J-wire Extraction Discovery Initiative

EDI-2

Sponsor	Royal Surrey NHS Foundation Trust (RSFT)
Funder	University of Surrey Doctoral College GUTS FBC ESICM
Protocol Version and Date	1.0 23 Jan 2024
Chief Investigator	Dr Ben Creagh-Brown
Statistician	University of Surrey Bioinformatics core facility

Signature Page

The undersigned confirm that the following protocol has been agreed and accepted and that the Principal Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the International Conference on Harmonisation Topic E6: Guideline for Good Clinical Practice (ICH GCP), any relevant SOPs, and other regulatory requirements.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest, accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature: Date:

..../...../.....

Name (please print):

.....

Position:

.....

Chief Investigator:

Signature: Date:

..../...../.....

Name: (please print):

.....

Table of Contents

1. Glossary

AE	Adverse Event
CRF	Case Report Form
DAMP	damage-associated molecular pattern
DAWN	Disabilities and wellness network
EC	Endothelial cell
ECBx	Endothelial cell biopsy
GCP	Good Clinical Practice
HTA	Human Tissues Act
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICU	Intensive care unit
ISF	Investigator Site File
PAMP	pathogen-associated molecular pattern
PI	Principal Investigator
R&D	Research, Development and Innovation department
REC	Research ethics committee
RNA	Ribonucleic acid
RSFT	Royal Surrey NHS Foundation Trust
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UOS	University of Surrey

2. Contacts

Chief Investigator	Ben Creagh-Brown (bencb@nhs.net) Intensive Care Unit, RSFT Reader (Clinical), School of Biosciences and Medicine, University of Surrey
Principal investigator	Charlie Piercy (Charles.piercy@nhs.net) Research, Development & Innovation, RSFT Intensive Care Unit, RSFT PhD student, School of Biosciences and Medicine, University of Surrey
Protocol Contributors	Paola Campagnolo (p.campagnolo@surrey.ac.uk) Senior Lecturer in Cardiovascular Molecular Biology, University of Surrey Christian Heiss (c.heiss@surrey.ac.uk) Professor of cardiovascular medicine, University of Surrey
Peer review	Dr Ib Jammer, Haukeland University Hospital, Bergen, Norway Professor John Laffey, National University of Ireland
Sponsor	Royal Surrey NHS Foundation Trust
Funder(s)	University of Surrey GUTS FBC ESICM
Statistician	University of Surrey Bioinformatics core facility

3. Protocol Summary

Title	Characterization of endothelial cells in multiple inflammatory pathologies
Short title	JEDI-2
Sponsor	Royal Surrey NHS Foundation Trust
Design	Prospective observational study of three cohorts: i) healthy volunteers, ii) surgical patients, iii) critically ill patients.
Primary objectives	To identify the changes in mRNA expression in endothelial cells from surgical patients and sepsis patients
Secondary objectives	Stratification of patients based on mRNA expression from endothelial cells. Characterisation of microparticles, histones and cell free DNA.
Target accrual	Part 1: 25 healthy volunteers, 20 patients with sepsis and 20 elective abdominal surgery Part 2: 20 patients with sepsis and 20 elective abdominal surgery
Inclusion criteria (both parts)	• ≥18 Years old • At RSFT for either and emergency admission to ICU, an elective surgery or as a healthy volunteer.
Exclusion criteria (both parts)	• Prisoners • Anticipated prohibitively difficult venous cannulation • Presenting with inflammatory diseases and disorders including but not limited to arthritis, peripheral artery disease, vasculitis, diabetes with end organ damage, cardiovascular disease and CKD. • Currently prescribed immunomodulatory medication or immunocompromised • Received chemotherapy within 2 weeks of predicted sampling. • History of recent trauma
Number of sites	1
Duration of recruitment	12-18 months
Duration of patient follow-up	Up to 28 days
Definition of end of trial	End of follow-up of last patient

4. Lay summary

The immune system is a complex network of cells and molecules that protects the body from infection and injury. When the immune system is activated, it produces inflammation, which is a natural response to help heal the body. However, too much inflammation can be harmful and lead to serious complications, such as sepsis, low blood pressure, organ failure and death.

The interaction of cells that line the blood vessels (endothelial cells, EC) with the immune system, is believed to be the root cause of these symptoms. When exposed to inflammation, the instructional molecules (RNA) inside the EC change. This leads to a change of operation promoting the severe symptoms previously mentioned.

Researchers have developed new safe techniques to collect these cells from the blood vessels of patients to study disorders like diabetes, heart disease and stroke. This technique involves gently inserting a metal guidewire into an arm vein to collect ECs.

This study plans to collect ECs from patients undergoing surgery or admitted to intensive care. We also plan to collect control samples from healthy volunteers. Samples will be collected over the duration of the patients to RSFT. The RNA will be removed from the cells and counted to highlight changes in instructions in the cells.

Data from this study will potentially highlight new pathways involved in inflammation and help classify how some patients will react to current treatments. To obtain this data, this study will be split into 2 parts. Part 1 focuses on collecting one sample from a patient when they are at their most unwell states and comparing that to a sample from a healthy person. Part 2 will focus on key mRNA molecules identified during Part 1 and identifying how their expression changes over time.

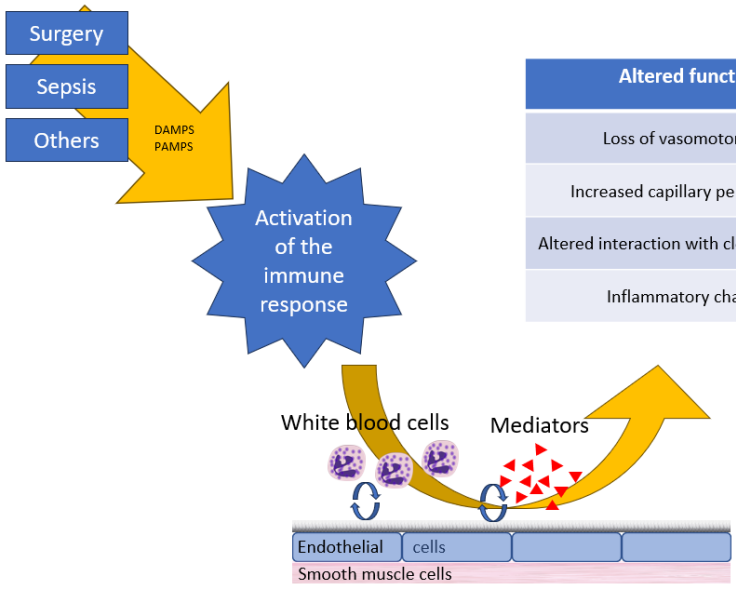
5. Background

The immune response is a complex process that is essential for protecting the body from infection and orchestrating the repair of injured tissues. Activation can be caused by severe infection (sepsis), or from significant injury to tissues - such as occurs during major surgery, trauma, pancreatitis or burns(1,2).

