JRMO Research Protocol for Interventional Studies

Full Title	**Investigating pathological mechanisms in non-alcoholic fatty liver disease: cross-sectional comparative study between patients and healthy controls**
Short Title	**Investigating mechanisms in non-alcoholic fatty liver disease**

Sponsor

Queen Mary, University of London

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IRAS Number **247449**

Sponsor (ReDA) Number **012414**

REC Reference **18/LO/1759**

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1. Contents

2. Glossary

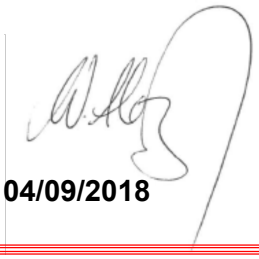
AE	Adverse Event
AR	Adverse Reaction
ASR	Annual Safety Report
BMI	Body Mass Index
CAP	Controlled Attenuation Parameter
CI	Chief Investigator
CRF	Case Report Form
CyTOF	Mass Cytometry
FNA	Fine Needle Aspirate
GLP-1	Glucagon Like Peptide 1
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HRA	Health Research Authority
JRMO	Joint Research Management Office
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic Steatohepatitis
NIHR	National Institute for Health Research
Participant	An individual who takes part in a clinical trial
PI	Principle Investigator
PIS	Patient Information Sheet
REC	Research Ethics Committee
T2DM	Type 2 Diabetes Mellitus

3. Signature page

Chief Investigator Agreement

The study as detailed within this research protocol will be conducted in accordance with the principles of Good Clinical Practice, the UK Policy Framework for Health and Social Care Research, and the Declaration of Helsinki and any other applicable regulations. I agree to take responsibility for the statistical analysis and oversight of this study.

Chief Investigator Name: **Dr William Alazawi**

Signature: 

Date: **04/09/2018**

4. Summary and synopsis

Short title	**Investigating mechanisms in non-alcoholic fatty liver disease**
Methodology	Cross-sectional study
Research sites	Barts Health NHS Trust, Homerton University Hospital NHS Foundation Trust Kings College Hospital, NHS Foundation Trust Bromley by Bow Centre
Objectives / aims	To identify key characteristics of the tissue resident and peripherally circulating immune-phenotype in addition to blood markers, metabolic profile, faecal and oral microbiota in non-alcoholic fatty liver disease within an ethnically diverse population
Number of participants	153
Inclusion and exclusion criteria	Over 18 years of age Confirmed Non-alcoholic Fatty Liver disease or Healthy Control Excluding participants with type 1 Diabetes Mellitus or immune-suppressant concomitant medication or auto-immune disease or excessive alcohol intake
Statistical methodology and analysis	Comparative and descriptive
Study duration	24 months

5. Introduction

5.1 Background and Rationale

Non-alcoholic fatty liver disease (NAFLD) is a major cause of morbidity and mortality worldwide. NAFLD is the hepatic manifestation of the metabolic syndrome and is strongly associated with type 2 diabetes mellitus, obesity, hypertension and hypercholesterolaemia. It affects 20-30% of the general population, 58% of overweight individuals, 70% of diabetics and almost 90% of obese individuals. [1].

NAFLD reflects a spectrum of disease from simple steatosis without inflammation and 12-20% of individuals with NAFLD who develop the aggressive form of disease, non-alcoholic steatohepatitis (NASH). This is characterised by liver cell death, inflammation and fibrosis which can lead to cirrhosis, liver failure and liver cancer[2]. It is recognised that NASH with fibrosis confers increased all-cause and liver-related mortality over simple steatosis or NASH without fibrosis.[2–4] [2] Cardiovascular disease dominates non-liver related mortality and this risk appears to be independent to the presence of metabolic syndrome.

