strength* will be assessed using a Biodex dynamometer, at 60 degrees/second (5 repetitions) and 180 degrees/second (15 repetitions) angular velocities.

Activity registration: sub-group of study participants

At the conclusion of the clinical examination, participants will be asked to wear a triaxial accelerometer (activPAL™ micro, PAL Technologies Ltd.) for a minimum of 5 days (maximum 7 days) immediately following the examination. The accelerometer will be attached mid-way between the hip and the injured knee according to the manufacturer's recommendations.

Imaging: sub-group of study participants

Patients recruited in Linköping (approximately 130 patients) will undergo extensive imaging assessment and collection of biosamples.

Plain weight bearing *radiographs* – the current gold standard for assessment of radiographic osteoarthritis – will be obtained using a slightly modified method of the Lyon-Schuss view (participants stand, bearing equal weight through each limb [36]) and a standardised axial patellofemoral joint view (Table 1).[37]

Magnetic resonance (MR) images will be obtained from both knees at baseline for diagnostic purposes, to confirm an acute ACL tear in the index knee and to examine the status of the contralateral knee. At follow up, only the index knee will undergo MR image acquisition. MR images will be acquired using a Philips Ingenia 3T scanner with a 16-channel knee-coil and will be obtained at baseline, 3, 6, 12 and 24 months (Table 1).

Table 1. MR imaging, radiographic assessment and collection of biological samples over the study period for patients recruited at the Linköping site

	Baseline	3 months	6 months	12 months	24 months	60 months
WB radiographs	X	-	-	-	-	X
Full clinical protocol	X*	-	-	-		-
Compositional protocol	X	X	X	X	-	-
Explorative protocol	X	X	X	X	X	X
Blood	X	X	X	X	X	X
Urine	X	X	X	X	X	X
Joint fluid**	X					

** Bilateral assessment*

*** Collection will be performed under anesthesia at the time of any surgery during follow up*

For *clinical evaluation and diagnostics*, the normal clinical protocol at the Linköping site will be followed (scan time 15 minutes, Table 2).

For *bone shape analyses*, a 3D PD sequence (scan time 6.5 minutes, Table 2) will be obtained.

For *compositional analysis* of cartilage and menisci, sagittal T2maps and T1Rho will be obtained (scan time approx. 16 minutes, Table 2).

For exploratory purposes, a new in-house developed sequence (Qmap) with the potential of reducing clinical and compositional scan time and adding explorative measures of T1-maps T2-maps and PD-maps will be obtained (scan time approximately 6 minutes, Table 2).[38]

Table 2. Detailed description of magnetic resonance imaging sequences

Clinical package	Sagittal PD, 3 mm slice thickness with 0.3 mm gap. TE=20 ms; TR=1800 ms, ETL 10; FOV 160x145, ACQ matrix 516x384=0.31x0.38mm, recon matrix 528. Scan time 2:58 min. Axial PD FatSat, 3 mm slice thickness with 0.3 mm gap. TE=35 ms; TR=3981 ms, ETL 15; FOV 140x140, ACQ matrix 332x330=0.42x0.42mm, recon matrix 512. Scan time 4:15min Sagittal PD FatSat, 3 mm slice thickness with 0.3 mm gap. TE=30 ms; TR=3400 ms, ETL 15; FOV 160x145, ACQ matrix 468x399=0.31x0.40mm, recon matrix 528. Scan time 3:56min. Coronal PD FatSat, 3 mm slice thickness with 0.3 mm gap. TE=30 ms; TR=3572 ms, ETL 16; FOV 160x140, ACQ matrix 516x332=0.31x0.42mm, recon matrix 528. Scan time 3:56min
PD FS 3D	Sagittal PD FatSat 3D, 0.63 mm slice thickness, TE=185, TR=1300, ETL=63, FOV=144x162, AQC matrix 228x226=0.63x0.63, recon matrix 448. Scan time 6:31min
T2map	Sagittal T2-map (T2 relaxation), 3 mm slice thickness with 0.3 mm gap. TE=n*10 ms; TR=2371 ms, ETL 8; FOV 160x140, ACQ matrix 456x280=0.35x0.50mm, recon 560. Scan time 5:53min
T1Rho	3D sagittal spin lock (T1Rho relaxation), 4 mm slice thickness. Spin lock time (1, 10, 20 and 40ms), (TE=3.3 ms; TR=6.4 ms, ETL 64; FOV 140x140, ACQ matrix 280x268=0.50x0.52mm, recon 352. Scan time 2:36min
Qmap	Sagittal Qmap (T1 relaxation, T2 relaxation, Proton Density), 3 mm slice thickness with 0.3 mm gap. TE=8.8/110 ms; TR=4217 ms, ETL 16; FOV 160x145, ACQ matrix 364x270=0.40x0.59mm, recon 576. Scan time 6:19min