Pattern recognition receptors. The innate immune response can be triggered through pattern recognition receptors. In infection, these receptors detect pathogen-associated molecular patterns (PAMPs)(3) and in sterile tissue injury, damage-associated molecular patterns (DAMPs)(4). Profound activation can cause systemic inflammation and, if excessive, may be harmful.

Sepsis and surgery. Sepsis is a dysregulated immune response to infection and is the most studied cause of systemic inflammation. It can be difficult to study due to its unpredictable nature and the uncertainty of defining the onset of infection(5). Planned major surgery causes similar systemic inflammation and many of its characteristic manifestations. Improving patients' outcomes from surgery is important to study in itself, but surgery is also a good model for studying sepsis – with known time of onset and a predictable evolution (2).

The endothelium is a single layer of cells that lines the inside of blood vessels. It is constantly exposed to a variety of stimuli, including blood flow, shear stress, and inflammatory mediators, and it responds to these stimuli by secreting a variety of factors that regulate vascular tone, coagulation, and inflammation. It is also a selectively permeable barrier between the extravascular and intravascular compartments and can alter its permeability acutely(6–8). Altered endothelial cell (EC) function has predominantly been studied in chronic diseases (9,10) but is implicated in acute life-threatening conditions including sepsis and acute respiratory failure (for example, from COVID or other respiratory infections). Figure 1 shows our concept that ties injury to alterations in EC function and how this is clinically manifest.



Altered function	Clinical features
Loss of vasomotor tone	Hypotension
Increased capillary permeability	Oedema
Altered interaction with clotting cascade	Coagulopathy and thrombosis
Inflammatory changes	Contributes to systemic inflammation

Figure 1: Conceptualised relationship between triggers of systemic inflammation, the immune response, the endothelial response, and the clinical features.

New method – EC biopsy (ECBx). Researchers studying endothelial dysfunction in humans have access to a range of commercially available cells for in vitro studies, but there are substantial limitations to their use. Unprecedented insights could be gained from serial sampling of ECs from individuals with acute conditions. The first successful attempt to obtain ECs from patients was reported in 1999 by Feng et al (11). Their technique involved inserting a stainless-steel J-shaped guidewires into major vessels and collecting the tissue adherent to the guidewire. The technique has evolved into using other vascular implements such as endovascular coils and stents to study the effects of diverse diseases (10,12). Believing that ECs from patients would offer improved insights into the vascular alterations that take place during systemic inflammation, we performed a pilot study (IRAS 310961).

We assessed the feasibility of ECBx in a surgical and intensive care setting. Discarded guidewires used during arterial and central line placements were collected from both surgical (n=16) and intensive care (n=16) patients. We also collected an additional biopsy from a peripheral arm vein in surgical patients only. ECs were then isolated from the guidewires and analysed using flow cytometry for absolute cell counts. From arterial lines we recovered an average of 536; from central lines we recovered an average of 598; from peripheral cannulation we obtained 736. One sample had RNA isolated which yielded a total

of 105ng of material. We also assessed the tolerability of the ECBx through collection of adverse event (AE) data and a short questionnaire. There were no serious AEs related to ECBx, with only four patients (25%) of patients experiencing minor bruising which resolved without any complication. Patient feedback from the pilot study was overwhelmingly positive. All said that the biopsy was bearable and they could tolerate additional ECBx (mean of 3 additional biopsies).

Biomarkers of EC function. It is not possible to directly measure the functions of the endothelium in patients but there are methods to gain insights. i) circulating levels of proteins reflect some aspects of EC function such as von Willebrand factor(13), Angiopoietin2(14) and angiotensin 2(15) ii) changes in circulating leukocytes (leukocytosis or leukopenia)(16) iii) micro particles released from EC can have pro-inflammatory, pro-thrombotic and vasoregulatory properties and also contain microRNAs (miRNAs), non-coding RNA molecules regulating genes (17,18) iv) circulating miRNA (19) V) measuring aspects of EC structure and function, including flow cytometry.

RNA sequencing of EC. We will process our patient-sourced ECs to preserve the mRNA for subsequent analysis. These analyses will provide insight into the EC function at the time of acquisition. RNA sequencing provides very detailed information about the relative abundance of many different transcripts. We can use knowledge of which transcripts are involved in which molecular pathways (for example a pro-inflammatory pathway like NFκB) to understand how putative pathways are expressed. Comparison of pathway expression between times within patient cohorts and between patient cohorts will allow us to see how EC respond in patients related to their clinical status.

Hospital. The Royal Surrey hospital intensive care unit (ICU) receives >600 patients per year who have had major surgery that typically has been prolonged (>6 hours) and often associated with major tissue injury and blood loss (~1L). These patients have systemic inflammation and the predominant feature of altered EC function is loss of vasomotor tone causing hypotension. This is a major reason why they are managed on the ICU where we have specific expertise in treating hypotension. As an acute hospital we also regularly treat patients with severe infections and admit >300 patients/y with sepsis to the ICU. These patients will invariably have features of systemic inflammation and altered EC function.

Research question

Using the ECBx technique, our research question is: “In patients with sepsis or following major surgery, are changes in EC expression of putative pathways related to the clinical manifestations of SI?”

Aims

To determine the relationship between changes in EC expression in putative pathways, and the clinical manifestations and patient outcome for patients with sepsis or following major surgery.

To identify the implicated pathways which will be useful to develop novel personalised therapies that may improve patient outcome.

Objectives for Part 1

- Recruit a cohort of healthy volunteers for method validation purposes. Undertake ECBx procedure (peripheral vein) to:
    - Optimise methodology for RNA extraction
    - Use as a control to compare with the patient groups.
- Use a small number of these samples to develop methods of cell culture.
- Concurrently recruit two patient cohorts: (A) planned major surgery, (B) acutely critically unwell patients (recently admitted to ICU with sepsis).
- For each:
    - Undertake one ECBx procedure (peripheral vein). Isolate EC from guidewire. RNA sequencing on EC. In accordance with the corresponding schedule of events.
    - Characterise clinical status of the cohorts – for example extent of vasodilation as reflected in the dose of vasopressor drug being delivered to maintain an adequate blood pressure.
    - Characterisation of blood sample specifically microparticles, histones and cell free DNA, leukocyte activity.
- Analyse RNA data to identify candidate gene expression changes that are linked to the change in phenotype

Objectives for Part 2

- Recruit a second cohort of patients with (a) sepsis and (b)those undergoing major abdominal surgery.
- For each:
    - Undertake serial ECBx procedure (peripheral vein). Isolate EC from guidewire. RNA sequencing on EC. In accordance with the corresponding schedule of events.
    - Characterise clinical status of the cohorts – for example extent of vasodilation as reflected in the dose of vasopressor drug being delivered to maintain an adequate blood pressure.
    - Characterisation of blood sample specifically microparticles, histones and cell free DNA, leukocyte activity.
- Use data from Part 1 to characterise the change in candidate gene expression.

6. Trial design

This is a two-part prospective observational cohort study comparing the mRNA changes in EC from two independent biological onsets.