Classically NAFLD is associated with an elevated Body Mass Index (BMI), a well-recognised measure of obesity. However, studies have identified that people of south Asian ethnicity develop NAFLD with a BMI outside of the 'obese' range. [5,6] Factors possibly involved in this observed phenomenon includes preponderance for insulin resistance [7] and a distinct genetic profile. [8]

Apart from performing a liver biopsy, there is no convenient or accurate way to distinguish patients who have NASH from those who have simple steatosis which does not progress. This is largely because the pathogenesis of NASH is not fully understood. Hepatic fat deposition, genetic abnormalities, oxidative stress, inflammatory cytokines and more recently bacterial dysbiosis have all been shown to be relevant, but, the mechanisms that link these phenomena to NASH have not been elucidated [9,10].For the same reasons, there are currently no drugs licensed for the treatment of NASH and prevention of its complications. Therefore the cornerstone of treatment is behaviour and lifestyle change, but adherence to such changes is limited and effect on long term outcomes has not been demonstrated. [11,12] However, public health measures such as education and legislation will take many years to impact on the burden of established liver disease in the population. Therefore, there is a large unmet clinical need for drugs to target the pathogenic processes that drive inflammatory liver diseases.

Therefore, what is needed is a better understanding of the mechanisms that are associated with NASH in order to develop effective biomarkers of disease stage and therapeutic targets. In recent years, it has become clear that NASH is driven by a combination of metabolic dysfunction and an aberrant immuno-inflammatory response resulting in liver cell injury, and fibrosis. In a multisystem disease such as NASH, many tissues are affected and a comprehensive understanding of the disease

cannot take place without simultaneously studying the different tissues involved, including liver, fat tissue, muscle and blood. Historically such co-ordinated tissue assessment has been very difficult, but the recent rise to prominence of bariatric surgery as a metabolic treatment intervention changes that.

Bariatric surgery (increasingly referred to as 'metabolic surgery') has wide-ranging benefits on insulin sensitivity, body weight and gut hormone profile such as glucagon like peptide-1. [13,14] Bariatric surgery is safe with morbidity and mortality comparable to hip-replacement, cholecystectomy and appendicectomy. [15] Cohort studies and case series have shown benefits in both liver biochemistry and histology. [16–18] The Lille bariatric cohort demonstrated resolution of NASH in 85% of patients with significant reduction in steatosis fibrosis. [19] It is well recognised that there is marked improvement in insulin sensitivity within days post bariatric surgery driven particularly driven by reduced basal glucose production and increased hepatic insulin sensitivity.[20] Furthermore, immediate and dramatic changes in gut hormones such as glucagon-like peptide 1 (GLP-1) increases pancreatic insulin secretion in response to glucose and reduced glucagon production contributing to attenuation of the metabolic syndrome phenotype.[21,22] Prior to bariatric procedures patient undergo two to three weeks of a low calorie diet which has been shown to reduce liver and intra-abdominal fat volume.[23,24] It has been suggested this improves visibility intra-operatively, moreover, has been shown to decrease 30 day post-operative morbidity and complications.[25] We aim to evaluate the impact of this intervention and bariatric surgery upon serological markers and the peripheral immunophenotype.

Although only 1% of patients eligible for such operations are currently offered surgery[26] the prominence of metabolic surgery in international guidelines for patients with obesity, diabetes and complications means that these numbers are rising and expected to rise further. This provides an opportunity for mechanistic research such as ours.

For patients with NAFLD whom bariatric procedures are not appropriate or not under the care of bariatric services the cornerstone of management is behaviour and lifestyle change, focussing on weight loss and exercise [12,27,28]. Weight loss reduces fatty acid supply to the liver, improves insulin sensitivity, and reduces circulating inflammatory cytokines which may be mediated by a change in the inflammatory and metabolic states of adipose tissue.[29]

A previous meta-analysis has revealed weight loss improves steatosis and cardio-metabolic risk factors including HOMA-IR, lipid profile and glycated haemoglobin (HbA1c).[30] More recently, a cohort study of 293 NASH patients encouraged to adopt lifestyle changes over 52 weeks showed a dose-response between weight loss and all histological parameters of NASH with benefit seen with weight loss greater than 5%.[12]

In this study, we will collect tissue, blood, stool, urine and saliva samples at the same time in order to enable a comprehensive disease phenotyping and ability to compare immunological, microbiological and metabolic features in patients with varying stages of NAFLD and healthy controls. To date there are no published data evaluating simultaneously-obtained samples from adipose, gut and liver tissue and peripheral blood in NAFLD. The important regulatory role of adipose tissue in systemic inflammation has been highlighted previously and demonstrated a hyperactive state

in obesity. [31,32]. Therefore, a significant proportion of the participants in this study will be recruited from the bariatric surgery services so that these multiple tissue samples can be collected at time of surgery with minimal additional risk to participants.

