Trial Protocol and Analysis

Does the release profile of nociceptin from immunocytes differ in healthy volunteers and critically ill patients with sepsis?

NCT03037281

25/6/2018

Full Study Title: Does the release profile of nociceptin from immunocytes differ in healthy volunteers and critically ill patients with sepsis?

Sponsor Reference No: 0554

Ethics Ref: 16/EM/0046 Date and Version No: 25/06/2018 v2.0

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Sponsor:	University of Leicester
Funder:	Biotechnology and Biological Sciences Research Council Royal College of Anaesthetists

Signatures: The approved protocol should be signed by author(s) and/or person(s) authorised to sign the protocol

Confidentiality Statement

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Signature Page

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Date: _____

(In cases of Multi-centre studies, this must be replicated for each site)

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1. AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	0.2	07/12/2015	Hebbes	Version number and title correction
2	1.1	19/2/16	Hebbes	
3	**2.0**	**25/6/18**	**Hebbes**	**Single blood sample of 30mls (replacing 2 separate samples)** **Amendments to PIS and consent form** **Amendment to cell types examined (basophils removed)** **Sepsis screening criteria updated to Sepsis-3** **Updated sample sizes (reduced from 20 to 8)** **Updated sampling timeframe; increased to within 48 hours of ITU admission** **Updated planned study end date to December 2018** **Updated secondary objectives – Not possible to measure N/OFQ levels using this assay as stated. Immune cell expression of N/OFQ and NOP by PCR as a surrogate**

2. SYNOPSIS

Study Title	Does the release profile of nociceptin from immunocytes differ in healthy volunteers and critically ill patients with sepsis?
Internal ref. no.	0554
Trial Design	Case control
Trial Participants	Healthy volunteers Critically ill septic patients
Planned Sample Size	**8 healthy volunteers** **8 critically ill septic patients**
Follow-up duration	From admission to critical care, until discharge to ward based case or death
Planned Trial Period	January 2016 – **December 2018**
Primary Objective	To determine differences in the release profile of NOFQ from immune cells in the blood (**neutrophils, eosinophils**) between patients with sepsis admitted to the intensive care unit and healthy volunteers
Secondary Objectives	**Determine differences in expression of N/OFQ and NOP genes in immune cells from septic and healthy volunteers using immunofluorescence and PCR as surrogates**

Primary Endpoint	The release of NOFQ will be determined by fluorescence of Chinese Hamster Ovary cells, expressing NOP, coupled to Calcium release and stained with a calcium sensitive fluorescent dye. Relative fluorescence (change / basal) will be measured following layering of immune cells, and degranulation. A relative fluorescence of >1.6 indicates a response, and the presence of NOFQ. Measures will be number of "responder" cells

	Quantitative evaluation of N/OFQ and NOP expression in eosinophils and neutrophils, with a comparison between septic and healthy volunteers
Secondary Endpoints	Cell count and differential proportion of immune cell populations Physiological scores Mortality in-hospital, at 30 days Length of ITU stay, and hospital stay

3. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
BP	Blood Pressure
CI	Chief Investigator
CRA	Clinical Research Associate (Monitor)
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Clinical Trials
EC	Ethics Committee (see REC)
EDTA	Ethylenediaminetetraacetic acid
GCP	Good Clinical Practice
GP	General Practitioner
GTAC	Gene Therapy Advisory Committee
ICF	Informed Consent Form
ISF	Investigator Site File
NHS	National Health Service
N/OFQ	Nociceptin / Orphanin FQ
NOP	Nociceptin receptor
NRES	National Research Ethics Service
PI	Principal Investigator
PIL/S	Participant/ Patient Information Leaflet/Sheet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SIRS	Systemic Inflammatory Response Syndrome
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
WCC	White Cell Count

4. BACKGROUND AND RATIONALE

Sepsis is defined as an overwhelming systemic response to infection (of any source), characterised by a systemic inflammatory response syndrome (SIRS), combined with suspicion, or evidence (from microbiological samples) of infection(Dellinger et al., 2013). Classification is according to organ dysfunction, as septic shock (circulatory failure, in the presence of sepsis), severe sepsis (liver, kidney, circulatory, respiratory or gastrointestinal failure in the presence of sepsis), or sepsis (system inflammatory response in the presence of infection). The mortality increases proportionately to the degree of sepsis, and, untreated is 40-50%.