Part 1 focuses on identification of mRNA expression changes in one timepoint of acutely inflamed patients in comparison to a healthy control.

Part 2 focuses on validating data from Part 1 in the same patient groups by analysing mRNA over several timepoints.

Part 2 will commence upon completion of the Part 1 data review. See section 9 for further details.

6.1. Study procedures

Part 1, Cohort 1: Healthy volunteers

	Screening	Sample	7-days Post sample
Consent[1]	X		
Demographics[2]	X		
Medical history [2]	X		
ECBx[3]		X	
Blood sampling[3]		X	
AE and SAE monitoring[4]	X	X	X

1 - Consent must be performed to any study procedures. A standard consent or deferred consent model will be used, see section 7.9 for further details

2 – Medical history and demographics will be collected from the available medical records on Surrey Safe Care and NHS SPINE

3 – Severity of illness and clinical characterisation will use standardised methods such as qSOFA score and data from patient record to define. See section 7.16 for further details

4 - Both samples to be collected at the same time. See section 7.15 for further details

5 – See section 9 for further detail

Part 1, Cohort 2: Surgical cohort

	Screening	24 hours post-surgery	48 hours post-surgery	28-day follow-up
Consent[1]	X			
Demographics[2]	X			
Medical history [2]	X			
Severity of illness scoring[3]	X	X	X	
ECBx[4]		X		
Blood sampling[4]		X		
Clinical characterisation[3]		X	X	
AE and SAE monitoring[5]	X	X	X	X
Survival outcome				X

1 - Consent must be performed to any study procedures. A standard consent or deferred consent model will be used, see section 7.9 for further details

2 – Medical history and demographics will be collected from the available medical records on Surrey Safe Care and NHS SPINE

3 – Severity of illness and clinical characterisation will use standardised methods such as qSOFA score and data from patient record to define. See section 7.16 for further details

4 - Both samples to be collected at the same time. See section 7.15 for further details

5 – See section 9 for further detail

Part 1, Cohort 3: Sepsis cohort

	Screening	On admission	24 hours post admission	48 hours post admission	28-day follow-up
Consent[1]	X				
Demographics[2]	X				
Medical history [2]	X				
Severity of illness scoring[3]		X	X	X	
ECBx[4]		X			
Blood sampling[4]		X			
Clinical characterisation[3]			X	X	
AE and SAE monitoring[5]	X	X	X	X	X
Survival outcome					X

1 - Consent must be performed to any study procedures. A standard consent or deferred consent model will be used, see section 7.9 for further details

2 – Medical history and demographics will be collected from the available medical records on Surrey Safe Care and NHS SPINE

3 – Severity of illness and clinical characterisation will use standardised methods such as qSOFA score and data from patient record to define. See section 7.16 for further details

4 - Both samples to be collected at the same time. See section 7.15 for further details

5 – See section 9 for further detail

Part 2, Cohort 1: Surgical cohort

	Screening	Pre-surgery	24 hours post-surgery*	48 hours post-surgery*	28-day follow-up
Consent[1]	X				
Demographics[2]	X				
Medical history [2]	X				
Severity of illness scoring[3]	X		X	X	
ECBx[4]		X	X	X	
Blood sampling[4]		X	X	X	
Clinical characterisation[3]		X	X	X	
AE and SAE monitoring[5]	X	X	X	X	X
Survival outcome					X

1 - Consent must be performed to any study procedures. A standard consent or deferred consent model will be used, see section 7.9 for further details

2 – Medical history and demographics will be collected from the available medical records on Surrey Safe Care and NHS SPINE

3 – Severity of illness and clinical characterisation will use standardised methods such as qSOFA score and data from patient record to define. See section 7.16 for further details

4 - Both samples to be collected at the same time. See section 7.15 for further details

5 – See section 9 for further detail

*Time and procedures subject to changed based on emerging data from part 1. See section 8 for further details

Part 2, Cohort 2: Sepsis cohort

	Screening	On admission*	24 hours post admission*	48 hours post admission *	28-day follow-up
Consent[1]	X				
Demographics[2]	X				
Medical history[2]	X				
Severity of illness scoring[3]		X	X	X	
ECBx[4]		X	X	X	
Blood sampling[4]		X	X	X	
Clinical characterisation[3]			X	X	
AE and SAE monitoring5	X	X	X	X	X
Survival outcome					X

1 - Consent must be performed to any study procedures. A standard consent or deferred consent model will be used, see section 7.9 for further details

2 – Medical history and demographics will be collected from the available medical records on Surrey Safe Care and NHS SPINE

3 – Severity of illness and clinical characterisation will use standardised methods such as qSOFA score and data from patient record to define. See section 7.16 for further details

4 - Both samples to be collected at the same time. See section 7.15 for further details

5 – See section 9 for further detail

*Time and procedures subject to changed based on emerging data from part 1. See section 8 for further details