The largest cohort of paired liver biopsies monitoring the effects of weight loss through lifestyle modification revealed that a significantly greater proportion of individuals had histological resolution of NASH if they lost greater than 5% of body weight.[12] Insulin resistance is a key component to NAFLD, therefore, the dramatic impact upon hepatic insulin resistance and gut hormones seen post bariatric surgery may display and very different pathological phenotype compared the same amount of weight loss attained through lifestyle changes. This study will enable evaluation of these immunological, microbiological and metabolic features of NAFLD at baseline and in participants who have lost at least 5% of body weight by lifestyle interventions versus a bariatric surgery. This comparison may reveal unique insight into the pathological mechanisms in NAFLD altered by the rapid improvement of the metabolic syndrome seen after bariatric surgery independent of the amount of weight loss.

The potential benefits of this study will include identifying components which could aid disease staging; reducing the need for invasive liver biopsy but also identify those individuals at risk of disease progression with associated elevated all-cause mortality. Collecting samples from the ethnically diverse population of East and South London will enable us to evaluate risk factors and pathological characteristics which may vary between ethnic groups. Furthermore, a greater understanding of the peripheral and tissue resident inflammatory responses could highlight potential therapeutic targets for future drug development.

5.2 Risks / benefits

We do not expect any significant additional risk to participants within this study. Blood samples would amount to no more than 55mls in total, substantially less than taken at time of a blood donation, which amounts to 400mls. Tissue biopsies will only be collected from participants undergoing a planned procedure such as a liver biopsy for existing clinical purposes or gastrointestinal surgery. A large review of the safety of liver 3806 biopsies without direct visualisation but guided by ultrasound revealed a low rate of haemorrhagic complication 0.32% or hypovolaemic shock 0.11%. [33] Furthermore, the Lille bariatric cohort did not report any significant risks associated with the 1496 intra-operative liver biopsies performed within their study.[19] Tissue samples taken at time of surgery have lower risk than percutaneous procedures as they are performed under direct vision of the operating surgeon with ability to ensure haemostasis post biopsy.

Participants will have an optional fine needle aspiration (FNA) of their liver at baseline and at follow up, once attaining 5%-7% weight loss. This procedure has been in use for more than 20 years as a research and clinical tool with better safety profile than standard liver biopsy.[34] A recent study revealed safe repeated fine needle aspiration from the same study participants with the only adverse event reported as pain post procedure requiring simple analgesia in two cases after 39 fine needle aspirates.[35] Furthermore, another research group has reported no serious complications in a series of more than 500 liver FNA procedures.[36]

As part of this study if participants identified to have evidence of the more aggressive form of fatty liver disease, NASH, other liver abnormality, or signs of hepatic fibrosis they would be referred for ongoing monitoring and management and benefit from specialist advice from the Hepatology clinics at the Royal London Hospital or King's College Hospital.

6. Study objectives

6.1 Primary objective

To determine the changes in tissue-specific and peripheral immuno-inflammatory and metabolic abnormalities in NALFD in people who achieve 5% to 7% weight loss by Roux-en Y gastric bypass surgery or lifestyle measures.

6.2 Secondary objective

1. Understand the association between variations in immune-profiling and existing markers of disease activity
2. Identify which markers predict disease progression / regression after 5% to 7% weight loss

6.3 Primary endpoint

To determine differences between specific immune cell populations in peripheral blood, liver and adipose tissue between two methods of 5% to 7% weight loss.

