Molecular pathways involved in knee pain: an observational study

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SYNOPSIS

Title	Molecular pathways involved in knee pain: an observational study
Short title	Molecular pathways involved in knee pain
Chief Investigator	Dr Ana M Valdes
Objectives	**Observational Study:** [1] To test whether there is an association between proteomic concentrations in the synovial fluid and pain hypersensitivity. [2] To identify proteomic signatures in synovial fluid of centrally vs peripherally driven knee pain. [3] To investigate the relationship of pain hypersensitivity and different proteomic profiles with radiologically-established joint structural changes and muscle strength. [4] To explore the correlation between knee inflammation (effusion, synovial hypertrophy or/and synovial hyper vascularity) and biomarkers of insulin resistance, stress, DNA markers, poor metabolism and knee pain.
Study Configuration	Observational cross-sectional study
Setting	University and secondary care
Sample size estimate	**Observational Study:** We propose to compare proteomic signatures in 140 knee pain cases. To achieve a Bonferroni $p < 1.25 \times 10^{[-5]}$ (adjusting for 4000 tests, i.e. the number of proteins in the Somalogic platform) effect sizes of beta=0.64 in units of standard deviations are needed to achieve 80% statistical power. Based on the effects seen on radiographic progression (standardized beta =0.62) these effects appear reasonable and achievable with the proposed sample size. **Reliability Sub-study:** Considering that the study will comprise two different sessions (n=2) featuring one measurement within each and with minimally accepted reliability of ρ0=0.5 and expected reliability of ρ=0.8, the minimum sample size has been calculated to be 22 participants. High variability is not expected however, to account for such an occurrence, data will be collected from 25 individuals.
Number of participants	Observational Study: 140 Sub-study: 25
Eligibility criteria	Inclusion criteria: • have the ability to give informed consent • be 45 years or over

	• have complaints of knee pain for 3-6 months with or without radiographically established OA (K/L scale score ≥ 1) • have complaints of knee pain for 3-6 months with or without satisfying the non-radiographic American College of Rheumatology criteria for knee OA • are willing to undertake knee synovial fluid aspiration • be able to speak, read, and write in English as all instructions and questionnaires are designed in the English language <u>Exclusion criteria:</u> • Inability to give informed consent due to cognitive impairment or otherwise – (capacity levels are already established under GP care) • Inability to understand key aspects of the study due to cognitive impairment or otherwise • Giving history of additional co-morbidities such as cancer, neurological conditions, inflammatory joint diseases including rheumatoid arthritis, diabetic neuropathies, fractures or other conditions causing greater disability than their knee pain • Acute soft tissue injury to the knee within last 3 months before potential recruitment Same criteria apply for the sub-study
Description of interventions	Clinical assessment for this study will include self-report questionnaires, and ultra-sound scanning, synovial fluid aspiration, blood samples. and faecal extraction as well as balance and muscle strength assessment through a sensitive platform and an isokinetic dynamometer respectively. The duration of the clinical assessment will be approximately 1.5 hours.
Duration of study	18 months Planned recruitment date: January 2020 Per participant appointment duration (Observational and Sub-study): 1 x 2 hours
Outcome measures	**Primary:** Pain measured by patient reported pain scoring. **Secondary:** Knee joint morphology on X-ray, Central aspects of pain in knee (CAP-Knee), Synovial fluid protein concentration, Cognitive function (CFQ), Frailty (FRAIL), Inflammatory markers on ultrasound (Synovial fluid, synovial hypertrophy and hypervascularity), Muscle Strength, , Biomarkers of insulin resistance, DNA markers, compromised metabolism, and stress generators (cortisol, gut microbiota), Physical functioning (Time up and go test, 30-second sit to stand test), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), General health questionnaire (EQ5D-5L)
Statistical methods	Standard parametric regression methods will be used to assess the levels of association between proteomic profiles, pain and inflammatory markers on ultrasound.