6.2. Study overview

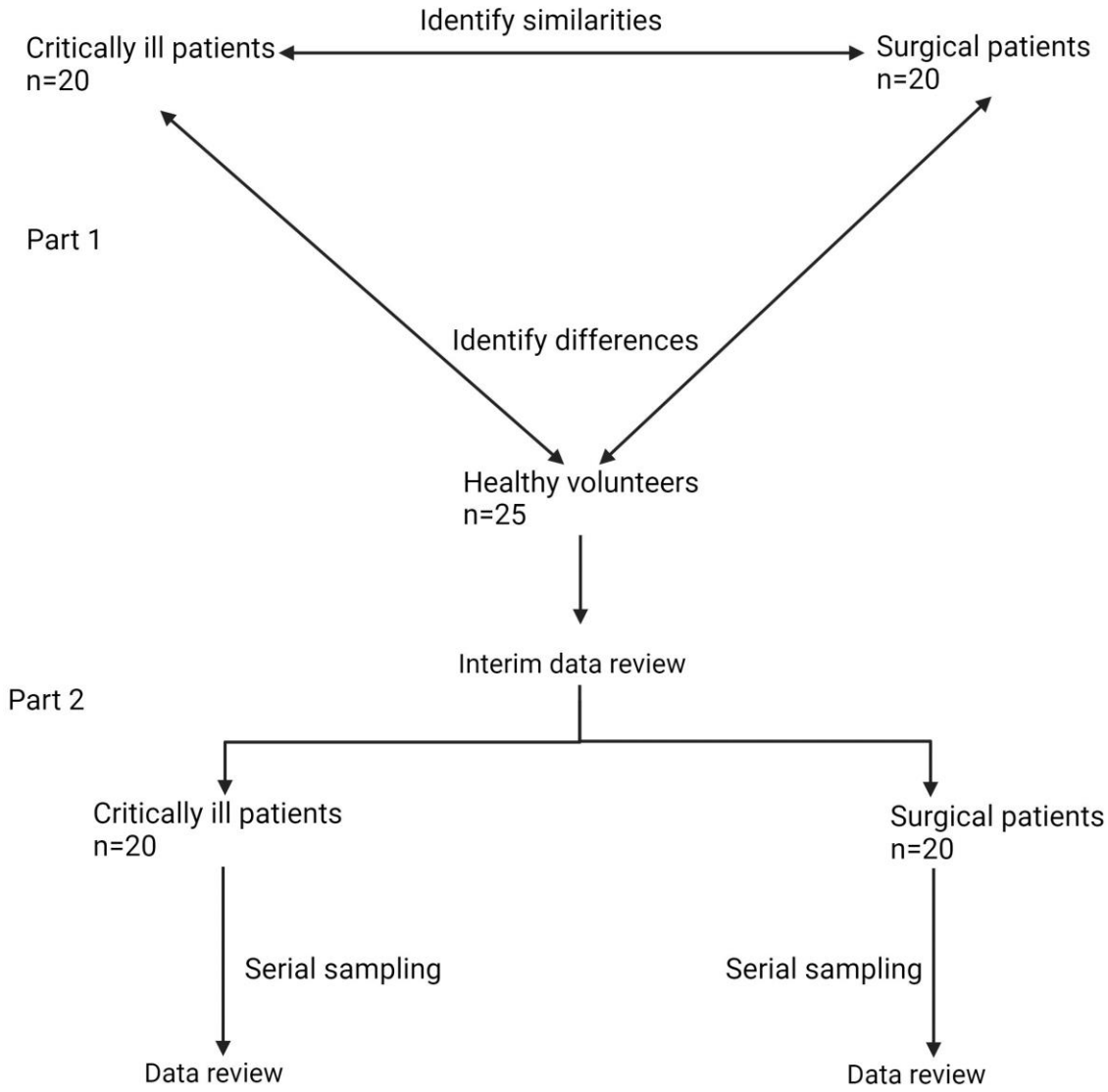


Figure 1: overview of the planned study. Part 1 – comparsion of an EC mRNA from 2 cohorts of patients taken at peak inflamation vs a healthy control. Data from this part will be reviewed to determine time points and genes of interest(see section 8 for further details). Part 2 - serial sampling looking at changes in gene expression from the genes of interest identified in Part 1.

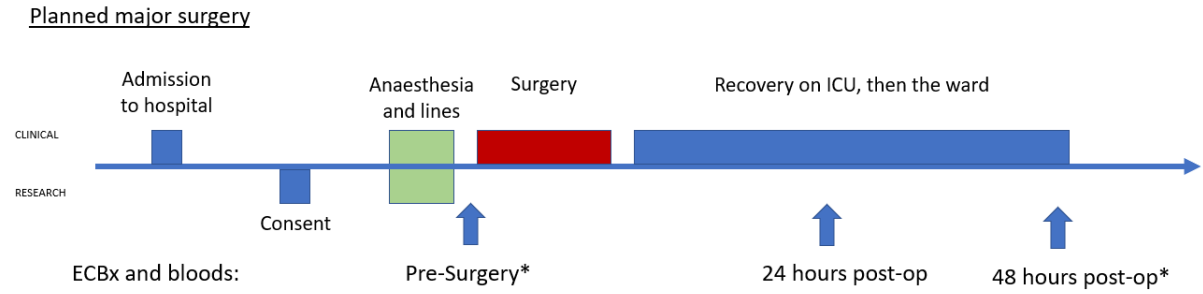


Figure 3: Schematic of major events for patients having planned major surgery. *Predicted time points for part 2 only.

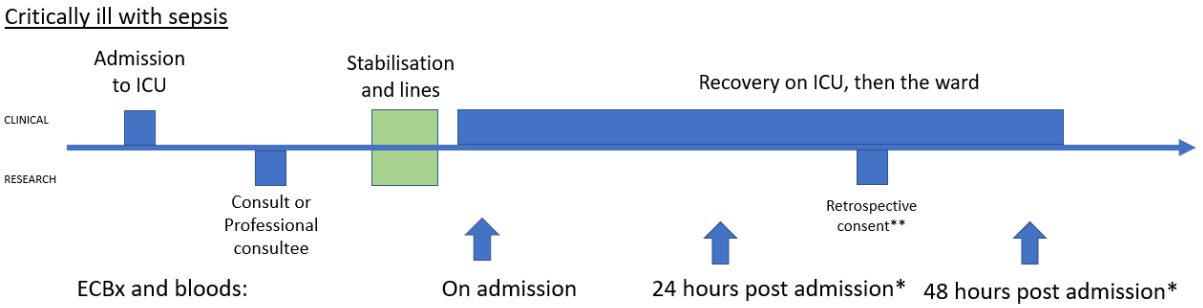


Figure 4: Schematic of major events for patients admitted to ICU with sepsis. *Predicted time points for part 2 only. **if required

7. Eligibility criteria

Eligibility criteria will be the same for both cohorts of patients in Part 1 and 2. Healthy volunteers eligibility criteria only applicable to Part 1.

7.1. Inclusion – Healthy volunteer

- Adult ≥ 18 years
- Able and willing to give consent

7.2. Exclusion – healthy volunteer

- Not currently a patient within the hospital
- Absence of inflammatory diseases and disorders including but not limited to arthritis, peripheral artery disease, vasculitis, diabetes, cardiovascular disease and CKD.
- Not on immunomodulatory medications, such as corticosteroids

- History of recent major trauma within the last 2 months (e.g., surgery or injury requiring hospitalisation)
-

7.3. Inclusion – Surgical patients

- Adult ≥ 18 years
- Patients admitted to RSFT for planned major surgery

7.4. Exclusion – Surgical patients

- Prisoners
- Anticipated prohibitively difficult venous cannulation
- Presenting with inflammatory diseases and disorders including but not limited to arthritis, peripheral artery disease, vasculitis, sepsis, diabetes with end organ damage, cardiovascular disease and CKD
- Currently prescribed immunomodulatory medication or immunocompromised
- Received chemotherapy within 2 weeks of predicted sampling
- Receiving vasopressor support prior to surgery
- History of recent major trauma within the last 2 months (e.g., surgery or injury requiring hospitalisation)

7.5. Inclusion – Critically ill patients

- Adult ≥ 18 years
- Emergency admission to ICU at RSFT
- Meets the sepsis 3.0 definition

7.6. Exclusion – Critically ill patients

- Prisoners
- Anticipated prohibitively difficult venous cannulation
- Presenting with inflammatory diseases and disorders including but not limited to arthritis, peripheral artery disease, vasculitis, diabetes with end organ damage, cardiovascular disease and CKD.
- Currently prescribed immunomodulatory medication or immunocompromised
- Received chemotherapy within 2 weeks of predicted sampling.
- History of recent major trauma within the last 2 months (e.g., surgery or injury requiring hospitalisation)

7.7. Justification for using healthy volunteers

We are using healthy volunteers in this study to compare baseline mRNA expression to sepsis patients and surgery patients. This is because we want to isolate the effects of sepsis and surgery from other factors, such as the presence of other medical conditions or medications. Healthy volunteers are less likely to have confounding factors that could interfere with the results of our study.