Collection and storage of biological samples: sub-group of study participants

All samples will be stored in a dedicated biobank at -70°C.

Joint fluid (haemarthrosis) will be aspirated from the index knee at baseline to ascertain joint bleeding (highly indicative of severe knee injury), according to clinical routine. Due to the pain and discomfort associated with knee joint arthrocentesis (especially in knees without effusion), additional longitudinal collection of joint fluid will only be performed in case of

surgical procedure (collection will be during surgery). Collected samples will be centrifuged at 2500G for 10 minutes and aliquoted into 0.7ml tubes for storage.

Venous blood samples will be collected at the same visit as image acquisition. Collected samples will be centrifuged and aliquoted according to a specific protocol for storage.

Urine samples will be collected at first morning void (preferred). Collected samples will be centrifuged at 1800G for 10 minutes and aliquoted into 1.0mL tubes for storage at -70°C.

Analyses will include, but are not limited to, those presented in Table 3. In addition, several markers of inflammation, such as IL-6, IL-8, IL-10, IL-12, TNF-α, INF-ɣ, will be analysed.

Table 3. Planned analyses of biomarkers

BIOMARKER	FLUID	PROCESS	TISSUE
ARGS-AGGRECAN	Serum Synovial fluid	Cartilage turnover	Cartilage
CTX-II	Urine	Type II collagen degradation	Cartilage Bone
CTX-I	Serum Urine	Bone turnover	Bone
COMP	Serum Synovial fluid	Cartilage degradation	Cartilage
C2C	Serum Urine	Type II collagen degradation	Cartilage
NTX-I	Serum Urine	Bone resorption	Bone

Decision making for choice of treatment and return to sport

Factors affecting the *decision of choice of treatment* (ACLR or not) will be evaluated by the patient, orthopedic surgeon and physiotherapist with questionnaires. Respondents will answer questions about why the particular treatment was chosen, if they perceive it was the right treatment choice, the agreement for choice of treatment between the clinicians and patient, about the patient's involvement and understanding of information, and about the communication between clinicians. The physiotherapist and patient also answer questions about patient rehabilitation.

Factors affecting the *decision for return to sports* (RTS) is evaluated at the time the patient replies that she/he is back to full sports participation of the goal sport/physical activity (with or without knee problems) based on the response to a question from the OSTRC questionnaire [33] ("have you had any difficulties participating in your sport activity due to your knee problems" with the response "full participation without or with knee problems"). Other questions capture the areas on how the decision for RTS was taken, possible criteria used to approve RTS, activity and participation modification.

Primary outcomes and statistical analyses

A suite of analyses is planned for each of the 5 main study objectives.

Study objective A: assessment of the biological, psychological and social factors, and their relationships to the natural corollaries and recovery after acute ACL injury

Specific aims:

1. Assess whether there is a relationship between knee status and self-reported function early (up to 8 weeks) following ACL injury, and the IKDC-SKF at 3 and 12 months follow-up.
2. Assess whether there is a relationship between knee status in the first 8 weeks following injury and functional performance at 12 months follow-up
3. Evaluate how physical activity, self-reported activity participation or as measured by activPAL, in the first 8 weeks after ACL injury is related to self-reported function and functional performance at 3 and 12 months after injury.
4. Investigate the prognostic relationship between returning to physical activity after ACL injury, and key biological, psychological and social factors.