6.4 Secondary endpoint

1. Expression of key immune system markers (genes and proteins) and immune cell population frequency and activation status
2. Number and type of bacterial species in stool and mouth samples.
3. Severity of NAFLD - based on histology and Fibroscan (transient elastography)
4. In a subset of patients, non-invasive assessment of liver function and metabolic control once 5% to 7% weight loss is achieved
5. Measure of quality of life at baseline and once achieved 5% to 7% weight loss
6. Healthcare utilization; populated from healthcare records

7. Study population

Participants will be identified from outpatient clinics of Bariatric, Gastroenterology, and Hepatology services at Homerton and King College Hospitals in addition to Gastroenterology outpatients from the Barts Health NHS Trust. An additional cohort will be recruited from the community weight loss program, Fit For Life, run in Tower Hamlets and Waltham Forest at the Bromley by Bow Centre.

Healthy control participants will be identified from patients undergoing outpatient assessment, gastroscopy or surgery for other non-malignant gastrointestinal disorders at hospital sites. Participants will have the opportunity to discuss the study with a member of the research team when they receive a copy of the patient information sheet. Only adults 18 years of age and above and not classified as a vulnerable adult will be recruited. Most participants will only need to attend one or two study visits including consent.

7.1 Inclusion criteria

- Age ≥18 years
- Confirmed non-alcoholic Fatty liver disease (diagnosed clinically, radiologically or histologically)
- If diabetic, Diagnosed with Type 2 Diabetes Mellitus

OR

- Healthy Control: no diagnosis of any liver condition including NAFLD
    - NAFLD excluded by Fibroscan Controlled Attenuation Parameter (CAP) score of <222 dB/m

7.2 Exclusion criteria

- Unwilling or unable to give informed consent
- Type 1 Diabetes Mellitus
- Other form of liver disease (other than NAFLD)
    - Viral hepatitis, Auto-immune hepatitis, primary sclerosing cholangitis, primary biliary cholangitis, haemochromatosis, Sarcoidosis, cystic fibrosis, sickle cell disease
- Taking medication associated with liver dysfunction (except methotrexate)
- Auto-immune disease which in the investigator's opinion may confound immune profiling
- Concomitant immunosuppressive medications (except methotrexate, short course oral steroids or inhaled corticosteroids)
- Currently pregnant
- Any major organ transplant (excluding corneal or hair transplant)
- Regular alcohol intake greater than 14 units a week for female participants and 21 units a week for male participants

8. Study design

This observational study will invite individuals from hospital outpatients (Gastroenterology and Bariatric services), community weight loss program or academic institutions associated with hospital sites to form the NAFLD and healthy control participants respectively. We plan to recruit 153 participants over an expected 24 month period. Opening this study at two bariatric surgery services we would expect to meet recruitment targets. However, if opportunistic recruitment does not meet expectations we will collaborate with other centres with whom we have existing relations (Imperial College and University College, London).

Participants will be approached at routine clinic appointments if pre-screened to meet study inclusion criteria. Community participants will have a short presentation at the beginning of their weight loss program where the study will be explained by one of

the research team with the opportunity to ask questions. All potential participants will be given a copy of the PIS at this visit. A combined screening/first study visit will then be arranged, usually by telephone or e-mail. Following consent, bariatric surgery participants or healthy control participants undergoing elective abdominal surgery will have a second study visit defined as the day of surgery for tissue sample collection. Patients will be asked to monitor their weight and when they reach 95% of starting weight, they will be asked to contact the study team to arrange a visit. All participants will have a final study visit once they have attained 5% to 7% weight loss, this is expected to be between one to six months after baseline visit. Participants who do not achieve at least 5% weight loss 6 months after the baseline visit will also be asked to attend the visit.

Healthy control patients will be identified from patients undergoing outpatient assessment, gastroscopy or elective surgery for other non-malignant gastrointestinal disorders at NHS organisations associated with the study; Kings College Hospital, Barts Health NHS Trust and Homerton University Hospital.

In order to exclude significant hepatic steatosis in healthy control participants and ensure study eligibility we will utilise the Controlled Attenuation Parameter (CAP) function of the Fibroscan. This is particularly important due to the recognised poor negative predictive value of liver ultrasound. [37] This will be carried out at the participant's screening visit after informed consent.