7.8. Incentivisation of healthy volunteers

Staff at RSFT and staff and students at UOS were interviewed on how to best incentivise healthy volunteers' participation and compensation for their time. A consensus agreement was reached suggesting that financial compensation would most appropriate. A median answer of £20 was suggested.

Healthy volunteers will be remunerated £20 for their participation in this study.

Trial procedures

7.9. Recruitment and screening

All patients will be identified from the electronic records systems at RSFT, by theatre lists or from referral by clinical staff. The study will use summary posters placed in staff areas to promote recruitment.

Screening of patients will be done by checking clinical notes and from verbal confirmation from the participant. Patients who decline to participate or fail to meet eligibility will have the reason documented on the screening log. The PI or delegate can confirm eligibility.

Healthy volunteers will be made up of members of staff and volunteers at RSFT and students at UoS. They will be identified by email advertisement from each individual organisation. All correspondence between volunteers and study team will be through the PI to maintain confidentiality. The wider study team will only be able to access anonymised date.

7.10. Consent

The PI retains overall responsibility for the conduct of research at their site, this includes the taking of informed consent of participants at their site. They must ensure that any person delegated responsibility to participate in the informed consent process is duly authorised, trained and competent to participate according to the ethically approved protocol, principles of GCP and Declaration of Helsinki. If delegation of consent is acceptable then details should be provided.

Informed consent or agreement must be obtained prior to the participant undergoing procedures that are specifically for the purposes of the trial and are out-with standard routine care at the participating site. Personal consultees must be contacted first before approaching a professional consultee.

The right of a participant to refuse participation without giving reasons must be respected.

The participants are free to withdraw at any time from the trial without giving reasons and without prejudicing their further treatment and will be provided with a contact point where they may obtain further information about the trial. Data and samples collected up to the

point of withdrawal will be used after withdrawal unless the participant has explicitly said so. The intention to utilise their data is outlined in the consent literature. Where a participant is required to re-consent or new information is required to be provided to a participant it is the responsibility of the PI to ensure this is done in a timely manner.

The PI takes responsibility for ensuring that all vulnerable participants are protected and participate voluntarily in an environment free from coercion or undue influence.

Where the participant population is likely to include a significant proportion of participants who cannot read or write, require translators, or have cognitive impairment, appropriate alternative methods for supporting the informed consent process should be employed. This may include allowing a witness to sign on a participant's behalf (in the case of problems with reading or writing), or allowing someone to date the form on behalf of the participant, or providing Participant Information Sheets in a format easily understood by the participant population (in the case of minors or cognitive impairment).

Cohorts:

Healthy volunteers will be consented after the have shown an expression of interest in participating. They can be approached at any time to sign consent after they have registered their interest.

Surgical patients will be consented prior to their surgery. They will be approached by a member of the clinical team to see if they would consider learning more about a potential research opportunity. If they are amenable, a member of the study team will introduce the study and present them with a PIS/ICF to review. This can be done prior to admission using telephone consent or in person on the day of surgery. There will be no deferred consent used.

Sepsis patients will be recruited on admission to ICU. Should the patient have capacity, they will be approached by a member of the clinical team to see if they would consider learning more about a potential research opportunity. If they are amenable, a member of the study team will introduce the study to them and present the PIS. While the patient may have full capacity to make a decision, the anticipated stressful nature of an intensive care admission may leave them in a state where they do not want to read or sign a document. In this case a witnessed verbal agreement to participate will suffice until the patient is ready to sign the consent form. In patients who lack capacity, we will use a deferred consent model. A personal or professional consultee will be consulted. Once the patient has improved, the study team will then seek to be granted consent, retrospectively. If they do not regain capacity, we will still analyse their samples.

Consent procedures can be completed by the PI or delegate. See figure 3 for visualisation.

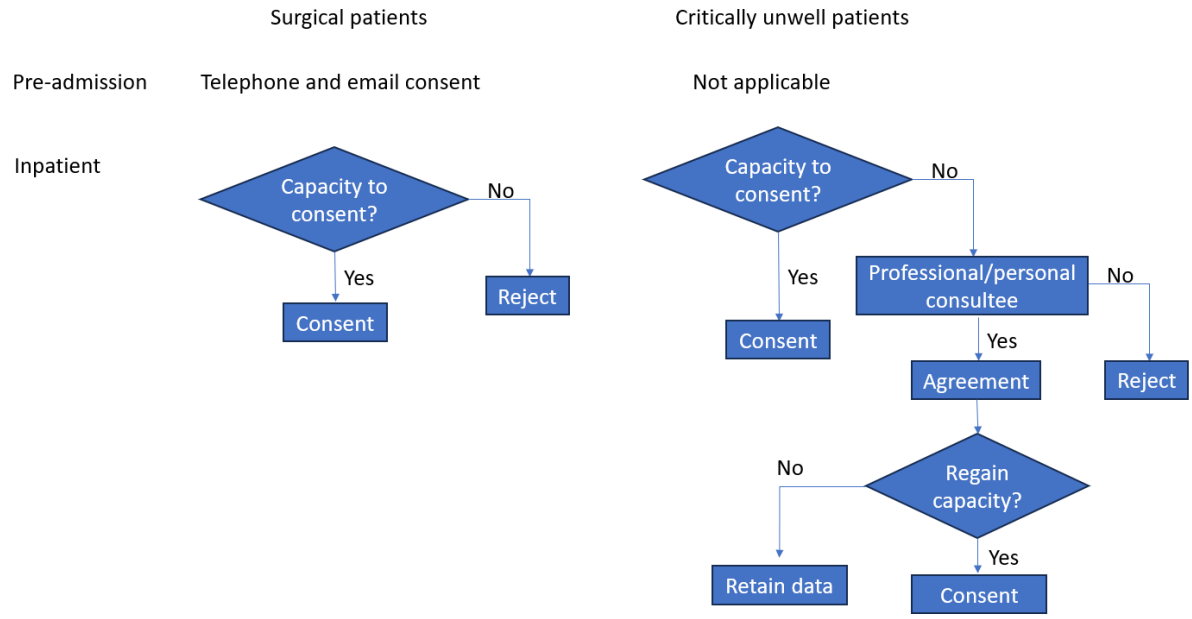


Figure 3

7.11. Baseline data and previous medical history

Information will be obtained from the medical notes following provision of consent by the participant. At time of enrolment we will collect demographic data, past medical history and current medical problems and current medications. Medical history and demographics in healthy volunteers will be collected as an oral history. The study will not look at medical records for this cohort. Data for patient cohorts will be collected from patient notes and from the summary care record from NHS SPINE.

7.12. Follow up

Follow up of patients will consist of obtaining their clinical status (Recovered/discharged from hospital/still in hospital/dead) from the patient's clinical notes. This information is to capture any significant clinical events happening after discharge from ICU. No formal contact will be made with the patient, unless there are any outstanding AEs that require resolving. Follow up will take place at 28 days (±2 days) post enrolment.

