ABPP Study

<u>Study Title:</u> Autoimmunity-informed Phenotyping in Chronic Non-Specific Low-Back Pain Sufferers

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Study Duration: Two years and six months

Study End: One year after study visit of final patient

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Study Team

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Clinical Queries

Clinical queries should be directed to Dr. Samuel Hewitt who will direct the query to the appropriate person.

Sponsor

The University of Liverpool is the research Sponsor for this Study. For further information regarding the sponsorship conditions, please contact:

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Internal Funding

Pain Research Foundation Grant awarded

STUDY SUMMARY

This protocol describes the ABPP Study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the Study. Problems relating to this Study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Framework for Health and Social Care Research (v3.2 10th October 2017). It will be conducted in compliance with the protocol, the EU General Data Protection Regulation 2016 and Data Protection Act 2018, and other regulatory requirements as appropriate.

GLOSSARY OF ABBREVIATIONS

HRA	Health Research Authority
REC	Research Ethics Committee
NS-LBP	Non-specific Low Back Pain
LBP	Low Back Pain
CRPS	Complex Regional Pain Syndrome
FMS	Fibromyalgia Syndrome
PIC	Patient Identification Centre

KEYWORDS

Non-Specific Low Back Pain

Autoimmune

Autoinflammatory

Phenotype

CRPS

Fibromyalgia

Quantitative-Sensory Testing

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1. INTRODUCTION

1.1 BACKGROUND

Low Back Pain (LBP) is defined as activity limiting low back pain that lasts for at least 1 day[1]; this is a common and often disabling condition. It is estimated that around 80% of the general population will experience an episode of acute back pain over their lifetime[2], while the natural course appears to often follow a pattern of recurrent episodes[2]. LBP has been identified as the number one cause of disability based on years living with disability and disability adjusted life years[1]. LBP can be divided into pain caused by a specific insult, such as a fracture or tumour, or pain lacking an identifiable precipitating factor. This latter non-specific LBP (NsLBP) is the most common, accounting for an estimated 90% of sufferers[3]. Treatment is largely supportive and a 'one-size fits all', which may contribute to outcomes being inconsistent[2]. The development of specific phenotypically distinct subgroups within the NsLBP population may help target therapies and has been identified as an area of urgent clinical need[4].

Clinical experience has suggested that there may be patients with NsLBP who present with symptoms and signs that overlap with either Complex Regional Pain Syndrome (CRPS) or Fibromyalgia (FMS). CRPS is characterised by sensory, autonomic and motor signs and symptoms, affecting a limb after trauma. Some of these features have been known to occur in the lower back, including following spinal surgery[5]. FMS is characterised by diffuse widespread pain and hyperalgesia, alongside fatigue and often depression, and retrospective studies have identified patients fulfilling FMS diagnostic criteria in back pain populations[6], with Brummet et al. reporting a prevalence of 42% meeting the American College of Rheumatology (ACR) survey criteria for FMS in their population[6]. The development of chronic widespread pain (CWP)/FMS in individuals with regional pain, at 12-month follow-up, has been estimated at 15-20%[7]. The ICD-11 diagnostic category of 'chronic primary pain syndromes' contains fibromyalgia (FMS) and complex regional pain syndrome (CRPS) as well as NsLBP[8]. We therefore wonder whether an NsLBP subgroup with a shared clinical presentation to these chronic pain conditions may present secondary to a shared pathological process.

NsLBP, CRPS and FMS all present with an unclear pathogenesis, but in each abnormal activation of the immune system has been implicated. Analysis of skin blister fluid at the affected limb in early CRPS shows elevated pro-inflammatory cytokines, highlighting the potential contribution of the innate immune system[9]. The injection of IgG or IgM from CRPS sufferers into mouse-injury models has reproduced many symptoms of CRPS, including hyperalgesia and limb swelling[10,25]. Very recently IgG transfer model studies have been performed at our lab and suggested similar results in FMS, however here not requiring the application of trauma[11]. The latter results have highlighted the important role of the adaptive immune system in both conditions. Systematic reviews of NsLBP have identified positive correlations between serum pro-inflammatory biomarkers and the presence and severity of NsLBP[12], as well as self-assessed pain levels[13], again highlighting a role for the innate immune system. To our knowledge adaptive immune mechanisms or autoimmunity have not been assessed in NsLBP. It appears possible that an underlying immune pathology may help explain the presence of this condition in a subgroup of NsLBP patients also presenting with CRPS or FMS–like symptoms.