There is range of published cut-off values to determine the presence of hepatic steatosis (215 dB/m- 268 dB/m).[37–40] For the purposes of our study we have utilised the data from a recent publication comparing CAP score in 349 individuals with NAFLD and 437 healthy controls as determined by proton-magnetic resonance spectroscopy.[37] At the cut-off of 222 dB/m, the sensitivity, specificity, positive predictive value and negative predictive value of CAP, for diagnosing NAFLD, were 93.1%, 45.1%, 57.5% and 89.1%, respectively.[37] This indicates it to be an appropriate cut-off for our study.

9. Study procedures

At the first study visit a researcher will obtain informed consent and determine that the participant meets all study inclusion and exclusion criteria to proceed. Consent will include agreeing to access NHS databases for medical information and access to existing tissue samples surplus to clinical requirements. The study researcher will guide the participant through the consent form and case report form (CRF). This will also incorporate the Short Form (36) Health Survey (SF-36) as a measure of participant self-reported health status. This is in addition to medical and drug history in addition to smoking, alcohol, dietary and exercise data. The same assessment will take place at the final study visit, once they have achieved 5% to 7% weight loss.

Data collection will include:

1. History and examination: family history, smoking and drinking habits, past medical history, drug history, demographics (race, gender, age) and life-style (quality of life, dietary, exercise and sleeping habits).
2. Anthropometric measurement including body weight, body height, waist circumference and Body Mass Index (BMI).
3. Transient elastography (Fibroscan)
4. Fasted clinical blood tests including liver function and metabolic profile.
5. Fasted research blood tests - 55mls (3 tablespoons) extra.
6. Urine, saliva and stool samples
7. Optional fine needle aspiration of the liver

Every effort will be made to arrange all study procedures at each time point in a single visit, however, if this is not possible, participants have the option to return at an alternative date for that component / those components.

If a participant is undergoing bariatric or upper gastrointestinal surgery for a non-malignant indication, a study investigator or research nurse will attend the day of their surgery and collect the tissue samples: liver and adipose tissue and gut tissue (if their operation includes bowel resection) having consented previously. All participants with NAFLD will be offered a follow up visit once they have achieved 5% to 7% weight loss with repeat assessment of immune-profile and liver specific tests. Additional consent pertaining to fine needle liver aspirate will be obtained immediately prior to this procedure. The standard NHS consent form will be used for this.

Study procedures by visit

Study intervention	***Visit 1***	***Visit 2 (Surgical & biopsy participants only)***	***Visit 3 (5%-7% weight loss achieved)***
Informed Consent	X		
Screening	X		
Medical history	X	X	X
Questionnaires	X		X
Blood sample	X	X	X
Urine, stool, saliva samples	X	X	X
Liver Fibroscan	X		X
Liver Fine Needle Aspiration ***(Optional)***	X		X
Liver Biopsy sample (If already clinically indicated)		X	
Intra-abdominal tissue sampling (At time of planned surgery)		X	

If the study procedures identify a significant liver abnormality, elevated liver function tests or elevated Fibroscan score. We will ensure the participant is informed by one of the study investigators and referred for further investigation and management within a liver clinic at either Barts Health or Kings College Hospital.

If a participant wishes to fully, or partially, withdraw from the study, they will be asked if they wish to have existing data and samples to be destroyed or whether they can be still used for the purposes of the study. Withdrawal of consent will be documented in the participants CRF.

10. Assessment and management of risk

We do not expect any significant additional risk to participants within this study. Blood samples would amount to no more than 55mls in total, substantially less than taken at time of a blood donation, approximately 400mls. Fresh tissue samples will only be collected from participants undergoing a planned procedure such as a liver biopsy for existing clinical purposes or bariatric surgery. A large review of the safety of liver 3806 biopsies without direct visualisation but guided by ultrasound revealed an exceptionally low rate of haemorrhagic complication 0.32% or hypovolaemic shock 0.11%. [33] Furthermore, the Lille bariatric cohort did not report any significant risks associated with the 1496 intra-operative liver biopsies performed within their study. [19] Tissue samples taken at time of surgery have minimal risk as they are performed under direct vision of the operating surgeon with ability to ensure haemostasis post biopsy.