The underlying organism triggering the sepsis has a role in determining the severity; and early treatment with appropriate antibiotics reduces mortality dramatically in a time dependent manner(Kumar et al., 2006). However, the control and the regulation of the immune system in the face of sepsis may also be implicated in survival.

Following the discovery and cloning of the fourth opioid receptor (NOP)(Wang et al., 1994), the use of reverse pharmacology led to the sequencing of the endogenous ligand Nociceptin(Meunier et al., 1995) and OrphaninFQ(Reinscheid et al., 1995) (N/OFQ). It has a role in the regulation of the pain response and reward behaviours, and is increasingly considered as a therapeutic target in sepsis. Opportunities to regulate the detrimental effects of immune system over-activation in sepsis would potentially improve outcomes for this patient group.

Evidence for the role of N/OFQ in the immune system

Clinical studies in humans have demonstrated increased levels of NOFQ in sepsis(Stamer et al., 2011; Williams & Thompson, 2008), following cardiopulmonary bypass(Thompson JP et al., 2013), and arthroscopy(Lee & Jeon, 2013). Administration of a NOP antagonist in the caecal ligation model of septic peritonitis in the rat was improved mortality.

There is evidence for the presence of N/OFQ and NOP in cells of the immune system at the molecular level. Binding studies are inconclusive, with some suggesting the presence of NOP on immune cells(Peluso, Gaveriaux-Ruff, Matthes, Filliol, & Kieffer, 2001), whereas other authors have failed to find any evidence of NOP binding (Williams et al., 2007).

Genetic studies have demonstrated the presence of NOP mRNA in human lymphocytes(Wick, Minnerath, Roy, Ramakrishnan, & Loh, 1996), monocytes(Peluso et al., 1998), and polymorphs(Fiset,

Gilbert, Poubelle, & Pouliot, 2003). This discrepancy may be due to differences in functional, isolated human cells, versus the use of cloned human cell lines, or may suggest that NOP mRNA exists but not functionally transcribed, or may be upregulated in sepsis.

mRNA for N/OFQ, and its peptide precursor pp-NOFQ has been demonstrated consistently in human splenocytes and leukocytes(Nothacker et al., 1996), lymphocytes(Arjomand, Cole, & Evans, 2002), and neutrophils(Fiset et al., 2003). Whilst mRNA is demonstrated in PCR studies, the functional expression has not been evaluated, nor the identity of the releasing cell.

Neither the significance nor source of nociceptin in sepsis is known. The measurable rise in plasma concentrations following septic or inflammatory stimuli may represent a consequence or cause of the underlying septic response, and may therefore represent a therapeutic intervention, or a biomarker for sepsis severity, or prognostication.

Risks and benefits

This study requires a single 30ml blood sample to be taken from septic patients, within 48 hours of initial admission to the intensive care unit, into an EDTA bottle, stored at 18-22 degrees centigrade and processed within 2 hours of sampling. A member of the investigative team competent at phlebotomy will collect these samples, either by venepuncture or by sampling from an indwelling arterial or central venous line using a standard aseptic non touch technique.

A single 30 ml sample will be collected by venepuncture from healthy volunteers.

The collection of this small volume of blood would be an additional procedure to the patient's care. However, collection of this small volume of blood would not be anticipated to have any deleterious effect. In order to avoid harm, data regarding allergies, medical problems and medication will be collected.