The role of psychosocial factors as predictive risk factors in the development of both FMS and NsLBP is well documented[6,2,3], while their role in CRPS is currently less conclusive but nevertheless of great interest[14]. We will assess these features, alongside other demographic data and disease characteristics (listed below), to develop a participant characteristic database, from which phenotypic subgroups may be identified.

Assessment including clinical examination and quantitative sensory testing (QST) of our study group will investigate the presence of CRPS-like features and comparison with other participants' characteristics; this will allow us to explore the contribution these factors have on symptom presentation.

Alongside this, we will use blister fluid to assess skin cytokines in the CRPS-like subgroup and serum samples and white blood cells to investigate the presence of altered inflammatory cytokines, autoantibodies and white blood cells in all patients, both during the current study and in future research.

Separately, we will ask 30 patients to return when they have a flare, and we will perform a 12-month follow-up of all participants, with the aim of identifying new-onset CWP/FMS.

Analysis of baseline clinical characteristics will then explore the clinical risk factors for CWP/FMS development. Serum and DNA samples from these patients taken at study visits will allow later exploration of the role of genetic and serum markers including autoantibodies for symptom presentation and development.

2. STUDY OBJECTIVES

Primary Objective: Phenotype chronic non-specific low back pain patients

Secondary Objectives:

1. Identify, quantify and phenotype the presence of a 'CRPS-like' presentation in a population of NsLBP sufferers
2. Identify individuals that report evidence of a conversion from regional LBP to chronic widespread pain/FMS symptoms at a 12-month follow-up, and consider features that may predict this development
3. Invite patients for a second visit during a flare up, to gain serum and white blood samples allowing later correlation of serum antibodies, mediator concentrations, and white cell subsets with disease flare parameters.
4. Investigate the presence of local (blister-fluid) alterations in cytokines at the lower back in CRPS-like NsLBP sufferers during an acute flair
5. Secure serum and DNA samples for later studies allowing analysis of i)subtype-specific serum/DNA markers, including autoantibodies, and ii) serum markers of conversion to CWP/FMS

3. STUDY DESIGN

Clinical Examination and Administration of Questionnaires with Human Biological Sampling

Participants will be identified from our patient identification centre (PIC) sites: these are physiotherapy led back pain clinics held at the Walton Centre and Aintree University Hospital, and consultant led pain clinics held at the Walton Centre. Individuals will be identified as potentially suitable for inclusion in the study by their treating healthcare professional, during a routine clinical visit. Study inclusion and exclusion criteria will be used as a guide and the treating healthcare professional will approach the potential participant in clinic, where they will be given an information leaflet regarding the study. Patients are invited to contact the study team using contact details in their patient information leaflets, at which point the study team will respond to any questions and arrange a study contact or note declination. In addition, the Walton Clinic Pain Management Registry (a registered Trust asset) will be searched for patients with back pain, and contacts will be passed on by Walton Centre staff to the study team. The CI for this study, Dr. Andreas Goebel, is also a member of the pain management program clinical care team. Suitable individuals will be contacted by letter through the study team. If no response is received within 2 weeks, then these patients will be contact by the study team by phone, no more than once. Study subjects will then be seen at the laboratories of the Clinical Sciences Centre, Aintree, as detailed below.