Primary outcome: Self-reported physical activity participation and IKDC subjective knee score at 12 months follow-up

Secondary outcomes: functional performance at 3, 6, 12 and 24 months follow-up, time to return to the goal physical activity

Statistical analysis: We will use generalised estimating equations (GEE) to assess longitudinal relationships between knee status and subjective knee function. The outcome variable will

be IKDC subjective knee form score. Predictor variables may include knee joint effusion, laxity and range of motion, and SANE.

We will use GEE to assess longitudinal relationships between knee status and functional performance. The outcome variables will be measures of hopping performance, strength and postural control. Predictor variables may include knee joint effusion, laxity and range of motion, and SANE.

We will use multilevel modelling to assess relationships between physical activity and knee status. The outcome variable will be IKDC subjective knee form score. Predictor variables may include physical activity (self-reported and objectively measured), knee status (extent of index injury (i.e. concomitant injuries), knee joint effusion, laxity and range of motion), age and sex.

We will use multilevel modelling to assess the prognostic relationship between returning to the goal physical activity and biopsychosocial factors. The outcome variable will be time to return to the goal physical activity. The predictor variables will be different biopsychosocial factors (collected with questionnaires and clinical examination). We will use factor analysis to guide which independent variables are entered into the model.

Study objective B: evaluation of the choice of treatment after ACL injury

Specific aims:

1. Describe factors that are important for the choice of treatment after an ACL injury, i.e. ACLR or non-ACLR, from patients, orthopedic surgeons' and physical therapists' perspective
2. To confirm the factors identified as important for treatment choice, using demographic and patient-reported data
3. Assess the relationship between factors (biological, psychological, social factors and factors that affected the choice of treatment) and satisfactory knee function (IKDC subjective knee form) at 12 months
4. Describe the decision-making process for treatment and evaluate patient satisfaction with the decision that was made

Primary outcomes: satisfaction with the treatment choice and the relationship to patient-reported outcome (IKDC) at 12 months after injury or ACL-R

Secondary outcomes: factors affecting treatment decision

Statistical analysis: we will summarise the treatment decision factors reported by patients and clinicians descriptively using frequency tables. We will confirm whether specific treatment factors exist for individual patients, by matching the patient's own demographic and/or patient-reported data to the relevant factor.

We will use factor analysis to determine the common constructs underlying the factors that are important for the choice of treatment. The smaller number of related groups of factors will be used in a subsequent multivariable model. We will run separate analyses for the factors cited as important for the decision for ACLR and the factors cited as important for the decision for non-ACLR.

Finally, we will use a multilevel model to estimate the relationship between biopsychosocial factors and self-reported knee function at 12 months. The outcome variable will be IKDC subjective knee form score at 12 months. The predictor variables may include clusters of biopsychosocial factors (identified in A - Assessment of the biological, psychological and social factors, and their relationships to the natural corollaries and recovery after acute ACL injury) and treatment choice clusters (independent variables). The model will be adjusted for treatment received (i.e. ACLR or non-ACLR).

Study objective C: evaluation of return to sport after ACL injury

Specific aims:

1. Describe the decision-making process for return to sport following ACL injury
2. Describe the criteria physiotherapists use in clinical practice to clear patients to return to sport after ACL injury
3. Validate the criteria used to clear patients to return to sport after ACL injury

Primary outcome: return to sport rate at 24 months follow-up

Secondary outcomes: time to return to sport, sports participation rates over time, incidence of new knee injuries

Statistical analysis: we will summarise the return to sport decision factors reported by patients and clinicians descriptively using frequency tables. We will also summarise the criteria used by clinicians to decide when the patient was ready to return to sport descriptively using frequency tables. To assess the discriminant validity of the criteria used to clear patients to return to sport after ACL injury, we will use logistic regression analyses to compare relevant outcomes (e.g. strength, effusion, range of motion) between participants who do and do not return to sport.