Patient population

The patient population for this study will be patients admitted to the intensive care unit with a diagnosis of sepsis. Healthy volunteers will be recruited from staff and students within the department of Cardiovascular Sciences at the University of Leicester.

There is a likely role for NOP and NOFQ in the regulation of the immune system in sepsis. Elevated plasma NOFQ in inflammatory conditions may be a cause or consequence of immune system activation, and could provide a new therapeutic target. This is the first assay developed to demonstrate NOFQ release from single cells.

Using this assay, we will determine the differences in functional NOFQ expression in immunocytes from healthy volunteers and septic patients.

5. OBJECTIVES

5.1 Primary Objectives

To determine differences in the release profile of NOFQ from immune cells in the blood (neutrophils, eosinophils) between patients with sepsis admitted to the intensive care unit and healthy volunteers.

5.2 Secondary Objectives

Determine the identity of cells of the immune system containing NOFQ in healthy volunteers and patients with sepsis admitted to the intensive care unit, and any differences between sepsis and severe sepsis

Determine NOFQ release in

Neutrophils

Eosinophils

Determine differences in expression of N/OFQ and NOP genes in immune cells from septic and healthy volunteers using immunofluorescence and PCR as surrogates

5.3 Exploratory End Point (where applicable)

6. STUDY DESIGN

6.1 Summary of Trial Design

Case control study to determine the types of immune cells capable of releasing NOFQ, and how this differs in patients diagnosed with sepsis/septic shock, and healthy volunteers using a fluorescence microscopy test. Differences in this release profile will be determined in septic patients and healthy volunteers, and correlated with mortality, length of hospital and intensive care stay and physiological scores.

Participants will be recruited sequentially; screening, recruitment, sampling and testing will occur concurrently. Patients with sepsis will be identified by discussion with the consultant responsible for the intensive care unit. Healthy volunteers will be approached within the Department of Cardiovascular Sciences, University of Leicester.

Following consent or assent, patients will be required to have a single 30ml sample of blood taken, within 48 hours of intensive care admission, after which no further active participation is required. They will be followed until hospital discharge or death, and these dates recorded, and may withdraw consent at any time. Their General Practitioner will be contacted at 30 days for follow up of mortality, and they will be informed of the patient's participation in the study.

Patient medical records will be accessed to 1) ensure that there is no contraindication to blood sampling, 2) they do not fall within the exclusion criteria, 3) apply physiological scores to grade severity, 4) ensure that the diagnosis of sepsis is valid.

Healthy volunteers will be required to have a single 30ml sample of blood taken following consent, and will be interviewed to ensure that they do not fall within the exclusion criteria and that there is no contraindication to blood sampling.

Once collected, all samples will be pseudoanonymised and assigned a case ID, such that participants will not be identifiable. The case ID and original participant details will be linked in a sample log, stored securely. For purposes of research governance, it would be possible to disclose the anonymisation, although the requirement for this is unlikely for a non-interventional study.

The duration of the study is from January 2016-December 2018.

6.2 Primary and Secondary Endpoints/Outcome Measures

To determine differences in the release profile of NOFQ from immune cells in the blood between patients with sepsis admitted to the intensive care unit and healthy volunteers

Determine the identity of cells of the immune system containing NOFQ in healthy volunteers and patients with sepsis admitted to the intensive care unit, and any differences between sepsis and severe sepsis
Determine NOFQ release in
Neutrophils
Eosinophils

Outcome measures

The release of NOFQ will be determined by fluorescence of Chinese Hamster Ovary cells, expressing NOP, coupled to Calcium release and stained with a calcium sensitive

fluorescent dye. Relative fluorescence (change / basal) will be measured following layering of immune cells, and degranulation. A relative fluorescence of >1.8 indicates a response, and the presence of NOFQ. Measures will be number of “responder” cells, fluorescent change by time, area under the time / fluorescence curve.
Cell count and differential proportion of immune cell populations
Physiological scores
Mortality in-hospital, at 30 days
Length of ITU stay, and hospital stay