This study will end one year after the study day of the final patient

100 individuals suffering from Chronic Non-specific Low Back Pain will be involved. Low Back Pain will be defined using the Delphi Definition: 'pain between the inferior margin of the 12th rib and inferior gluteal folds that is bad enough to limit usual activities or change the daily routine for more than 1 day. This pain can be with or without pain going down into the leg. This pain does not include pain from feverish illness or menstruation'.[2]

3.1 STUDY OUTCOME MEASURES

Primary Outcome: Ns-LBP patient characteristics as defined by clinical examination and depression, pain and stressor questionnaires

Secondary Outcomes:

- Somatosensory profiles, as determined by quantitative sensory testing (QST)
- Presence of bilateral sacral tissue oedema in Ns-LBP
- Altered sweating pattern in Ns-LBP
- Cytokine levels in local blister fluid of Ns-LBP patients presenting with a 'CRPS-like' picture during an acute flare
- Conversion rate from regional Ns-LBP to chronic widespread pain/FMS at 12 month follow-up, using ACR 2016 criteria

4. PARTICIPANT ENTRY

4.1 PRE-REGISTRATION EVALUATIONS

Where possible consent will be gained remotely, to minimize the risk of COVID exposure and for patient convenience. Potential participants will be contacted via secure video link following their initial contact with the study team. Prior to this contact via secure video link, consent forms will be posted to individuals. During this contact patient identification will be confirmed via the display of photographic ID (passport or drivers license). The study will be explained to the individual, using the patient information

leaflet provided previously, and adequate time given to respond to questions. Consent will then be gained by the participant signing the provided consent form and posting this, via return mail, to the study team. Patients unable to establish a video link will instead be directly invited for their study visit, and all activities, including consent signing will take place then. Explicit consent for the use of samples in future research will be obtained. Patients will also be asked whether they would like for their details to be forwarded to other study teams involved in current or upcoming research. Participants will then have the study questionnaire booklet sent to them via email or post for completion before the planned study visit. Study subjects will be seen in the laboratories of the Aintree University Hospital – located at the Clinical Sciences Centre (a facility belonging to the University of Liverpool) 50m opposite of the Walton Centre. A summary table of participant visits and evaluations is included below.

Participant Visit	**Required Assessments/Data**
Remote Meeting (where a remote meeting is technically not possible this is integrated into the 1st Visit below)	Assess suitability of participant for inclusion into study Explain study and respond to questions Gain informed consent and records via electronic means Questionnaire booklet sent via email or post for home completion
1st Visit (study day)	Pain-related examination Assessment of CRPS-like picture (QST, tissues oedema, etc) Questionnaire submission Blood samples
2nd Visit (10 individuals with pronounced CRPS-like picture, 30 patients during flare)	Blister fluid sampling (only from the 10 patients with pronounced CRPS-like picture) Blood samples
Phone Follow-up (all participants)	Telephone based questionnaire
1 year Follow-up Visit (estimated 10-20 individuals with CWP/FMS symptoms on telephone follow-up)	Examination and ACR-based assessment/diagnosis of FMS Blood samples

Table 1: summary of participant contacts and required data collected at each one

4.2 INCLUSION CRITERIA

- 18 years or older
- Presence of non-specific LBP without the presence of another condition to explain the pain (ie. cancer, primary musculoskeletal conditions, sciatica)
- Chronic NsLBP defined as pain persisting for >12 weeks, with pain occurring >4 days/week
- LBP as defined topographically and temporally by a modified Delphi approach[32]
- Average weekly pain intensity >= 6/10 on numeric rating scale (NRS) scoring[15]
- LBP that causes at least severe disability (>=41% result as defined by the Modified Oswestry Low Back Pain Disability Questionnaire (MODI)[15])
- Pain may radiate down the buttocks or backs of legs but usually not below the knees
- In patients with features of more widespread, non-specific pain, the low-back pain must be the predominant complaint or pain and the pain intensity of these other pains should not exceed 3/10 on NRS scoring

4.3 EXCLUSION CRITERIA

- Pregnant or Breast-Feeding patients
- Patients with immune deficiency or taking immune modulating drugs
- Patients with an acute systemic disorder
- Language other than English as first language

4.4 WITHDRAWAL CRITERIA

If a participant wishes to leave the study early they may do so without any concern regarding future or on-going treatment. Their data, serum and blood cells, should any exist, may still be used for the purposes of the study.