ABBREVIATIONS

VA	Versus Arthritis
CAP-Knee	Central Aspects of Pain in the Knee
CFCF	Consent for Contact Form
CFQ	Cognitive Failures Questionnaire
CI	Chief Investigator overall
CNS	Central Nervous System
CPA	Computerised Pressure Algometer
CS	Central Sensitisation
CSB	Clinical Sciences Building
cmRCT	Cohort Multiple Randomised Controlled Trial
CPM	Conditioned Pain Modulation
DAP	Data Analysis Plan
DCU	Data Collection Unit
FFQ	Food Frequency Questionnaire
FRAIL	Simple Frailty Questionnaire
GCP	Good Clinical Practice
GP	General Practitioner
HADS	Hospital Anxiety and Depression Scale
ICC	Intraclass Correlation Coefficient
ICF	Informed Consent Form
ICOAP	Intermittent and Constant Osteoarthritis Pain
JA	Joint Academy
K/L	Kellgren and Lawrence system for classification of osteoarthritis of knee
KPIC	Knee Pain in the Community
MLR	Multiple Linear Regression
MSK	Musculoskeletal
MSK-HQ	The Versus Arthritis Musculoskeletal Health Questionnaire
MSK-USS	Musculoskeletal Ultrasound Scan
MVC	Maximum Voluntary Contraction
NHS	National Health Service
NRS	Numerical Rating Scale
NUH	Nottingham University Hospital Trust
OA	Osteoarthritis
PA	Posterior-Anterior
PIS	Participant Information Sheet
PPI	Patient and Public Involvement
PPT	Pressure Pain Threshold
PRU	Patient Response Unit
QST	Quantitative Sensory Testing
REC	Research Ethics Committee
R&D	Research and Development department
SAE	Serious Adverse Event
SF	Synovial fluid
TS	Temporal Summation
TUG	Time Up and Go Test
USGA	Ultrasound-guided Aspiration
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index
30CST	30-second Sit to Stand Test

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STUDY BACKGROUND INFORMATION AND RATIONALE

Osteoarthritis (OA) is the most common cause of disability in the elderly population and most individuals suffering from osteoarthritis are managed in the primary care setting (1). Knee OA is the most common form of arthritis and the most common cause of knee pain in the world (2). The rate of knee arthritis is as high as that of cardiac disease and is the most common problem in individuals over the age of 65 (3). In the United Kingdom, 10% of 65 to 74-year-old individuals consult their general practitioners about OA per year (4). Out of the entire population, 4% attend their general practitioners as a result of knee OA, and half of them (2%) consult their general practitioner for the first time or with the acute flare of knee arthritis (1).

We will recruit 140 individuals with OA-related knee pain, and we will record their pain levels on the Numerical rating scale (NRS) at a single time-point. The validity and sensitivity of NRS have been established numerous times (14-18).

Metabolic evidence from body secretions can provide significant insight about the course of OA, as the presence of glucosamine and chondroitin sulfate in faecal samples has shown to improve the symptoms of the condition and delay its progression (56). Nutritional information and metabolic evidence from faecal samples can be used to explore associations of gut microbiota with pain sensitivity and stress levels (57, 58).

The widely utilised Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) will be also used for the evaluation of knee OA. It is a self-administered questionnaire made of 24 items and consists of three subscales covering pain, stiffness and physical function. It has been used extensively and is considered a valid and reliable tool (59-63). Frailty has been also found to be a predictor of disability (66) and a determinant of treatment outcomes (67-69) and the Simple Frailty Questionnaire (FRAIL) is considered a valid and reliable tool for that purpose (70).

Both the 30-second sit to stand test (30CST) and the ‘time up and go’ (TUG) test will be used to see if patients have improved their lower limb fitness levels. 30CST has shown excellent reliability and validity (63, 71, 72). TUG has been widely used in clinical setups and is a valid tool to assess necessary functional mobility (73-79).

Musculoskeletal Ultrasound scan (MSK-USS) will be also conducted on the knees of participants to establish if they have inflammation of the synovial membrane. There is enough evidence that inflammation is present in all stages of OA (108-111). Synovitis or inflammation of synovial fluid is associated with pain, disease severity and, OA progression (108, 112). Synovitis manifests as synovial membrane thickening, increased vascularity, and/or joint effusion (108, 113-115). During MSK-USS, the presence of synovial fluid, synovium hypertrophy, and Power Doppler will be also assessed. Synovial hypertrophy, synovitis and knee effusion are linked with arthritis in the knee and associated with knee pain in osteoarthritis (116-121). Power Doppler provides a reliable and accurate method for visualising blood flow in the synovial tissue, and histological findings support the value of this technique (119, 122, 123). An ultrasound scan is considered to be a valid and reliable instrument for the assessment of synovial disease (113, 119, 120, 124) and synovitis is strongly associated with osteoarthritis as mentioned earlier. The synovial fluid will be aspirated (subject to participant consent), in order to establish a phenotype which is strongly associated with OA. Studying synovial fluid biomarkers alongside clinical, radiographic and ultra-sonographic characteristics is one strategy to improve resolution and stratification into targetable OA phenotypes (125). Synovial fluid aspiration will be ultrasound guided as it increases the accuracy of needle placement

compared to blind needling (95.8% versus 77.8%, $p < 0.001$) (126) reduces procedural pain by 43%, improves effusion detection by 200%, and volume of synovial fluid aspirated by 337% compared with blind synovial fluid aspiration (127). Ultrasound guidance also reduces procedural pain (43% reduction) in knees with no palpable effusion and increases the responder rate and therapeutic duration by 107% and 36% respectively (128-132).