7.13. Withdrawal criteria

It is within the remit of the treating physician to withdraw their patient from the study, if they believe that it is causing them significant harm or inappropriate to perform the procedure. The trial team will also withdraw patients from the study if the patient has suffered an serious adverse event (SAE) as a consequence of the trial procedures. In both cases it must be explicitly documented in the eCRF the reason/s for withdrawal. Participants who are prematurely withdrawn may be replaced at the decision of the study team.

It is the responsibility of the trial site to ensure that samples are appropriately labelled in accordance with the trial procedures to comply with the 1998 Data Protection Act. Biological

samples collected from participants as part of this trial will be transported, stored, accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities shall at least meet the requirements as set out in the 2004 Human Tissue Act and the 2006 Human Tissue (Scotland) Act.

7.14. End of study

End of study will be defined as when the project has recruited all patients successfully, the last patient has completed follow-up and all adverse events have been resolved.

7.15. Interventions

ECBx is a technique that has been developed at RSFT for the safe and effective retrieval of EC for research. The procedure involves cannulating a peripheral arm vessel and gently passing a metal guide wire in to collect EC. The procedure is very low risk and very well tolerated in patients. AE data from our pilot study support this statement. Of the 16 patients who received an ECBx, only 4 experienced minor bruising. All bruises resolved within a week of the procedure, did not require any additional treatment nor extend hospitalisation. No evidence of phlebitis or infection at the site of cannulation noted. More importantly the procedure did not restrict vascular access for standard of care.

Participants in Part 1 will receive only 1 biopsy.

Participants in Part 2 will receive up to 3 biopsies.

Some patients may also receive arterial lines and central lines as part of their standard of care. These wires will be collected if available and used for *in vitro* modelling.

A paired blood sample will be collected at the same time as the ECBx to look for biochemical markers of endothelial dysfunction, micro particles, Leukocyte function and circulating RNA. Serum and Plasma will also be collected and used to recreate the *in Vivo* phenotype, *In Vitro.*

Participants in Part 1 will have 1 blood samples of 25 ml collected from a line or venepuncture at the same time as the ECBx.

Participants in Part 2 will have 3 blood samples of 25 ml each collected from a line or venepuncture at the same time as the ECBx.

Tissue samples, blood for leukocyte analysis, and serum and plamsa for *in Vivo* phenotyping will be sent to Charlie Piercy at the University of Surrey, where they will be prepared in room 35AY04 and then stored at -80^{o}C in 37AY04.

Serum for microparticle analysis and circulating RNA will be sent to University of Cologne Germany and covered in a separate MTA.

7.16. Clinical characterisation and severity of illness scoring

To assess severity of illness we will use qSOFA score and NEWS2. These assessments can be performed by looking at routinely collected information as part of standard of care and provide insight into how sick the patient currently is.

For clinical characterisation we will look at the patients' medical records. We will collect information on every aspect of their admission including but not limited to:

- Blood – changes in blood biochemistry, whole blood counts, blood gasses, etc
- Diagnosis – what the patient is initially diagnosed with and the change over the course of their admission
- Targeted therapies – use of medications like noradrenalin to treat hypotension, inotropes to modulate heart contractions and antibiotics for infection
- Concomitant medications – use of other medications that are not specifically given for the indication of sepsis or post-surgical hypotension.
- MDT support – data from physiotherapy, dietetic and Speech and language therapy.

7.17. Protocol deviations

Major deviations from this protocol include mishandling of EC biopsy sample or blood, late processing of sample or incorrect storage of samples. These will be documented in the patients CRF and these samples may be excluded from analysis.

Minor deviation from this protocol is any other deviation not listed above. This will be documented and use to describe any outliers to the data in the final analysis

8. Progression to part 2

Progression to part 2 will only take place after review of data and adverse events from part 1 has been completed. The focus of the review will be to assess the variability of the data collected from part 1. If the variability is as predicted or less, no changes to the protocol will be made. If the variability is greater than expected, then the power will be recalculated. Based on the updated sample size, we will then reassess requirement for the current predicted sampling for part 2.

Progression to part 2 will happen after an amendment to the protocol has been made confirming sample size requirement and sampling requirements.

9. Adverse events

All patients will be monitored in ICU as part of their routine clinical care pathway will potentially experience a range of serious events, including organ dysfunction and death. None of these events are plausibly related to the study and therefore they will not be reported. They will however, be recorded and included in the analysis of this study.

For healthy volunteers, any adverse event related or non-related to the procedure will be recorded as an AE. Healthy volunteers will be followed-up at 1-week post procedure to confirm any adverse events. Any adverse events outstanding at a week post procedure will be followed-up until resolution.

In those patients in whom we have undertaken EC samples using a guidewire in a peripheral vein, we will monitor for complications related to this procedure. Our collaborators (combined experience of >20 years using these techniques) do not report any serious adverse events related to the procedure. This is supported by the AE reporting from our pilot study, JEDI-1 (IRAS 310961). Nonetheless we will be vigilant specifically for: inflammation of the vein (phlebitis), thrombosis of the vein (thrombophlebitis), or infection around the vein or venous puncture site (cellulitis) for the 3 days following the procedure.

9.1. Reporting procedures

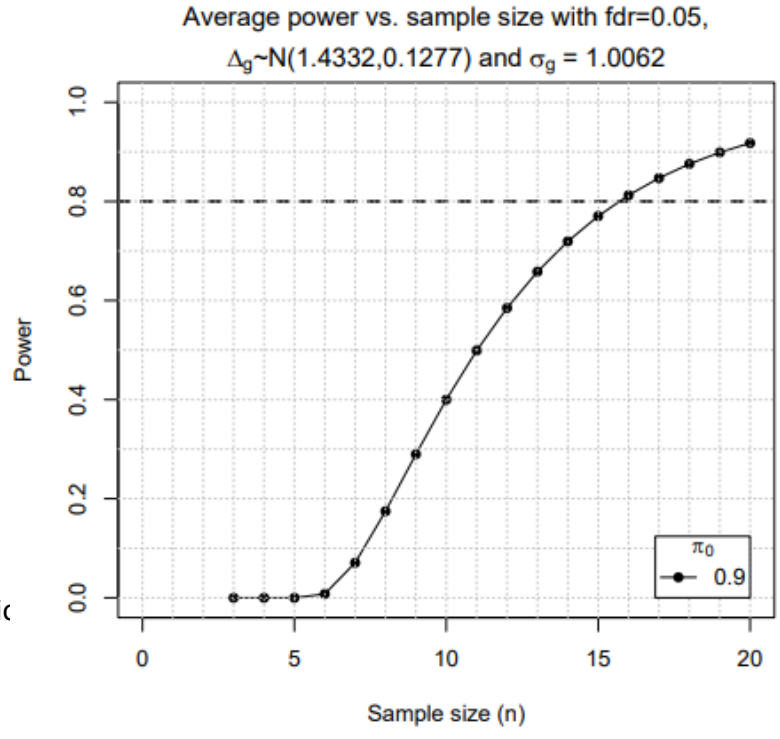
Adverse event data will be collected from the moment consent is signed until follow-up is completed. Adverse events will be reported twice a year to the study sponsor to demonstrate compliance with the regulatory guidelines.

