PROPOSED PROTOCOL

LOOKING FOR BIOMARKERS IN HUMAN JOINT FLUID

SPONSOR: IMPERIAL COLLEGE LONDON

STUDY CENTRE: IMPERIAL COLLEGE NHS TRUST

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Aims:

1. To utilise metabonomic analysis to analyse human joint fluid in tandem with other body fluids

2. To analyse joint fluid from osteoarthritic joints to identify potential biomarkers of the disease and further understanding of the cartliage destruction pathways

3. To attempt to further understand population variances in joint fluid composition and if this composition correlates to arthropathy of joints

4. To study the lubrication and wear properties of the joint fluid in tandem with metabonomic analysis to better understand these properties; with a particular view to the impact of wear upon prosthetic joints

5. To study joint fluid for biomarkers of bacterial colonisation and analyse in tandem with microbiological analysis in an attempt to correlate results

Background:

Joint fluid represents a source of possible disease detection largely uninvestigated by analytical chemistry to date. We aim to chemically investigate the composition of joint fluid. Joint fluid has many key functions.

1. It is involved in lubrication of cartliage. In osteoarthritis the cartliage is lost leading to bone on bone wear. The mechanisms of the loss of cartliage and how the composition of joint fluid affects this are poorly understood.

2. In the case of joint replacement corrosion and wear of the components is an important concern. It has a direct effect upon the lifetime of the any prosthesis. It also has a bearing upon destructive process seen in some joints as a result of biological handling of corrosion and particulate wear.

3. The presence of infection in any joint, replaced or not is a destructive and aggressive disease process. It is difficult to treat due to the joint fluid being a happy medium for microbial growth with poor antibiotic penetration.

To analyse the joint fluid composition, a metabonomic approach will be used. Metabonomics is relatively in its infancy. It is the study of profiling biological biproducts combined with powerful computer analysis. Previous strategies for identifying metabolites have fallen foul of an inability to detect at low levels in complex samples with too many compounds to distinguish satisfactorily. This necessitated complex and lengthy chemical purification techniques. Utilising modern NMR analysis overcomes these challenges. Body fluids can be analysed directly. Once targetted compounds have been found, further study using alternative analysis techniques like mass spectroscopy can be used to help identification.

Microbiological assays will attempt to amplify any organisms present in the joint fluid by growth. These organisms will then by subjected to a series of selected stresses to identify them. Furthermore, polymerase chain reaction assays may be used to identify the genetic component of any organisms found within the joint fluid.

Tribology testing will be carried out to determine lubrication and wear properties of synovial fluid samples. A laboratory test device will be used to rub implant materials under simulated artificial joint conditions. Only a small amount of fluid is required for the tests. At the end of the test wear of the implant material surface will be measured. The synovial fluid test sample will be recovered and wear products (particles, metal ions in solution) analysed.

Once the human joint fluid has been characterised for composition, lubrication and microbiological content, the results will be correlated statistically in an attempt to identify which compounds in the fluid are key to these properties.

Any results will be published in a peer review journal and components of the research may be used for the submission of a research thesis. Joint fluid represents a source of possible disease detection largely uninvestigated by biochemistry to date. As such there may be many potential biomarkers to joint disease.

Collaborations:

The study will be coordinated by the Department of Musculoskeletal Research, Imperial College London. The research centre will be the Imperial College NHS Trust. Analysis will be conducted within the department of microbiology at Imperial College NHS Trust and with the collaboration of the Department of Surgery and Cancer, Imperial College London.

Duration:

We aim to achieve ethical approval and begin collecting samples from patients at the beginning of 2015. We would hope to complete joint fluid analysis before the close of 2017. Identification of relevant target markers would be completed before 2018. Any proposal of clinical application and use would require extensive validation, testing and replication. As a result we would not expect clinical application within 10 years of detection.

Method:

Blood, urine and joint fluid will be collected from suitable and consenting patients. The collection will be focused from the accident and emergency, rheumatology and orthopaedic departments. In addition control subjects may be recruited to allow potential background matrix subtraction.

Samples will be analysed using NMR and computer modelling analysis to identify key metabolites

Samples will be stored at 4^{o}C for no more than 48 hours before analysis. Samples will then be stored at -80^{O}C, should any further analysis be required.

All details of subject background will be collected. These include diet, medical history, drug history, exercise history and joint symptoms

Power Calculation

The previous evidence has demonstrated that power calculations in metabonomic methods of analysis has proved difficult. This is due to the large and varied amount of data that is generated1 This is due to the methodology and subsequent reliance upon powerful, computer generated statistical calculations to provide significance. However, from previous evidence and experience we would expect the numbers to be relatively low and thus are hoping to sample 40 patients for each arm of the study.

Subjects:

Subjects will be recruited in the clinical setting to donate joint fluid, blood and urine. These patients will be recruited in several scenarios.

1. Anyone receiving a diagnostic aspirate of joint.

2. Patients undergoing joint arthroscopy

3. Patients undergoing joint replacement

4. Patients undergoing prosthetic joint revision surgery

No patient under 16 years or age or older than 80 years will be entered for the study. Any female patient who is pregnant will be excluded. Any patient medicated with anti-coagulants will be excluded.

Analysis:

Body fluids will be analysed using unextracted direct NMR analysis. Statistical multi-variate analysis methods will then be employed to determine co-variates and significant metabolites.

Parallel analysis of the lubrication properties will be performed using a thin film optical interferometric test device.

Joint fluid will be analysed using standard microbiological assays and polymerase chain reaction for any organisms present and their genetic make-up. These results will be correlated using the multi-variate analysis to identify how the composition of the joint fluid corresponds.

Complications:

No adverse events are expected arising from the sampling of body fluids. A low risk of both intra-articular infection and haemoarthrosis exists. It is possible that subjects may experience minor bruising from blood sampling. Should any complications occur they will be immediately reported to the study coordinator who will then inform the study centre and sponsor. Any adverse events will be dealt with at Imperial College or the treating hospital.

Reporting:

The authors will attempt to publish those results in a peer review journal of relevance.

Regulatory Issues:

Ethics Approval

The investigators are seeking approval from the North West London REC2. The study must be submitted for Site Specific Assessment (SSA) at each participating NHS Trust. The Chief Investigator will require a copy of the Trust R&D approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

Consent

Samples will be taken from patients fulfilling the inclusion criteria on arrival. Consent to enter the study must be sought from each participant only after a full explanation has been given and time allowed for consideration. In all situations samples will be collected and not analysed until consent is gained. The right of the participant to refuse to participate without giving reason is to be respected. Irrespective of whether consent is gained, the treatment of the patient shall not be affected either in process or decision making. Patients will be followed up for a period of six months after discharge from hospital. All participants are free to withdraw at any time from the study without prejudicing further treatment.

Confidentiality

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

Indemnity

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study. Imperial College London Healthcare NHS Trust's research is covered by the standard NHS Hospital Indemnity for negligent or wrongful harm and this is provided under clinical negligence scheme for Trusts clinical risk management NHS Litigation Authority (NHSLA) for NHS Trusts in England.

Sponsor

Imperial College Healthcare NHS Trust will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

Audits

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).