Optionally, isometric quadriceps strength will be also assessed to establish current strength levels of vastus medialis muscle and see whether muscle strength associates with centrally driven pain or proteomic synovial concentrations. Quadriceps muscles strength deficits are associated with knee osteoarthritis (133). Isometric testing will be done at 30 and 60 degrees of flexion as per the protocol of a previous study (134). Hand grip strength will also be measured as an option

Blood samples will be also extracted to assess the biomarkers (including serum levels and gene expression levels of various molecules) and to establish insulin resistance (135, 136).

Collection and study of these parameters can provide more insight into the traits of knee pain, allow the examination of possible correlations to each other, and highlight potential detrimental effects of them on knee joint health.

STUDY OBJECTIVES AND PURPOSE

PURPOSE
The primary purpose of this study is to explore the potential molecular links between pain and structure on knee pain using synovial fluid proteomics. A secondary purpose is to explore the association of knee pain with biomarkers of stress and metabolism

HYPOTHESIS
Among individuals with moderate to severe knee pain different proteomic signatures will be seen in synovial fluid of the affected knees.

PRIMARY OBJECTIVE
1. To identify proteomic signatures in synovial fluid of the knee joint in individuals with knee pain.

SECONDARY OBJECTIVES
1. To test whether there is an association between proteomic concentrations in the synovial fluid and pain.
2. To link the changes in pain and physical activity to biomarkers of insulin resistance, stress, poor metabolism, inflammation and pain.
3. To investigate the relationship of pain and different proteomic profiles with radiologically-established joint structural changes and muscle strength.
4. To explore the correlation between knee inflammation (effusion, synovial hypertrophy or/and synovial hyper vascularity), biomarkers of insulin resistance, DNA markers, stress, poor metabolism, gene expression and knee pain.

DETAILS OF PRODUCTS

We will be using ultrasound to assess participant knee joints.

<u>Ultrasound:</u>

An ultrasound will be used to image both knee joints using a Toshiba Aplio SSA-770A machine with a multi-frequency (7 – 12 Hz). This equipment belongs to the university and is CE marked.

Known Side Effects

There are not any known side effects from ultrasound. There can be a small amount of pain from the aspiration site.

STUDY DESIGN

Study Configuration

A single-centre observational cross-sectional study in a university and hospital setting with participants suffering from knee pain.

Primary endpoint

The primary outcome measure is pain measured by patient reported scores.

Secondary endpoint

- Synovial fluid protein concentration (synovial fluid)
- Knee joint morphology on X-ray
- Central aspects of knee pain (CAP-Knee)
- Inflammatory markers on ultrasound (Synovial fluid, synovial hypertrophy and hypervascularity)
- Muscle Strength (Dynamometer)
- Balance (Balance platform)
- Biomarkers of insulin resistance (blood, urine) and metabolic rate (faeces)
- Gene expression levels of molecules in pain
- Physical functioning (Time up and go test, 30-second sit to stand test)
- Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)
- General health questionnaire (EQ5D-5L)

All these endpoints will be measured at a single time-point (baseline) and at a follow up time point 120 days for those who subsequently go on to have surgery

Stopping rules and discontinuation

As the study involves only a single clinical assessment and does not involve investigational medicinal products or medical devices it is not envisaged that circumstances will arise that require termination or discontinuation of this study.

Study Management

The study will be managed in Academic Rheumatology, Clinical Sciences Building by the Research Manager under the supervision of the Chief Investigator who has overall responsibility for the study and shall oversee all study management.

Other members of the team are Professor David Walsh (Director of Pain Centre Versus Arthritis (VA)), Dr Tony Kelly (Research Metrologist), Dr Daniel McWilliams (Post-doctorate Research Fellow), Vasileios Georgopoulos (Post-doctorate Research Fellow) and Dr Bonnie Millar (Musculoskeletal Project Manager). Professor Ollivere (Professor of Orthopaedic Surgery) will provide oversight of research procedures conducted within NHS facilities. Mr Ben Bloch (Consultant Orthopaedic Surgeon) will facilitate recruitment from NHS waiting lists. Dr Jessica Nightingale (NUH Orthopaedic Clinical Research Manager) will support recruitment and research delivery from NHS areas.

The study management group will meet regularly and have documented quarterly meetings from the start of the study. The group will also monitor and supervise the progress of the study

and advice on recruitment rate and unforeseen logistical issues to ensure that its objectives are achieved.

The data custodian will be the CI who will also have overall responsibility for the study and shall oversee all study management.