Participants will have an optional fine needle aspiration of their liver at baseline and at follow up, once attaining 5% to 7% weight loss. This procedure has been in use for more than 20 years as a research and clinical tool with better safety profile than standard liver biopsy.[34] A recent study revealed safe repeated fine needle aspiration from the same study participants with the only adverse event reported as pain post procedure requiring simple analgesia in two cases after 39 fine needle aspirates.[35] Furthermore, another research group has reported no serious complications in a series of more than 500 fine needle liver aspirates.[36] In this study, liver FNA will be only undertaken by individuals with adequate prior experience to minimise risk to participants.

We expect small risk to study researchers undertaking analysis of biological samples from this study. Standard mitigating precautions including personal protective equipment, sealed containers opened only in fume hoods within containment level 2 laboratory facilities will be adhered to. Further information can be found in section 15 regarding laboratories. All research staff dealing with samples will have to demonstrate up to date safety training either from their employing NHS trust or academic institution.

11. Statistical considerations

Patients undergoing assessment for bariatric surgery will predominantly be classified as obese, BMI >30Kg/m^2 (>27.5Kg/m^2 for South Asian patients) or more likely severely obese, BMI greater than 35 Kg/m^2. It is recognised that up to 90% of obese individuals will have concurrent NAFLD.[1] A recent meta-analysis reports the incidence of NASH among NAFLD patients to be 59.1%[41] although in a bariatric cohort with more risk factors, a higher proportion is expected. A previous NAFLD study in 109 predominantly Caucasian bariatric patients revealed that almost 90% of patients undergoing bariatric surgery had evidence of NASH with at least stage 1 (out of 4) fibrosis [19] .

In this exploratory, basic science observational project, formal power calculations are not possible. However similar work by our group suggests that 40 patients in each of the following groups: NASH with mild fibrosis (stages 0-1) and significant fibrosis (stages 2-4) will be informative. Given that we cannot determine the stage of disease before surgery, and assuming a conservative NASH rate of 75% of all patients and a

relatively even split of patients into the two categories and 20 healthy control participants we estimate that a total sample size of 153 will enable us to recruit with 20% contingency for dropout.

Techniques involved in analysis of biological samples will include flow cytometry, quantitative polymerase chain reaction, RNA sequencing of immune cell populations, 16S ribosomal RNA sequencing of the microbiome and enzyme-linked immunosorbent assays on serum or plasma samples.

The data will be analysed using widely used statistical packages Graphpad Prism and Statistical Package for the Social Sciences (SPSS) utilising descriptive statistical tests. Specific techniques may require additional software such as CytoBank for CyTOF data and HistoCAT for imaging mass cytometry.

12. Ethics

The Principal Investigator must ensure that the study will be carried out in accordance with the ethical principles in the Research Governance Framework for Health and Social Care, Second Edition, 2005 and its subsequent amendments as applicable and applicable legal and regulatory requirements.

None of the Investigators declare any conflicts of interest with regards to this study.

12.1 Annual Safety Reporting

The CI will send an Annual Progress Report to the REC and the sponsor using the HRA template on the anniversary of the REC “favourable opinion”.

13. Public Involvement

The work of the group has been discussed with the North thames Diabetes lay Panel and our results will be reported back to them at their quarterly meetings.

14. Data handling and record keeping

14.1Data management

Data pertaining to study participants will be recorded directly recorded into CRF which in turn would be transcribed into an anonymised electronic excel database stored as per the conditions below.

- Confidentiality

  Personal data will be stored in password-protected formats on hospital networks. We will follow local Trust guidelines on the storage and transfer of

data and all databases / spreadsheets will be compliant with the Data Protection Act, NHS Caldecott Principles, The Research Governance Framework for Health and Social Care, and the conditions of Research Ethics Committee Approval.

- Record Retention and Archiving

Data will be stored in codified, password-protected formats. We will follow local Trust guidelines on the storage and transfer of data and databases / spreadsheets will be Caldicott compliant. The UK Policy Framework for Health and Social Care Research requires that research records are kept for 20 years after the project has completed and will be archived in the Trust Corporate Records Centre.