SAEs will be reported within 24 hours of the study team being made aware. SAEs will be reported to the sponsor and chief investigator who will report to the regulatory bodies.

10. Statistics and data analysis

10.1. Part 1 Sample size calculation

Power analysis using simulations generated by the package ssizeRNA suggests that a sample size of 16 can detect a fold change of 2 with power 0.8 when 20,000 genes are tested at a false discovery rate of 0.05 if the proportion of non-differentially expressed genes is 90%, the average number of reads mapped per gene is 10 and dispersion 0.1. **For part 1 we will recruit 20 patients per cohort to ensure that we get enough useable mRNA for further analysis. We will also recruit 25 healthy volunteers, 20 for analysis and 5 to validate the method.**



10.2. Part 2 sample size calculation

Using the sample size calculation method for a two-sample t-test with the provided parameters (effect size of 2, significance level of 0.05, power of 0.8, and a variability of 2) the analysis suggests that approximately 17 patients are needed. Variability is based on an expected result from part 1. Power analysis will be re-calculated after the part 1 data review. **For Part 2, 20 patients per cohort will be recruited to ensure that data for analysis.**

10.3. Planned recruitment rate

Scicluna et al, 2017 (Lancet), highlighted potential endotypes based on leukocyte transcriptomics. Of the four theoretical endotypes highlighted, the most common and most severe was prevalent in ~30% of the population(20). Data from the VOLUME CHASER study on the use of vasopressor in patients with septic shock also shows that up to 23% of patients will require high continuous administration of vasopressor(21).

Only patients requiring organ support will be transferred to ICU. ICNARC data from Q1 2023 for RSFT ICU showed that there were 22 high risk sepsis admissions (1.6 patients a week). There are over 20 major abdominal surgery cases a week admitted to ICU each week as part of their standard of care.

Based on data collected from the pilot study we estimate to be able to enrol at a rate of 1.2 patients a week. All patients will be recruited at one site by one member of the research team. **Based on this we estimate that we will successfully recruit all patients within for part 1 within 12 months and part 2 within 8 months.**

10.4. Statistical analysis plan

RNA extracted from endothelial cells will be sequenced and analysed using packages available on R. Preprocessing steps, including quality control, trimming, filtering, and alignment, will precede the analysis. Differential gene expression and pathway enrichment analyses will be performed using R packages such as DESeq2 and edgeR. The results will be presented through clear visualizations, such as heatmaps and volcano plots, providing a detailed overview of gene expression patterns.

11. Data handling

11.1. Data collection tools and source document identification

ICH E6 section 1.51, defines source data as "All information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical trial

necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies)."

The basic concept of source data is that it permits not only reporting and analysis but also verification at various steps in the process for the purposes of confirmation, quality control, audit or inspection. A number of attributes are considered of universal importance to source data and the records that hold those data. These include that the data and records are:

- Accurate
- Legible
- Contemporaneous
- Original
- Attributable
- Complete
- Consistent
- Enduring
- Available when needed

For this study, all data collected will be entered into an eCRF and all data stored digitally. All data will be collected on a tablet computer and stored centrally and securely on EDGE. The eCRF will be considered the source for all data in this study. Any data such as ultrasonography, ECGs or assessments that have physical sources will be translated into the eCRF. These assessments will be available as a hard copy in the patient notes.

11.2. Data handling and record keeping

GCP requires that sponsors operating such systems validate the system, maintain SOPs for the use of the system, maintain an audit trail of data changes ensuring that there is no deletion of entered data, maintain a security system to protect against unauthorized access, maintain a list of the individuals authorized to make data changes, maintain adequate backup of the data, safeguard the blinding of the trial and archiving of any source data (i.e. hard copy and electronic). Data is transformed during processing, and it will be possible to compare the original data and observations with the processed data. Sponsors are responsible for ensuring compliance with the requirements outlined above when tasks are subcontracted. There should be no loss of quality when an electronic system is used in place of a paper system.

11.3. Access to data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections- in line with participant consent.

Data can be freely shared with members of the collaborative group. Data may be shared with external researchers and industry if there is a consensus agreement by the collaborative group. This will documented in the PIS.

11.4. Archiving

Archiving will be authorised by the sponsor following publication of results. All trial related media and documents will be stored by Kelly's in accordance with RSFT SOPs for a minimum of 5 years. Destruction of any essential documents will require authorisation from sponsor.

12. Monitoring, audit & inspection

Trial monitoring and protocol compliance will be led by R&D at RSFT. All visits will be conducted on-site by a delegate of R&D following RSFT SOPs. They will inspect the site files at initiation and close out of this study. They will also conduct a data quality check on the first patient/s recruited. If data collected is compliant with GCP and meets Trust standard, no further data quality checks will take place.

The University of Surrey will be responsible for auditing protocol compliance with the HTA for all samples transferred to the Stag Hill Campus.

13. Ethical and regulatory considerations

13.1. Public and Patient Involvement

As part of JEDI-1, we asked all patients involved in the study their opinions on multiple aspects of the study. The first area we focussed on was the consent procedure. We were concerned that the patients may feel they did not have enough time to make an informed decision given that they are usually consented within an hour of their procedure. All 26 patients asked felt that they had enough time to make an informed decision and were not overwhelmed by our approach.

Secondly, we appreciate that this is an invasive procedure and additional procedures may not be too welcome. We asked our patients if they would be happy to have an additional biopsy, and if yes how many additional biopsies could they manage. All patients said that they would be happy with an additional biopsy, with a mean of 3 additional biopsies – 4 in total.

13.2. Co-enrolment

Co-enrolment will be sought from any studies that are recruiting patients in relevant clinical areas. There are no observational studies that we will not co-enrol with, but we need to ensure that the other study teams do not mind co-enrolment. We will consider interventional trials on a case-by-case basis. All co-enrolment agreements will be stored in the site file.

13.3. Protocol compliance

Prospective, planned deviations or waivers to the protocol are not allowed must not be used e.g. It is not acceptable to enrol a participant if they do not meet the eligibility criteria or restrictions specified in the trial protocol

Accidental protocol deviations can happen at any time. They must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately. Frequent deviations from the same root cause will be investigated to limit the effect it has on any data collected.

Minor protocol deviations are attributed to events that have minimal impact on the data set. These include but are not limited too samples being taken outside of specified nominal timepoint, a non-delegated individual performing a task they are not assigned and variance in sampling depth.

Major deviations are attributed to events that have a measurable impact on the data set and may necessitate a replacement participant. These include but are not limited too, enrolling a patient that is not eligible for the study, delayed transfer of sample to analytical laboratory and incorrect handling of sample in analytical laboratory.