Duration of the Study and Participant Involvement

Study Duration and Schedule of Events:

The trial is expected to start in April 2020, and enrolment will end in in the late summer of 2022. After about two months from the end of recruitment, all data will be put together for coding and further processing. Data analysis will be performed by a co-investigator supervised by the CI.

Participant Duration:

Those individuals who agree to participate will be asked to attend one session, at a time of their convenience in order to receive all relevant examination and samples extraction

Therefore, in total, individuals will be asked to attend a single session for no more than 2 hours.

End of the Trial

The end of the study will be on the last participant visit. The end of the study overall will be when the results will be written up and publication submissions will be attempted.

Selection and Withdrawal of Participants

Recruitment

There are several potential recruitment pathways for this study:

1) A selection of eligible people for the study will be invited from existing databases (Knee Pain in the Community (KPIC) and Investigating MSK Health and Well-being (IMH&W) Databases) held at Academic Rheumatology of participants with knee pain who have agreed to be contacted for future studies. The databases can be accessed by a member of the research team who will also contact the potential participants to ask if they are interested. We aim to recruit 140 participants and out of those we also aim to recruit 25 to participate in the sub-study.
2) Participants will also be recruited from consultant waiting lists and clinics for Total Knee Arthroplasty (TKR) at Nottingham University Hospitals NHS Trust. An invitation pack will be posted to eligible participants who are on the waiting list for TKA, these pack will be sent by the participants treating orthopaedic surgeons or a member of their administrative team. Interested individuals will be invited to declare their interest by mailing their reply slip via a prepaid envelope directly to the study team.

The study team in Academic Rheumatology will phone interested participants to further establish eligibility and to screen them about x-ray scanning. From those who had an x-ray in the previous 12 months and their x-ray was undertaken within the Nottingham University Hospital (NUH) Trust, verbal consent will be requested overt the phone for a member of the study team with an honorary contract with NUH to access the relevant-only information in order to establish whether the existing scan is relevant to this research.

Study visits will take place either in the Clinical Sciences Building in Academic Rheumatology, or in an NHS clinic room at the Queens Medical Centre site. Participants who satisfy the eligibility criteria upon the telephone screening will be invited to attend a study visit, at a day of their convenience. That visit will be considered as their single study session. The purpose of the visit will be to further establish suitability and gain full consent about knee aspiration and all other proceedings (samples, ultrasound, functional assessment) before any study activities are undertaken. Participants will be eligible for inclusion to the study if they are willing to undergo knee synovial fluid aspiration and demonstrate radiographic evidence of osteoarthritis (K/L= 1 or above) or if they satisfy the American College of Rheumatology clinical criteria for knee OA (139) even in the absence of radiographic findings (e.g. knee pain and three or more of the following: over the age of 50, less than 30 minutes of stiffness in the morning, no palpable warmth on synovium, bony tenderness, bony enlargement, crepitus on active motion).

Participants will be free to leave the study at any time without giving any reason. Participants who indicate in their questionnaire that they are willing to receive information about future knee pain studies may withdraw this indication at any time, and this will be altered appropriately on the study database. Participants will be made aware via the questionnaire that all data will remain confidential and will be anonymised before use in further analysis.

It will be explained to the potential participant that entry into the study is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time, but attempts will be made to avoid this occurrence. In the event of their withdrawal, it will be explained that any data collected so far cannot be erased, and we will seek consent to use the data in the final analyses where appropriate (via the information sheet and consent form). Those who withdraw from the study will be made aware that this will not in any way affect their future care.

Participants who have agreed to participate in the study will be asked directly by a member of the research team whether they would like to participate also in the sub-study. Those who will agree to participate will be provided with a separate Participant Information Sheet (PIS) composed specifically for the sub-study and they will have to sign an additional informed consent form (ICF).

Inclusion criteria

For individuals to be included in the study they must:

- have the ability to give informed consent
- be 45 years or over
- have complaints of knee pain for 3-6 months with or without radiographically established OA (K/L scale score ≥ 1)
- have complaints of knee pain for 3-6 months with or without satisfying the non-radiographic American College of Rheumatology criteria for knee OA
- are willing to undertake knee synovial fluid aspiration

- be able to speak, read, and write in English as all instructions and questionnaires are designed in the English language

Exclusion criteria

Individuals who meet one or more of the following criteria will be excluded:

- Inability to give informed consent due to cognitive impairment or otherwise – (capacity levels are already established under GP care)
- Inability to understand key aspects of the study due to cognitive impairment or otherwise
- Giving history of additional co-morbidities such as cancer, neurological conditions, inflammatory joint diseases including rheumatoid arthritis, diabetic neuropathies, fractures or other conditions causing greater disability than their knee pain
- Acute soft tissue injury to the knee within last 3 months before potential recruitment

Participant Withdrawal

Participants may be withdrawn from the study either at their own request or at the discretion of the investigator. The participants will be made aware that this will not affect their future care. Participants will be made aware (including via the information sheet and consent form) that should they withdraw the data collected to date cannot be erased and may still be used in the final analysis.