5. ADVERSE EVENTS

5.1 DEFINITIONS

Adverse events will primarily be related to those normally associated with the process of blood taking. These include pain, haematoma formation/bleeding and infection at the site of needle insertion. Other adverse events may be related to the formation of skin blisters and blister fluid collection. Itching and irritation at the site, hyperpigmentation, infection and the uncommon event of scarring may occur. We do not anticipate any serious adverse events occurring as a result of the study procedure.

All patients will be informed of these risks and consent gained.

5.2 REPORTING PROCEDURES

5.2.1 Non serious AEs

Participants will be encouraged to report non-serious adverse events if they feel it necessary. These will be considered and recorded by the principle investigator if deemed appropriate. Routine adverse events of the procedures, such as bruising or discomfort at the site of needle insertion may not need to be recorded.

5.2.2 Serious AEs (SAE)

We do not anticipate any serious adverse events occurring as a result of the study.

If an SAE occurs, an SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to pre-existing conditions and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the local Research Ethics Committee where in the opinion of the Chief Investigator, the event was:

•'related', i.e. resulted from the administration of any of the research procedures; and

•'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs.

6. ASSESSMENT AND FOLLOW-UP

Participants will be seen in a dedicated study room at the Aintree Clinical Sciences Centre. Study visit assessment will be carried out by the principal investigator, Dr. Sam Hewitt. They will be examined with a focus on non-specific low-back pain and alternative diagnoses ruled out. They will then complete a series of questionnaires, listed below, aimed at collecting information on pain experience (including pain quality), mood and cognitive parameters, coping mechanisms and experience of daily stressors, and life events now and in the past. Samples of blood will be taken, about 180ml in total, for immediate processing, the storing of white blood cells, storing of EDTA for later genetic analysis (5ml full blood), and immediate serum-separation, aliquoting and freezing for later analyses (see below). All samples will be stored frozen for future analysis of serum mediators, serum immunoglobulin, white cell immunophenotyping and genetic analysis.

A CRPS-like clinical picture will be investigated using a combination of the following: a battery of standardized sensory tests, Quantitative Sensory Testing (QST), with a standardized protocol based on the German Research Network on Neuropathic Pain (DFNS)[24]. While a diagnosis of CRPS cannot be confirmed by means of QST, the typical pattern of thermhypaesthesia and mechanical and pressure hyperalgesia helps to support a diagnosis[28,29]. The use of these QST protocols is well established in LBP[30,31]. Sweating, the presence of tissue oedema and anatomical pain distribution will also be assessed, as these are features of CRPS. A qualitative analysis of bilateral local tissue oedema at the sacrum of patients will be performed by the researcher and noted. Sweating at the lower back will be quantified using the Minor's starch-iodine test[16], in which the colour intensity of a starch-iodine preparation, when applied to the lower back in standardized conditions, may give a numerical indication of the intensity of sweating. The use of the painDETECT questionnaire[27] will gather data on the anatomical site of most severe pain.

We will correlate clinical findings with the Budapest Criteria for diagnosis of CRPS[26] to determine whether the NsLBP would completely fulfill diagnostic criteria, but a lack of fulfillment would not exclude a participant from follow-up if they have signs deemed significant by the researcher.

We will also analyse serum samples from 30 healthy volunteers as comparison. These samples will be obtained from healthy control samples stored at the Liverpool Bio-Innovation Hub (LBIH) Biobank. Volunteers will have agreed, as part of their original LBIH consent, that donated samples may be used in other regulated research projects. The samples will be stored at the Liverpool Biobank. Ethical approval for this is through the Liverpool Bio-Innovation Hub.

COVID-19 Considerations: due to social distancing measures and COVID-19 diagnosis implications we have made some amendments to our assessment methods. We will exclude patients who have been previously diagnosed with COVID-19 due to the unknown effects this may have on the immune system. We have already excluded those with immune deficiencies and pregnancy, therefore reducing the risk of transmission to vulnerable groups. While we still require a face-to-face study visit for assessment and blood sampling, we may initially undergo a secure video/audio meeting to assess a patient's suitability for inclusion in the study and gain consent remotely, to avoid unnecessary exposure for participants. Participants will be sent the study questionnaire booklet to complete before their study visit, through the post or via e-mail as per preference, to minimize the time spent at the study site. The principle investigator and participants at study visit days will both wear facemasks and adhere to up to date infection control advice to minimize risk of transmission.