Study objective D: knee problems in the short- and long-term after acute ACL injury

Specific aim:

1. Describe the rate and nature of knee problems (new acute knee injury, gradual onset knee injury and osteoarthritis) after index ACL injury
2. Assess whether there is a relationship between biological, psychological and social factors, and new knee problems after acute ACL injury

Primary outcome: new acute knee injury

Secondary outcomes: gradual onset knee injury, osteoarthritis

Statistical analysis: we will use a time-to-event analysis to estimate the rate of new acute knee injuries (may include new ACL tears, new meniscus tears), the rate of radiographic osteoarthritis and the rate of symptomatic osteoarthritis. The predictor variables may include concomitant injury to other knee structures at index ACL injury, treatment (ACLR or non-ACLR), sex, age and clusters of biopsychosocial factors (identified in A - Assessment of the biological, psychological and social factors, and their relationships to the natural corollaries and recovery after acute ACL injury).

We will use a multi-level modeling approach to assess whether there is a relationship between biopsychosocial factors and gradual onset knee injuries. The independent variables may include clusters of biopsychosocial factors (identified in “Assessment of the biological, psychological and social factors, and their relationships to the natural corollaries and recovery after acute ACL injury”), treatment, sex and age.

Study objective E: identification of proxies for early detection of osteoarthritis

Specific aims

1. Identify imaging-based proxies of early radiographic and symptomatic osteoarthritis
2. Identify change in specific local and/or systemic molecular biomarkers (biological proxies) and investigate their relation to imaging based structural change and patient relevant outcomes
3. Investigate the temporal relation between symptoms, structure and biology after knee injury

Primary outcome: radiographic osteoarthritis at 5-years follow-up

Secondary outcomes: MR-defined at 2-years follow-up; symptoms as defined by IKDC subjective knee form and SANE at 2- and 5-years follow-up

Statistical analysis: We will use a multi-level modelling approach to relate predictor variables that may include imaging-based and biologically-based proxies, and possible risk factors (e.g. concomitant injury to other knee structures at index ACL injury, treatment (ACLR or non-ACLR), new meniscus injury, activity participation) to the primary and secondary outcomes. We will adjust for potential confounders that may include sex, age, and body mass index.

Sample size calculation

For regression analysis in the different parts, using approximately 10 independent variables for each outcome, at least 130 participants will be included.[39] For part B and C, evaluating decision for treatment and RTS, we need geographically spread collected data in order to be generalisable. Since there might be different routines and common praxis among different clinics even in the same geographical area, it is important to include different clinics when collecting data. We are collecting data from seven different counties, and several clinics within these counties, spreading from south to north of Sweden. We expect to collect data regarding decision making from at least about 25 orthopedic surgeons (about 10% of all surgeons performing ACL reconstructions over Sweden) and at least 45 physiotherapists (there is no registry for the number of physiotherapists treating ACL injured patients in Sweden).

Patient and Public Involvement

Participants will receive a short summary of the results following completion of the study.

Timeline

Patient recruitment started in October 2016 and will continue until June 2018.

Ethics and dissemination

Being included in this study will not influence which treatment the patient will receive. The study is approved by the regional Ethical committee in Linköping, Sweden (Dnr 2016/44-31 and 2017/221-32).

Results will be presented at national and international conferences and submitted for publication to peer-reviewed journals. Participants will receive short summary of the study.

Figure legends

Figure 1. Flow chart of the NACOX study follow-up plan. w1, w2 and m3, m6 etc: denote weeks or months after injury. Each box denotes when a questionnaire will be sent; shaded boxes indicate extended questionnaires. Time points for clinical examination (blue arrows), Magnetic Resonance Imaging (MRI) and lab and x-rays (black arrows) are indicated. Baseline questionnaire, clinical examination, MRI, lab and x-ray are within 6 weeks after the injury.