7. TRIAL PARTICIPANTS

7.1 Overall Description of Trial Participants

Patients admitted to the intensive care unit with a diagnosis of sepsis will be identified by discussion with the responsible consultant and nursing staff. For the purposes of this study, patients must have a diagnosis of sepsis by Sepsis-3 criteria; with microbiological evidence or suspicion of infection (positive blood culture, urine dipstick, compatible history or examination, radiographic evidence)

Patients (or their personal consultee where the patient lacks capacity) will be provided with a Participant Information Sheet and consent/assent taken if the participant (or their personal consultee) agrees. As an early blood sample is required, the time to read and consider the PIS is limited.

Healthy volunteers will be approached within the Department of Cardiovascular Sciences, and provided with the PIS, with consent taken by one of the investigating team.

7.2 Inclusion Criteria

For septic patients;

1. Participant is willing and able to give informed consent for participation in the study, or if lacking capacity, a next of kin or advocate is willing and able to give assent for participation in the study. Must be able to read and understand English.
2. Male or Female, aged 18 years or above.
3. Diagnosed with sepsis (see 7.1) and admitted to the intensive care unit.
4. Able (in the Investigators opinion) and willing to comply with all study requirements.
5. Willing to allow his or her General Practitioner and consultant, if appropriate, to be notified of participation in the study.

Healthy Volunteers;

1. Participant is willing and able to give informed consent for participation in the study. Must be able to read and understand English. 2. Male or Female, aged 18 years or above and be

3. In good health.
4. Have had no course of medication, whether prescribed or over-the-counter, in the four weeks before first study dose and no individual doses in the final two weeks other than mild analgesia, vitamins and mineral supplements or, for females, oral contraceptives
5. Able (in the Investigators opinion) and willing to comply with all study requirements.
6. Willing to allow his or her General Practitioner and consultant, if appropriate, to be notified of participation in the study.

7.3 Exclusion Criteria

1. Conditions which may make phlebotomy hazardous to the participant (such as significant bleeding disorders or anaemia, or allergy), or to the investigator (blood viral infection).
2. Any significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate in the study.
3. Participants who have participated in another research study involving an investigational product in the past 12 weeks.

8. STUDY PROCEDURES

8.1 Informed Consent

Following screening and identification (see 8.2), participants or their personal consultee (where the participant lacks capacity) will be approached by the investigating team. Practically, the consenting procedure and blood sampling should occur within the first 48 hours of ITU admission. If required, the participant will be allowed as much time as wished up to 48hours post intensive care admission to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the informed consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator as detailed on the Delegation of Authority and Signature log for the study. The original signed form will be retained at the study site within the Trial Master File (TMF) or Investigator Site File (ISF). A copy of the signed Informed Consent will be given to participants and a copy retained in the participant medical notes.

For those patients lacking capacity (as defined by the Mental Capacity Act 2005), their friend or relative (personal consultee) will be asked to assent for them, as per the process above. If the patient later regains capacity, they will be approached in order to confirm consent for the study. If they withdraw their consent, their data and samples will be destroyed. If the patient dies without regaining capacity, they will be included in the trial, and samples retained.

At the time of consent/assent, a Case Report Form (CRF) will be completed, including safety information (medications, past medical history, allergies), demographic information (age, gender), and study information (lactate, blood pressure, oxygen requirement, pulse, and likely source of sepsis). These data will already exist on admission to the intensive care unit. After consent and provided that there are no exclusions (see 7.3), a blood sample (1 x 30ml EDTA) will be collected by the investigator, either by venepuncture, or by sampling an indwelling arterial line or central venous catheter, using a standard aseptic non touch technique.

8.2 Screening and Eligibility Assessment

Participants will be identified by discussion with the consultant in charge of the intensive care unit, nursing staff and screening medical records.