Measurements

- Full QST profile as defined by the DFNS protocol[24] *(see appendix)*
- A qualitative assessment of bilateral sacral tissue oedema or cutaneous sweating will be performed and noted by the researcher.
- Quantitative assessment of sweating will use the Minor's starch-iodine test[16]
- Pain distribution will be analysed by the use of the pain drawing supplied in the painDETECT questionnaire, supplemented by direct questions about any more widespread pains.

Demographics and Back-Pain related Data

Age and Gender

Ethnicity

BMI

Past Medical History

Past and Current Medications

Pain Duration

Average Income

Highest Educational level achieved

Depression severity and risk

Markers of life stress exposure

Clinical course of pain

Tissue swelling in low back pain

Alcohol and smoking use

Waddell Score[17]

Suction blister fluid cytokine levels

Anatomical location of most severe pain

Presence of widespread, secondary pain

Presence and degree of sweating at lower back

QST profiles

Questionnaires

Present Pain Intensity (0-10 scale)

Hospital Anxiety and Depression Scale (HADS)[19]

Keele STarT back screening tool[18]

Brief Pain Inventory[22]

Short-Form McGill Pain Questionnaire[21]

Coping Strategies Questionnaire

Pain Self-Efficacy Questionnaire[20]

Daily Stressors Questionnaire

EQ-5D[23]

painDETECT Questionnaire[27]

Revised Illness Perception Questionnaire (IPQ-R) – timeline questions[35]

Touch Experiences and Attitudes Questionnaire (TEAQ)[37]

Second Study Visit

10 individuals identified with exceptionally prominent cutaneous symptoms or signs will be invited back for a second visit. These will be identified by the presence of mechanical and pressure hyperalgesia, thermhypaesthesia, sweating and evidence of tissue oedema. These participants identified as presenting with a 'CRPS-like' picture will be asked to re-attend, around the time of an acute 'flare' or painful episode, in order to mimic an early CRPS presentation. However if the participant reports that the pain could not get any worse or that they would be unwilling to return during an acute flare due to pain/functional limitation, and they are deemed suitable for a second-visit, the sampling of blister fluid may be conducted at the first study visit. A 100-microliter sample of suction blister fluid will be gathered from a maximum of 3 blisters, located at specific areas at the lower back, based on pain distribution and any cutaneous symptoms/signs. A protocol for this procedure, based on the process detailed by Huygen et al.[9] is detailed in the attached appendices below (section 14.2) and will be followed by the principle investigator. These samples will be analysed for inflammatory cytokine levels using a Luminex® bead array.

A 180ml serum blood sample will be taken from each participant, at a similar day-time as the first visit sample to control for 24-hour variation of cytokines, for storage and future analysis into mediators and serum immunoglobulin antibodies, as well as possible correlation of biomarkers with clinical pain intensity. From this sample an aliquot of PBMC isolation will also be obtained and stored. Samples will be stored in the Rheumatology Freezer at the Clinical Sciences Centre.

A further 30 patients, who have no CRPS-like features at baseline, will also attend for a second visit, during a time of pain flare. These participants will have samples of blood taken only. This patient group will be selected based on i) their report on visit 1 of a pain pattern that presents in flares typically lasting longer than 48h and occurring at least once per year on average, to make it feasible for them to attend for a second visit, and ii) their report that they are not in a pain flare at the first visit.

Follow-up

In order to investigate the conversion of regional NsLBP into a chronic widespread pain/FMS picture, participants will be followed up 12-months after the initial visit. Participant details will be kept on file and individuals will be followed-up by telephone consultation to assess their LBP and any alterations in symptom presentation, with specific focus on new-onset CWP/FMS symptoms. Individuals who report these symptoms will then also be invited back for a further study visit, where they will be assessed for the presence of fibromyalgia using the ACR diagnostic criteria questionnaire (2016), and a further 180ml serum blood sample will be taken for processing and purposes as described above. We anticipate that there will be 10-20 patients in this group, based on estimates of conversion rates[7]. Participants who had been included in the blister-fluid sample population will not be invited back for further consideration in this CWP/FMS population, but patients not in the blister-fluid group, who returned during a flare, can be invited.