Informed consent

All participants will provide written informed consent. The ICF will be signed and dated by the participant before they enter the trial. The Investigator will explain the details of the trial and provide a Participant Information Sheet, ensuring that the participant has sufficient time to consider participating or not. The Investigator will answer any questions that the participant has concerning study participation.

Informed consent will be collected from each participant before they undergo any interventions (including physical examination and history taking) related to the study. One copy of this will be kept by the participant, one will be kept by the investigator, and a third will be retained in the participant's hospital records.

The same process will be reiterated with a separate study specific information sheet and consent form for those individuals who will agree to participate in the sub-study and undergo the additional assessment.

Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the study at any part, continuing consent will be obtained using an amended Consent form which will be signed by the participant.

Future contact

The consent form includes an optional clause asking participants to confirm whether they are happy for their personal details to be retained in order to be informed about relevant future studies within the Pain Centre for which they might be suitable. If they consent, their data will be stored on a secure database on a University of Nottingham server and will be kept up-to-date in accordance with Data Protection Act, 2018. If not, their contact details will be destroyed at the end of this study after it is no longer necessary to contact them.

Study Regimen

Participants seeking care for their knee pain will be informed of the study both orally and in writing. Those who qualify for the study will be asked to come to Academic Rheumatology for a single session. All research activities will be undertaken on the same session which is expected to last no longer than 2 hours. During the single session, participants will sign the informed consent form (ICF) and will be asked to go through the study procedure. All participants will have blood samples extracted. All participants will have a knee ultrasound scan by a trained member of the study team to assess their knee and locate the best needle insertion point and go through the ultrasound-guided aspiration (USGA) procedure. Participants will then have their pain sensitivity, strength, balance and function measured by special tests (sit to stand, time up and go). Outcome measures about pain mechanisms (CAP-Knee), OA (WOMAC), cognitive function (CFQ), frailty (FRAIL), and health (EQ5d-5L) will be completed by participants at home online or on paper copies. Participants will be also asked if they wish to donate a faecal sample and if they agree they will be given a collection kit to take home along which will be shipped back by post.

To summarise, the total study duration, including recruitment and the assessment visit, will be 18 months. The key stages are detailed below:

- Individuals who agree to attend for a clinical assessment will participate for a single appointment. This appointment will last approximately 2 hours.
- They will be seen at the Academic Rheumatology Unit, Clinical Sciences Building, Nottingham City Hospital or in an NHS clinic room at Queens Medical Centre.
- Transport (taxi) will be arranged if required and any travel expense will be reimbursed.
- Our Research Nurses will get a 15ml blood sample from the participant
- Once blood samples have been collected participants may be offered a light breakfast and drink refreshment before a brief assessment to confirm medical history and current medications.
- Questionnaires such as WOMAC, EQ5D-5L , CFQ, CAP-Knee, FRAIL and Numeric VAS will be collected.
- Physiological measurements such as 30 seconds sit to stand (30CST), time up and go (TUG), balance and muscle strength assessments will be taken at this point.
- Diagnostic ultrasound of the most painful knee will be conducted and ultrasound guided synovial fluid aspiration will follow.
- Follow up questionnaire will be posted or conducted through the telephone in line with the questionnaires at the initial visit.

Muscle, Strength, and Balance Measurements

A sequence of four physiological measures will be undertaken:

Time up and Go: The participant will start in a seated position. The participant will stand up upon therapist's command, walk 3 meters, turn around, walk back to the chair and sit down. The time will stop when the participant is seated. The subject can use an assistive device. If the assistive device is used, it will be documented.
NOTE: A practice trial will be completed before the timed trial

30 seconds sit to stand. The 30-Second Chair Test is administered using a folding chair without arms, with a seat height of 17 inches (43.2 cm). The chair, with rubber tips on the legs, is placed against a wall to prevent it from moving. The participant is seated in the middle of the chair, back straight; feet approximately shoulder width apart and placed on the floor at an angle slightly back from the knees, with one foot slightly in front of the other to help maintain balance. Arms are crossed at the wrists and held against the chest. Demonstrate the task both slowly and quickly. Have the participant practice a repetition or 2 before completing the test. If a participant must use their arms to complete the test, they are scored 0. The participant is encouraged to complete as many full stands as possible within 30 seconds. The participant is instructed to fully sit between each stand. While monitoring the participant's performance to ensure proper form, the tester silently counts the completion of each correct stand. The score is the total number of stands within 30 seconds (more than halfway up at the end of 30 seconds counts as a full stand). Incorrectly executed stands are not counted. The 30-second chair stand involves recording the number of stands a person can complete in 30 seconds rather than the amount of time it takes to complete a pre-determined number of repetitions.