Potential participants (or their personal consultee where participants lack capacity) will be approached by the investigating team for the consent/assent procedure. A participant information sheet (see appendix) will be provided, and the opportunity to ask questions about the study. Due to the need to obtain an early sample on Day 1 of intensive care admission, provided the participants or advocates agree, they must sign the latest approved consent form prior to further study activities.

<u>Demographics</u>

The date of birth and gender will be recorded.

Medical History

Details of illness likely to present a hazard to the participant (significant anaemia, bleeding disorder, allergies) or the researcher (transmissible blood viral infections) during blood sampling will be recorded.

The details of the current intensive care admission will be documented on the CRF including reason for admission, and medications on admission.

Physical Examination

A physical examination – or access to the documented observations will be sought to confirm the diagnosis of sepsis (7.1 - lactate, blood pressure, oxygen requirement, pulse urine output and likely source of sepsis).

Laboratory Tests

No new laboratory tests will be required for the screening and eligibility phase, although tests already completed as part of the routine care for the patient, including Full Blood Count, Urea and Electrolytes, Liver Function Tests, Blood Gas Analysis, CReactive Protein and the results of microbiological culture will be accessed in order to determine the presence of sepsis (7.1).

Radiology / Imaging Procedures

No new radiology or imaging procedures will be undertaken for the purposes of this study. If radiological evidence of infection (such as a chest infection or pneumonia) exists, this will be screened to determine eligibility and the presence of sepsis (as defined in 7.1).

8.3 Baseline Assessments

Baseline assessment will include severity of assessment, measured using existing patient data collected as part of routine care. The purpose of this assessment is to determine the presence of **sepsis/septic shock**.

- Lactate
- Blood Pressure, Pulse
- Arterial blood gas
- Blood glucose
- Microbiological samples (blood, urine, sputum, fluid for culture) - Presence of vasoactive medications and inotropes

8.4 Randomisation and Codebreaking (if applicable)

This study will be conducted as a case control protocol and is non-interventional.
Samples will be pseudoanonymised once collected; the code for the Case ID will be stored securely to enable codebreaking for governance purposes.
Subject numbers will be assigned sequentially as each subject enters the study.

8.5 Subsequent Assessments

Patients will have blood sampled (as per the protocol) within 48 hours of their intensive care admission. If a patient is readmitted to the intensive care unit with another diagnosis of sepsis after previous enrolment, they will not be re-enrolled and will be excluded from further participation.
The General Practitioner for enrolled patients will be contacted to inform them of their participation. Healthy volunteers will have a single sample of blood taken.

8.6 Definition of End of Trial

The end of trial is when the mortality follow-up is complete from the last recruited patient.

8.7 Discontinuation/Withdrawal of Participants from Study

Each participant has the right to withdraw from the study at any time. In addition, the investigator may discontinue a participant from the study at any time if the investigator considers it necessary for any reason including:

Ineligibility (either arising during the study or retrospective having been overlooked at screening)
Significant protocol deviation
Consent withdrawn Lost
to follow up
The reason for withdrawal will be recorded in the CRF.
If the participant is withdrawn due to an adverse event, the investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised.

8.8 Source Data

Source documents are original documents, data, and records from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g., there is no other written or electronic record of data All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code, not by name.

9. TREATMENT OF TRIAL PARTICIPANTS

9.1 Description of Study Treatment

The trial is non-interventional. Aside from blood sampling (as described in the protocol above), there will be no drugs or interventions administered.

9.2 Storage of Study Equipment or Related apparatus

The study requires phlebotomy equipment with no special storage requirements. Blood samples will be stored at 18-22□C following sampling. Storage for use in related studies will be at -20□C. Samples for PCR will be stored at -20◦C.

9.3 Compliance with Study Treatment

As this is a non-interventional trial, there will be no measures of medication or intervention compliance.

If patients become ineligible, their data will be retained but any samples will be destroyed. If a patient withdraws consent prior to the completion of their involvement, their data and samples will be destroyed.