In order to maintain patient autonomy, individuals will be re-consented for continued involvement in the study during this follow-up telephone call. Identity will be confirmed using the participant details from the first study visit.

Blood analyses

White blood cells (PBMCs) and serum will be analysed for the role of serum-immunoglobulin and mediators in causing low back pain, and the frequency and specificity of white blood cell subpopulations. These assessments will be carried out over the next 10 years. Full blood will also be used for analysis for genetic SNPs that may contribute to causing low back pain.

7. STATISTICS AND DATA ANALYSIS

This is an exploratory study therefore no formal sample size calculations were performed. Discussion with the relevant expert physiotherapists indicated that an estimated 1 in 10 patients with chronic NsLBP will present with exquisite hyperalgesia and/or sweating. 1-year conversion rates from regional pain to a chronic widespread FMS picture have been estimated at 15-20%[7], giving an expected 10-20 individuals reporting new-onset widespread pain at follow-up. The recruitment of 100 participants is preferred for a number of additional reasons. It enables researchers to become familiar with the selected phenotype and detect deficiencies in inclusion criteria and collected data, and it enables a sufficient number of individuals from both sexes to be included, an issue with smaller studies. 100 is also a suitable number to detect distinctions within the group, and will allow us to perform further analysese of subgroups within the total cohort. Previous work into genetic analysis in pain conditions has determined that 100 patients is a sufficient size to identify the genetic basis of extreme pain phenotypes, using the functional SNP allele discovery method as detailed in the analysis of persistent CRPS[34].

<u>Primary Outcome:</u>

- Frequency distribution of patient questionnaire responses
- Frequency distribution of clinical examination findings

<u>Secondary Outcomes:</u>

- Mean values for individual threshold determinations of QST parameters and calculation of Z-values for comparison with healthy population parameters
- Frequency distribution of tissue oedema and low back pain sweating
- Frequency distribution of anatomical pain sites
- Independent t-tests and fishers exact tests to identify relationships between categorical and continuous data

8. REGULATORY ISSUES

8.1 ETHICS APPROVAL

The Chief Investigator will obtain approval from the Research Ethics Committee and Health Research Authority (HRA), through the IRAS system. Approval for analysis of the healthy volunteer samples (from the APIF study) will be through LBIH ethical approval already established. 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.

8.2 CONSENT

Consent to enter the study will be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration (at least 24h after receiving the PIL). Signed participant consent will be obtained. The right of the participant to refuse to participate without giving reasons will be respected. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

8.3 CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and will abide by the EU General Data Protection Regulation 2016 and Data Protection Act 2018.

8.4 INDEMNITY

The University of Liverpool holds Indemnity and insurance cover with Griffiths & Armour, which apply to this study.

8.5 SPONSOR

The University of Liverpool will act as Sponsor for this study. It is recognised that as an employee of the University the Chief Investigator has been delegated specific duties, as detailed in the Sponsorship Approval letter.

8.6 FUNDING

Internal sources are currently funding this study.

A funding grant from the Pain Research Foundation for a total of £14,575 has been awarded.

Re-imbursement

Participants will be reimbursed for their travel and parking fees, up to a maximum of £30 per visit. This will be included in the patient information leaflet and participants will be informed of reimbursement before consenting to the study.

8.7 AUDITS

The study may be subject to inspection and audit by the University of Liverpool under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Framework for Health and Social Care Research (v3.2 10th October 2017).