13.4. Data protection and patient confidentiality

All investigators and trial site staff are compliant with the requirements of the Data Protection Act 1998 and General Data Protection Regulation (2018) with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

All clinical data collected will be anonymised before it is shared with any members of the trial team that are not affiliated with the NHS or RSFT. Patients will receive a 3-letter code identifying which group they are in and a 3-digit number based on when they joined the study. The codes are:

Part 1

Surgical patients – SUR001-SUR020

Critically unwell patients – ICU001-ICU020

Healthy volunteers – HEL001-025

Part 2

Surgical patients – SUR0101-SUR120

Critically unwell patients – ICU101-ICU120

All personal data will be stored on RSFT EDGE clinical research platform. This validated system can be only be accessed by study team members within the NHS. The study will be further restricted so that only the team at RSFT will have access to any patient identifiable information.

RSFT information governance and data protection team will monitor compliance of confidentiality and data protection. They are also responsible for any freedom of information requests and any complaints on data handling.

All clinical information collected will be entered into a password secured eCRF compliant with ICH GCP E6 R2 (2018). The eCRF will be translated into a password protected document

and shared via a private cloud space amongst the study team who will have read-only access. Data will be stored on the cloud space until the end of the project, then downloaded onto an encoded USB and stored for 15 years after the end of the study. Charlie Piercy will act as custodian of the data.

13.5. Financial and other competing interests

The study team have no financial or competing interest to declare.

13.6. Indemnity

As part of the sponsorship from R&D, this study will be placed under RSFTs indemnity policies. They will support the study and lead on any issues arising from this research ascertaining to indemnity and insurance. It is the responsibility of the trial team to notify the sponsor if any of these issues arise.

13.7. Dissemination policy

The data arising from this trial will be owned jointly between the study team named in this protocol. Upon completion of the study, the data will be analysed and written up in several formats. A copy of the full trial publication will be made available to both UoS, RSFT and GUTS FBC. We will work with DAWN at RSFT to create a lay summary and dissemination strategies to maximise the impact of our work with as many people as possible, including participants on the trial. We will also present the work as an abstract or presentation at conference with a focus on ESICM and EVBO.

13.8. Authorship eligibility guidelines

The study team named in the protocol will be named as authors on any subsequent publications arising from this work. However, the study team also acknowledge that there will be a lot of others who make contributions to the success of this work. Significant contributions which have a measurable outcome on the output of the project will be included in the authorship.

14. References

1. Boomer JS, Green JM, Hotchkiss RS. The changing immune system in sepsis. Virulence. 2014 Jan 1;5(1):45–56.

2. Bossy M, Nyman M, Madhuri TK, Tailor A, Chatterjee J, Butler-Manuel S, et al. The need for post-operative vasopressor infusions after major gynae-oncologic surgery within an ERAS (Enhanced Recovery After Surgery) pathway. Perioper Med. 2020 Sep 7;9(1):26.

3. Gruda MC, Ruggeberg KG, O'Sullivan P, Guliashvili T, Scheirer AR, Golobish TD, et al. Broad adsorption of sepsis-related PAMP and DAMP molecules, mycotoxins, and cytokines from whole blood using CytoSorb® sorbent porous polymer beads. PLOS ONE. 2018 Jan 25;13(1):e0191676.

4. Pantalone D, Bergamini C, Martellucci J, Alemanno G, Bruscino A, Maltinti G, et al. The Role of DAMPS in Burns and Hemorrhagic Shock Immune Response: Pathophysiology and Clinical Issues. Review. Int J Mol Sci. 2021 Jan;22(13):7020.

5. Jarczak D, Kluge S, Neirhaus A. Sepsis—Pathophysiology and Therapeutic Concepts. Front Med. 2021 May 14;8.

6. Cahil PA, Redmond EM. Vascular endothelium – Gatekeeper of vessel health. Atherosclerosis.

7. Dolmatova E, Wang K, Mandavilli R, Griendling K. The effects of sepsis on endothelium and clinical implications. Cardiovasc Res. 2021 Jan 1;117:60–73.

8. Joffre J, Hellman J, Ince C, Ait-Oufella H. Endothelial Responses in Sepsis. Am J Respir Crit Care Med. 2020 Feb 26;202(3).

9. Masaki N, Feng B, Bretón-Romero R, Inagaki E, Weisbrod R, Fetterman J, et al. O-GlcNAcylation Mediates Glucose-Induced Alterations in Endothelial Cell Phenotype in Human Diabetes Mellitus. J Am Heart Assoc. 2020 Jun 6;9(12).

10. Cooke DL, McCoy DB, Halbach VV, Hetts SW, Amans MR, Dowd CF, et al. Endovascular Biopsy: In Vivo Cerebral Aneurysm Endothelial Cell Sampling and Gene Expression Analysis. Transl Stroke Res. 2018 Feb 1;9(1):20–33.

11. Feng L, Stern DM, Pile-Spellman J. Human endothelium: endovascular biopsy and molecular analysis. Radiology. 1999 Sep;212(3):655–64.

12. Sheth SA, Verma A, Malhotra K, Tang X, Tateshima S, Jahan R, et al. Human Endothelial Cell Collection from the Middle Cerebral Artery in Acute Ischemic Stroke. J Stroke Cerebrovasc Dis. 2018 Mar 1;27(3):669–72.

13. Rusu L, Minshall RD, Rusu L, Minshall RD. Endothelial Cell von Willebrand Factor Secretion in Health and Cardiovascular Disease. In: Endothelial Dysfunction - Old Concepts and New Challenges [Internet]. IntechOpen; 2018 [cited 2023 Oct 2]. Available from: https://www.intechopen.com/chapters/59581

14. Akwii RG, Sajib MS, Zahra FT, Mikelis CM. Role of Angiopoietin-2 in Vascular Physiology and Pathophysiology. Cells. 2019 May 17;8(5):471.

15. Birk M, Baum E, Zadeh JK, Manicam C, Pfeiffer N, Patzak A, et al. Angiotensin II Induces Oxidative Stress and Endothelial Dysfunction in Mouse Ophthalmic Arteries via Involvement of AT1 Receptors and NOX2. Antioxidants. 2021 Aug;10(8):1238.

16. Farkas JD. The complete blood count to diagnose septic shock. J Thorac Dis. 2020 Feb;12(Suppl 1):S16–21.

17. Mesri M, Altieri DC. Endothelial cell activation by leukocyte microparticles. J Immunol Baltim Md 1950. 1998 Oct 15;161(8):4382–7.

18. Reid VL, Webster NR. Role of microparticles in sepsis. Br J Anaesth. 2012 Oct 1;109(4):503–13.

19. Shu Z, Tan J, Miao Y, Zhang Q. The role of microvesicles containing microRNAs in vascular endothelial dysfunction. J Cell Mol Med. 2019 Dec;23(12):7933–45.

20. Scicluna BP, Vught LA van, Zwinderman AH, Wiewel MA, Davenport EE, Burnham KL, et al. Classification of patients with sepsis according to blood genomic endotype: a prospective cohort study. Lancet Respir Med. 2017 Oct 1;5(10):816–26.

21. Roberts RJ, Miano TA, Hammond DA, Patel GP, Chen JT, Phillips KM, et al. Evaluation of Vasopressor Exposure and Mortality in Patients With Septic Shock*. Crit Care Med. 2020 Oct;48(10):1445.