Muscle strength Assessment: Isometric testing will be done at 30 and 60 degrees of flexion as done in the previous study and the participant will be in sitting position with hips and knees strapped to keep the position standardised.

Balance: Static balance and postural sway either in the medial-lateral or antero-posterior direction will be assessed using the RS Scan force plate. The participant will be asked to stand on the plate looking straight forward for 30 seconds in two conditions: first with their eyes open and then with eyes closed. Medial-lateral, antero-posterior and total sway will be recorded.

Ultrasound & Ultrasound-guided Aspiration

During the ultrasound scan, the supra-patellar pouch, medial and lateral recess of the knees will be assessed for synovial thickening, synovial fluid/effusion and for positive power Doppler. An ultrasonic probe will be used to direct ultrasonic waves onto the knee joint during sonography, and a computer converts the signals received so that they can be presented on the screen. During skin application, a sterile probe cover and sterile acoustic gel will be used. There is no radiation exposure to ultrasound due to the lack of radioactive rays and no detrimental side effects.

Once the best aspiration location is identified and the location of the needle insertion marked, the skin will be prepared with a cleansing agent such as iodine or chlorhexidine. The needle may be introduced into the skin either parallel to the probe or perpendicular to the probe (Figure 1). When the needle runs parallel to the probe it will be seen in full length. If it is advanced perpendicular to the probe the needle is only represented by a hyperechoic spot in the ultrasound image.

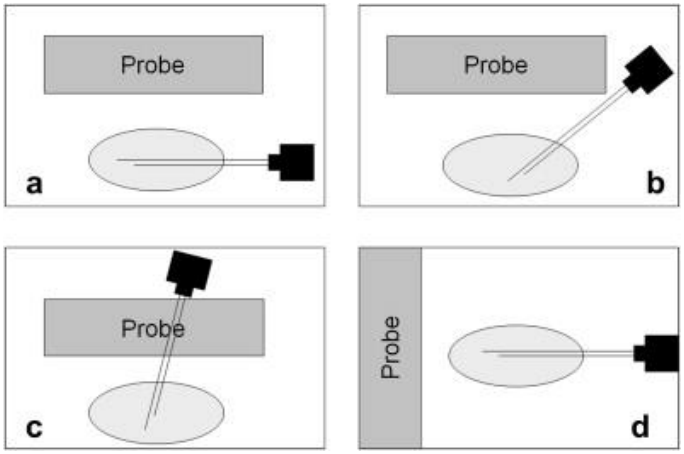

Figure 1. Approaches of ultrasound guided injections with possible positions of needles and probes (141).

Once the aspiration is completed, the needle will be removed, the skin cleansed, and a bandage will be applied. A woven elastic bandage or knee immobilizer may be applied to reduce post-procedural swelling and discomfort (140).

Compliance

Compliance will be judged by attendance for study-related appointments and during the study visit based on completion of key study procedures. Participants will be replaced only if they refuse to undertake ultrasound-guided synovial fluid extraction and quantitative sensory testing. Individuals will be given a contact number to contact us if they have any query or wish to reschedule an appointment.

Criteria for terminating the study

As the study involves only a single assessment and does not involve investigational medicinal products or medical devices it is not envisaged that circumstances will arise that require termination of this study.

Transport and Storage of the Tissues

The samples will be stored at University of Nottingham premises. Whole blood (5ml) samples will be stored in a refrigerator (at -20°C) within the Clinical Sciences Building. Remaining blood (5ml) will be centrifuged as soon after venesection as possible and the serum stored, with the urine, at -80C within freezers in University of Nottingham premises at the Clinical Sciences Building (CSB), City Hospital for future biochemical and molecular studies. Excess samples (DNA, serum, saliva, urine and faeces) that remain after the study analyses have been undertaken will remain stored within the CSB for possible future research studies related to OA and/or pain provided that participants are agreeable and sign the optional clause on the consent form. These CSB facilities come within the remit of the Research Tissue Bank (DI Dr William Dunn- Licence Number 12265). Where participants do not agree to the future use of the samples, any samples remaining after the analysis will be destroyed in accordance with the Human Tissue Act, 2004 and GDPR.

Samples will be stored in the linked anonymised format in the CSB and labelled using a randomly generated unique participant identifier to permit accurate linkage to clinical data and the consent form. The master database will be held by the CI in an encrypted password file. After 7 years all personal data linked to the samples will be destroyed.