14.2Source data

Source data will include the CRF incorporating the questionnaires completed at time of first visit in addition to participant's hospital medical records, NHS databases and General Practitioner records (if consent given to access these). The total accumulated data will be collated in an anonymised electronic database.

14.3Confidentiality

Information related to participants should be kept confidential and managed in accordance with the Data Protection Act, the General Data Protection Regulation (GDPR), NHS Caldecott Principles, the UK Policy Framework for Health and Social Care Research, and the conditions of Research Ethics Committee favourable opinion. Only data outlined in the protocol will be collected from study participants and anonymised within the study database. Only members of the research team will have access to healthcare records and source documents.

15. Laboratories

15.1Central laboratory

Recipient Scientist: Dr William Alazawi, Queen Mary University of London

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15.2Sample collection and preparation

Research samples will be collected at all study sites pseudo-anonymised and labelled with the patient study number. Material transfer agreements will be arranged with each study site with regards to the transfer of participant samples. All sample processing will occur at the primary site run by the chief investigator.

15.3Laboratory procedures

These may include flow cytometry, mass cytometry (CyTOF), imaging mass cytometry, tissue culture, nucleic acid and protein detection, mass spectrometry, bacterial sequencing and identification, metabolic profiling.

Working with human tissue products (blood, tumour tissue, urine, stool, saliva, plasma, serum) has inherent risks; tissue, whether screened or unscreened, can carry infectious agents and so will require careful handling.

All researchers planning to work with human tissue must ensure that they:

- Are trained in all necessary procedures.
- Are vaccinated against Hepatitis B.
- PPE (gloves, lab coat and eye protection) is worn.

Study personnel must:

- Avoid the use of sharps, but if unavoidable, follow safe sharps practice; If possible, no sharps should be used when handling blood samples that have been collected elsewhere.
- Where possible, process samples in a Safety Cabinet.
- Only work in designated areas
- All other work with samples should be carried out in at Containment Level 2

15.4Sample storage and transfer

All human tissue/product samples must be separately transferred or stored in watertight plastic containers and labelled appropriately (contents, date collected, name of researcher and a biohazard label). The laboratory is equipped with a spill kits for infectious material.

The triple packaging system described by the WHO must be used for all infectious substances (Category A and B), exempt substances and non-infectious GM substances.

This consists of three layers as follows:

Primary receptacle: water-tight and leak proof with enough absorbent material to absorb all fluid in the event of a breakage.

Secondary receptacle: a durable, water-tight and leak-proof packaging to enclose and protect the primary receptacle. Several cushioned primary receptacles may be placed in one secondary package but sufficient absorbent material should be used to absorb all fluid in a breakage. Goods of an unrelated type must not be packed together.

Outer packaging: secondary receptacles are placed in a durable outer packaging with suitable cushioning material to protect from outside influences.

Samples will be stored in either minus 80 freeze or liquid nitrogen storage to minimise degradation of participant samples. Only laboratory personnel trained in the safe use of these facilities will be permitted to undertake the storage of samples received.

All biological fluids or cells/tissue suspended in fluid which requires disposal will be treated with 10% distel for 24 hours before disposal down laboratory drain. All laboratory equipment used will undergo de-sterilisation by autoclaving or, if disposable, placed in clinical waste bags for appropriate disposal.

We do not expect to have sample left over, but if there is, this will be transferred to the Digestive Diseases Tissue Bank

16. Interventions and tools

16.1Devices

Fibroscan – transient elastography device. Manufactured by Echosens

Operated by individuals who have completed the certified training provided by manufacturer.

Owned by Site: who has responsibility for appropriate device maintenance under contract with Echosens.

16.2Techniques and interventions

Percutaneous liver biopsy – performed under ultrasound guidance as per Barts Health standard operating procedures

Intra-operative tissue sample collection. Under direct vision of operating surgeon with biopsy equipment with ability to achieve post biopsy haemostasis utilising diathermy electrocoagulation.

16.3Tools

Patient Questionnaires
Short Form (36) Health Survey (SF-36), measure of self-reported health status

17. Safety reporting

17.1Adverse Events (AEs)

An AE is any untoward medical occurrence in a participant to whom an intervention has been administered, including occurrences which are not necessarily caused by or related to that intervention. An AE can therefore be any unfavourable or unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with study activities.