Figure 2. Reported outcomes at different time points after injury or reconstruction. $KOOS_4$, Knee Injury and Osteoarthritis Outcome Score, subscales for pain, symptoms, function in sport and recreation and knee-related quality of life; RTS, return to sport; *, answered by the patient, orthopedic surgeon and physiotherapist; (x), only some questions are asked during these timepoints; [1], question answered when the decision for ACLR is made; [2],question answered by the patient and physiotherapist when the patient has returned to full sports participation; [3],assessed only for non-ACLR; [4],only the subscales "life style" and "social and emotional" of the ACL-QoL; QoL, quality of life.

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Authors' contributions:

JK wrote the first draft and coordinated the preparation of the protocol together with CA. HG, HTG, MH, AS and RF were responsible for different parts of the protocol writing. All authors revised and accepted the final manuscript of the protocol.

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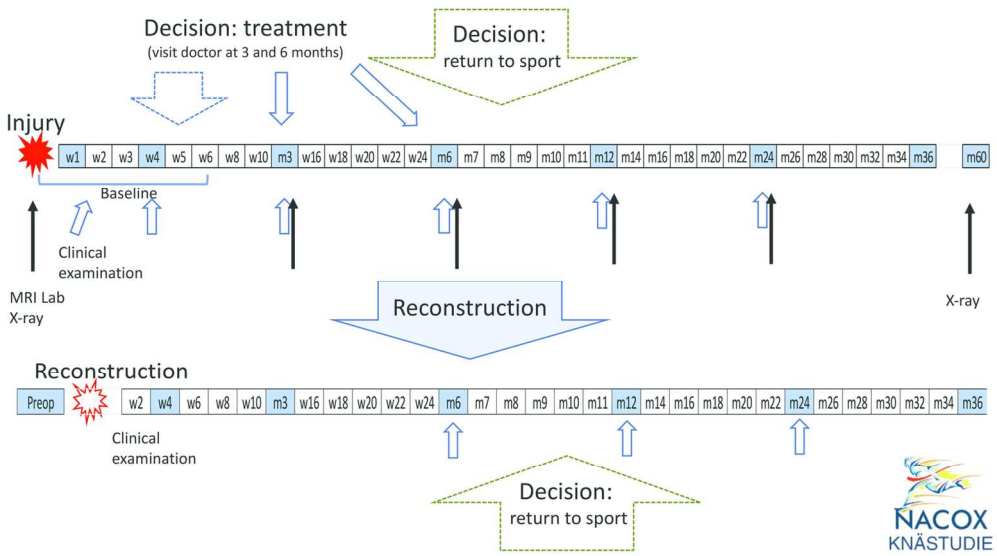
Competing interests statement

None declared



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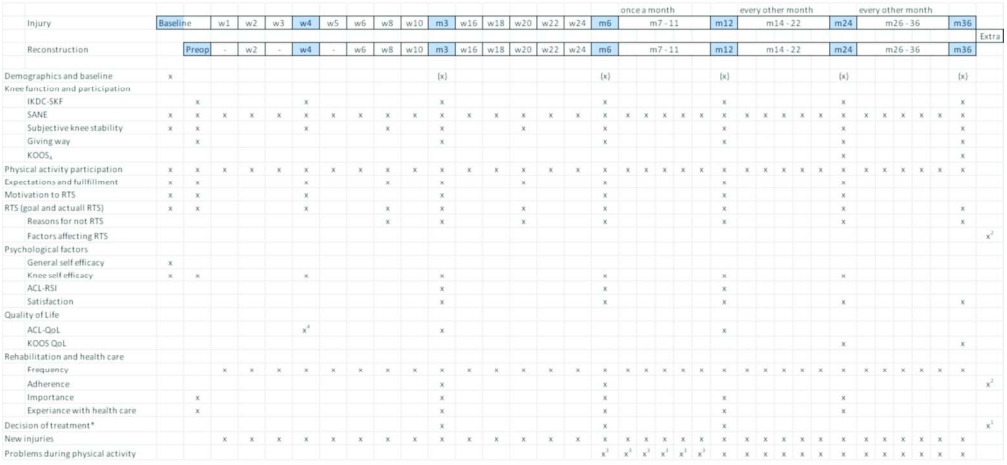

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