Laboratory Analysis

Whole blood may be sent to a specialist company for total genomic DNA extraction and processing using standard protocols. The DNA will be used to identify genes that identify with pain progression and severity. Bacterial DNA will be extracted from faecal samples to identify bacterial lineages associated with pain and inflammation. Cortisol will be extracted from saliva samples. Protein and inflammatory markers will be extracted from urine samples. Faecal samples will be stored until all samples are collected (as this is optional) and then they will be sent to the laboratory. Similarly, biochemical/biomarker analyses may be undertaken by other academic or commercial groups. Such investigations will be undertaken on anonymised samples under usual Material Transfer Agreement arrangements. The study will be registered with the Human Tissue Management Group of the UoN. Storage of these tissues will be managed, transported and handled according to Standard Operating Procedure (SOP) HTMG 004. All shipments will contain a complete inventory of all samples, along with the name of the person responsible for sending the samples. Companies which will be used for the analysis should process the samples within seven days upon receipt or companies with HTA licence will be considered. It is anticipated that samples will be destroyed following analysis. There is no plan for long-term storage of such samples by those companies and no samples are expected to be transported back to UoN.

Missed Appointments

Missed appointments are an avoidable cost and resource inefficiency which impacts upon research outcomes. Health care service colleagues are increasingly utilising reminder systems to manage these negative effects. A systematic review (145) found consistent evidence that all types of reminder systems are effective at improving appointment attendance across a range of care settings and patient populations. Therefore, a reminder mechanism (a reminder telephone call, a polite reminder e-mail and where the participant has provided their mobile telephone number, a reminder text message) will be considered.

STATISTICS

Methods

The chief investigator, co-investigators and the study statistician will evaluate the findings and analysis will be carried out by the co-investigator. The data will be entered into MS Access and analysed using with STATA, SPSS (Statistical Packages for Social Scientists, version 19) and R software. Data will allow exploration of potential relationships between objective sensory measures and molecular findings and/or pain. Several factors that putatively mediate the primary or secondary outcomes are inter-correlated so investigation of their interaction must be ascertained. Simple univariate unadjusted correlations (Pearson/Spearman) and linear regression modelling will be used to examine associations between pain and molecular findings in the synovial fluids at baseline. Multiple linear

regression (MLR) modelling (backward stepwise regression) will be conducted to assess the impact of the significant independent variables and their interactions with the dependent variable.

The results of the study will be available to the study management group. All data will be stored in Academic Rheumatology and will be backed up on the University of Nottingham servers.

Sample size and justification

We propose to compare proteomic signatures in 140 knee pain cases. To achieve a Bonferroni $p < 1.25 \times 10^{[-5]}$ (adjusting for 4000 tests, i.e. the number of proteins in the Somalogic platform) effect sizes of beta = 0.64 in units of standard deviations are needed to achieve 80% statistical power. Based on the effects seen on radiographic progression (standardized beta = 0.62) these effects appear reasonable and achievable with the proposed sample size.

ADVERSE EVENTS

As all procedures are being carried out following standard methods, the occurrence of an adverse event as a result of participation within this study is not expected however, participants will be asked to contact the study site immediately in the event of any serious adverse event which is not expected. Any adverse event will be recorded and closely monitored until resolution, stabilisation or until it has been shown that the study treatment/intervention is not the cause. The CI shall be informed immediately of any serious adverse events and shall determine seriousness and causality in conjunction with any treating medical practitioners.

Any serious adverse event (s) related to this study will be recorded and reported to the REC as part of annual reports. Unexpected serious adverse events will be reported within timeframes to the REC and CI will be responsible for all adverse event reporting.

ETHICAL AND REGULATORY ASPECTS

Involvement of Participant's General Practitioner

Participant's GPs will not be informed that their patient has agreed to participate in the study.

Ethics Committee and Regulatory Approvals

The study will not be initiated before the protocol, consent forms and participant information sheets have received approval / favourable opinion from the Research Ethics Committee (REC), and the respective National Health Service (NHS) Research & Development (R&D) department. Should a protocol amendment be made, that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms, and participant information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately notifying the REC as soon as possible and approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately, and the REC will be informed.

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical Practice, and the UK Department of Health Policy Framework for Health and Social Care, 2017.

Informed Consent and Participant Information

The process for obtaining participant informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant shall both sign and date the Consent Form before the person can participate in the study.

The participant will receive a copy of the signed, and dated forms and the original will be retained in the Study records.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No study-specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study and will discuss with them, whether they wish to continue with the study. If applicable, they will be asked to sign revised consent forms.

If the Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Consent Form by the REC and use of the amended form (including for ongoing participants).