17.2 Adverse Reaction (ARs)

An AR is any untoward and unintended response in a participant to an intervention. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to the intervention qualify as adverse reactions. The expression 'reasonable causal relationship' means in general that there is evidence or an argument to suggest a causal relationship.

17.3Notification and reporting of Adverse Events and Reactions

If the AE is not defined as serious, the AE will be recorded in the study documents and the participant followed up by the research team. The AE will be documented in the participants' source documents, the Case Report Form (CRF), and, where appropriate, medical records.

17.4Serious Adverse Events (SAEs) or reactions

A serious adverse event (SAE) is defined as an untoward occurrence that:

- Results in death,
- Is life-threatening,
- Requires hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability or incapacity,
- Consists of a congenital anomaly or birth defect, or
- Is otherwise considered medically significant by the investigator.

SARs will be reported to the REC where in the opinion of the Chief Investigator the event was serious and:

- Related (it may have resulted from administration of any of the research interventions), and
- Unexpected (the type of event is not listed in the protocol or other Reference Safety Information as an expected occurrence).

17.5Notification and reporting of Serious Adverse Events

Serious Adverse Events (SAEs) that are considered to be 'related' and 'unexpected' will be reported to the sponsor within 24 hours of learning of the event, and to the REC within 15 days in line with the required timeframe. Host sites will reports SAEs to the CI via NHS email or direct telephone contact to ensure data security. The SAE maybe reported to the PI at local sites who will directly notify the CI within the 24 hour timeframe.

17.6 Urgent Safety Measures

The CI will take urgent safety measures if necessary to ensure the safety and protection of the clinical study participant from immediate hazards to their health and safety. The measures will be taken immediately. The approval of the REC prior to implementing urgent safety measures is not required. However the CI will inform the sponsor and Research Ethics Committee (via telephone) of this event immediately.

The CI will inform the REC in writing within 3 days, in the form of a substantial amendment. The sponsor (Joint Research Management Office (JRMO)) will be sent a copy of the correspondence with regards to this matter.

17.7 Annual Safety Reporting

The CI will send the Annual Progress Report to the REC using the HRA template (the anniversary date is the date on the REC "favourable opinion" letter) and to the sponsor.

17.8 Overview of the Safety Reporting responsibilities

The CI is the medical assessor on behalf on the sponsor and will review all events reported. The CI will ensure that safety monitoring and reporting is conducted in accordance with the sponsor's requirements.

18. Monitoring and auditing

The sponsor or delegate retains the right to audit any study, study site, or central facility. Any part of the study may be audited by the funders, if requested. As there is no investigative intervention in this study no regular monitoring schedule will be devised. Reports of SAE or SAR will trigger discussion with the study sponsor whether a trial monitoring should be commenced. Any concerns regarding delivery of the study highlighted at the trial committees can also instigate formal monitoring procedures by the JRMO.

19. Trial committees

The study management group will include:
Dr William Alazawi
Dr James Brindley
Ms Johanna Preston
Mr John Loy
Professor Francesco Rubino
Ms Claire Dunne
Patient representative – to be confirmed

The Trial Committee (quorum is 3 members of the above list) will meet prior to recruitment, at 3 months and 6 months into recruitment and 1 year after initiation of the study. If recruitment is below target, additional meetings will be convened.

20. Finance and funding

The Association of Physicians of Great Britain & Ireland
Provided funding for laboratory experiments

c/o BCD M&E
3 Jubilee Way,
Faversham Kent
ME13 8GD
01227378874

21. Indemnity

The insurance that Queen Mary University of London has in place provides cover for the design and management of the study as well as "No Fault Compensation" for participants, which provides an indemnity to participants for negligent and non-negligent harm.

22. Dissemination of research findings

Results of this study will be published in peer-reviewed scientific journals, conference presentations, internal reports and presented locally at meetings within Barts Health NHS trust Queen Mary, University of London. If a study participant wishes to learn more about the research findings they can discuss this in more detail with the research team and given copies of published data.

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