9. STUDY MANAGEMENT

The day-to-day management of the study will be coordinated through the principle investigator, Dr. Samuel Hewitt, with help from the Chief Investigator, Dr. Andreas Goebel, and the administrator Ms. Hayley McCullough

10. END OF STUDY

The study will end one year after recruitment of the final participant

11. ARCHIVING

Serum and blister fluid samples will be stored for 10 years in the locked rheumatology freezer, 3rd floor clinical sciences centre on the grounds of Aintree Hospital, and white blood cells will be stored in the same laboratory in liquid nitrogen. Explicit consent for use of samples in future research will be obtained at initial assessment. Physical data will be stored in a locked cupboard, while digital data will be archived using a password protected, encrypted computer at the Pain Research Institute (PRI). Data will be stored for 10 years and then destroyed by the PI. Samples and patient data will be pseudoanonymised using unique reference codes, with a single linking document to be stored securely, as for the other digital data, at the PRI. Data will only be accessible by the CI or PI. These practises adhere to those required by the University of Liverpool.

12. PUBLICATION POLICY

Study results will be released through posters and oral presentations at conferences and through journal publications.

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14. APPENDICES

14.1 Quantitative Sensory Testing in Low Back Pain Protocol

Quantitative sensory testing (QST) uses pressure or temperature stimuli to investigate the state of an individual's peripheral and central nervous systems' contribution to pain processing. Altered pressure-pain thresholds have been identified in CRPS sufferers, and linked with a peripheral antibody effect[22]. Work in Non-specific LBP has been inconclusive, with some suggesting a possible prognostic benefit for pressure-pain modelling in predicting the development of chronic Ns-LBP[14] while others remain unclear[13]. Regardless, the use of QST in LBP and CRPS is well documented in the literature and within the Chronic LBP population has been suggested to be of use in classifying distinct somatosensory phenotypes[30,31]. A standardised protocol for the characterisation of somatosensory phenotypes in patients with neuropathic pain has been developed by the German Research Network on Neuropathic Pain[24]. Reference data for the application of QST parameters to the trunk has also been developed and may serve as a useful reference point for establishing abnormal values[33].

The standardised assessment protocol consists of 13 different thermal and mechanical

tests, summarised briefly as: thermal detection thresholds for cold and warmth perception (CDT: cold detection threshold and WDT: warm detection threshold), paradoxical heat sensations (PHS) involving alternating warm and cold stimuli, thermal pain thresholds for hot (HPT: heat pain threshold) and cold (CPT: cold pain threshold) stimuli, mechanical detection thresholds for touch (MDT) and vibration (VDT), mechanical pain sensitivity involving thresholds for blunt pressure (PPT: pressure-pain thresholds) and pinprick (MPT: mechanical pain thresholds), mechanical pain sensitivity (MPS) as determined by stimulus-response to pinprick, dynamic mechanical allodynia (DMA) using brush-evoked stimuli, and wind-up ratio (WUR) using pain-summation to repeated pinprick stimuli. Loss (negative) and gain (positive) of function will be assessed.

Impairments in descending pain processing is reported frequently in fibromyalgia and chronic widespread pain, while findings in chronic low back pain remain contradictory. Conditioned pain modulation (CPM), through the testing of pressure pain thresholds at the lower back before and after a cold pain stimulus, will be assessed as well.

14.2 Blister Fluid Protocol

The presence of peripheral autoinflammatory factors in the skin of CRPS sufferers was investigated by Huygen et al.[9], who identified elevated TNF-alpha and IL-6 in blister fluid. Goebel et al. have shown that injecting IgG serum concentrations from CRPS sufferers into animal model skin reproduces peripheral symptoms. In those suffering from NSLBP with pronounced peripheral symptom presentation, we wonder whether a similar peripheral immune component can be identified. We will use an adapted protocol for blister fluid production taken from Huygen et al.[9].

Blisters will be induced using the suction technique. Plexi-glass chambers with 3 round openings, each opening being around 10mm in diameter, will be attached to the affected area in the lumbar region and to an unaffected area on the extensor aspect of the forearm, with the chamber connected to a vacuum pump. An initial negative pressure of 300mmHg will be applied, which will be reduced to 250mmHg after 15 minutes, and then reduced to 200mmHg after another 15 minutes. This negative pressure will be maintained for 2 hours, until 3 blisters per site are formed.

The blisters will be punctured and fluid collected before being pooled in their respective containers for centrifuging and storage at -80^{o}C until analysis.