Records

Study Forms

Each participant will be assigned a study identity code number, and this will be used on the study forms as well as other trial documents and the electronic database. This will be a randomly generated unique participant identifier.

Study forms will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and Participant Study Number, to permit identification of all participants enrolled in the trial, in accordance with regulatory requirements and for follow-up as required.

Study forms shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded in a Study Personnel Log. All paper forms shall be filled in using a black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated.

The Chief or local Principal Investigator shall sign a declaration ensuring the accuracy of data recorded in the study form.

Sample Labelling
Each participant will be assigned a unique study identification code number for use on the samples, consent forms and other study documents and the electronic database.

Source documents
Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, study records, and. A study form may also completely serve as its own source data.

Direct access to source data/documents
The study form and all source documents, including progress notes and copies of laboratory and medical test results, shall be made be available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities.

Data Protection
All trial staff and investigators will endeavour to protect the rights of the trial's participants to privacy and informed consent and will adhere to the Data Protection Act, 2018. The study forms will only collect the minimum required information for the purposes of the trial. Study forms will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the trial staff and investigators and relevant regulatory authorities (see above). The computer held data including the trial database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one-way encryption method). Information about the trial in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information.

Electronic data will be backed up every 24 hours to both local and remote media in an encrypted format

QUALITY ASSURANCE & AUDIT

Insurance and Indemnity
Insurance and indemnity for clinical study participants and study staff are covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but study participants may have recourse through the NHS complaints procedures.

The University of Nottingham as research sponsor indemnifies its staff, research participants and research protocols with both public liability insurance and clinical trials insurance. These policies include provision for indemnity in the event of a successful litigious claim for proven non-negligent harm.

Study Conduct

Study conduct will be subject to systems audit for inclusion of essential documents; permissions to conduct the study; CVs of study staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, random selection of suitable participants for Phase II and Phase III, timeliness of visits); accountability of study materials and equipment calibration logs.

Study Data

Monitoring of study data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. This will be managed by the direct study team.

Entries on study forms will be verified by inspection against the source data. A sample of the forms (10%) will be checked on a regular basis for verification of all entries made. In addition, the subsequent capture of the data on the study database will be checked. Where corrections are required these will carry a full audit trail and justification.

Study data and evidence of monitoring and systems audits will be made available for inspection by the REC as required.

Record Retention and Archiving

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Code of Research Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least seven years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The study master file held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all anonymised study databases and associated meta-data encryption codes.

Discontinuation of the Trial by the Sponsor

The Sponsor reserves the right to discontinue this study at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice as appropriate in making this decision.

Statement of Confidentiality

Individual participant medical or personal information obtained as a result of this study are considered confidential, and disclosure to third parties is prohibited with the exceptions noted above.

Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files.

Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

Data generated as a result of this study will be available for inspection on request by the participating physicians, the University of Nottingham representatives, local R&D Departments and the regulatory authorities.

PUBLICATION AND DISSEMINATION POLICY

The study results will be submitted to Versus Arthritis, regulatory authorities, and peer-reviewed journals for publication. In addition, the results will be presented at national and international conferences. Study participants' identity will not be disclosed when publishing the results. Study participants will also be informed of the results if requested.

USER AND PUBLIC INVOLVEMENT

On 17/05/2017 6 representatives from the Patient and Public Involvement (PPI) MSK group were asked about their views on this project. They all agreed on the need to identify molecular markers to distinguish patient subgroups, not only if this can be conducive to drug development, but more generally they were very appreciative of a stratified medicine approach, and to them it was mostly an issue related to the discrepancies between pain and joint damage seen in their own X-rays. All group members (6/6) indicated their willingness to participate in studies that are conducive to understanding molecular subtypes of OA. Almost all (5/6) would be happy to have synovial aspirations for such a scientific project and would prefer US guided aspiration. They also expressed the view that having diagnostics that can better direct treatment based on molecular test from synovial aspirations or blood should be part of standard practice within the NHS.

The latest versions of the participant facing documentation (PIS, consent form, invitation letter and flyer) has all been forwarded to three PPI representatives who have reviewed and commented on them

STUDY FINANCES

Funding source

This study is part-funded by Versus Arthritis and the Kennedy Trust via their Step-Up OA consortium fund.

Participant stipends and payments

Participants will not be paid to participate in the study. However, all travel expenses to and from Academic Rheumatology, Clinical Sciences Building, Nottingham City Hospital or Queens Medical Centre will be covered.

SIGNATURE PAGES

Signatories to Protocol:

Chief Investigator: (name)_____

Signature:_____

Date: _____

Co- investigator: (name) _____

Signature:_____

Date: _____

Trial Statistician: (name)_____

Signature:_____

Date: _____

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