10. SAFETY REPORTING

10.1 Definitions

10.1.1 Adverse Event (AE)

An AE or adverse experience is:

Any untoward medical occurrence in a patient or clinical investigation participants, which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study, whether or not considered related to the study.

10.1.3 Severe Adverse Events

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

10.2 Reporting Procedures for All Adverse Events

As this is a non-interventional study, AE's and SAE's are unlikely, and therefore AS/SAE reporting is not applicable to this type of study.

11. STATISTICS

11.1 Description of Statistical Methods

Fluorescence regions of interest, defined as all CHO cells adjacent to and surrounding the immunocyte of interest will be marked by the researcher. Basal fluorescence – the mean fluorescence determined per cell (region of interest) for the first 10 seconds of imaging. Relative fluorescence (change in fluorescence / basal) will be determined for each cell, at each time point.

Cells expressing a relative fluorescence of □1.8 at any point will be classed "responders"; other CHO cells adjacent to the immune cell, and with relative fluorescence <1.8 will be classed as nonresponders. The proportion of responders will be compared using $\square^2$ with Fisher's exact test between healthy volunteers and intensive care patients.

Presence of nociceptin peptide, receptor, and identifying immune cell markers will be assessed qualitatively by fluorescence microscopy.

Presence of nociceptin peptide, and receptor encoding DNA will be assessed using quantitative PCR with comparison of CT values between groups.

11.2 The Number of Participants

This is a pilot study to develop this assay for use in comparing patient groups, and will be used to develop further understanding of the discriminative power of this assay.

Therefore, it is not possible to give a definitive number of participants. **8** participants per group is deemed practical and realistic.

11.3 The Level of Statistical Significance

A conventional level of statistical significance of $p<0.05$ will be used.

11.4 Criteria for the Termination of the Trial.

The trial will be terminated when the number of participants is >5 per group and the nociceptin release profile has been determined for septic and healthy volunteers.

11.5 Procedure for Accounting for Missing, Unused, and Spurious Data.

Where data is missing and cannot be reconciled, the case to which it related will be excluded from the analysis. All data will be retained, and through the ethics process, permission is sought to reuse this data in subsequent studies. The significance of the nociceptin release profile in sepsis is unknown, and data collected via the CRF for the purposes of this study may give additional information in relation to this.

11.6 Procedures for Reporting any Deviation(s) from the Original Statistical Plan

Depending on the sensitivity of the test, or the characteristics of the fluorescence observed, it may be necessary to examine other measures. Where necessary, this will be reported in any written report, and will be submitted as a protocol amendment.

11.7 Inclusion in Analysis

All participants giving consent for venepuncture will be included in the data analysis. For those patients who lacked capacity to consent, and for whom assent from next of kin was gained, if permission was withdrawn when they regained capacity, samples and data would be destroyed.

12. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

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.

13. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures.

The University of Leicester as sponsor operate a risk based audit programme to which this study will be subject.

14. CODES OF PRACTICE AND REGULATIONS

14.1 Ethics

Describe ethical considerations relating to the trial. Include general and study specific ethics considerations.

14.2 Sponsor Standard Operating Procedures

All relevant Sponsor SOPs will be followed to ensure that this study complies with all relevant legislation and guidelines

14.3 Declaration of Helsinki

The Investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended October 2000, with additional footnotes added 2002 and 2004).

14.4 ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

14.5 Approvals

Once Sponsor authorisation has been confirmed, the protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), regulatory authorities (MHRA in the UK), and host institution(s) for written approval.

Once Sponsor authorisation has been confirmed, the Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

14.6 Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participants ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by trial staff and authorised personnel. The study will comply with the Data Protection Act which requires data to be anonymised as soon as it is practical to do so.

14.7 Other Ethical Considerations

This study will require the recruitment of patients admitted to the intensive care unit. These patients may lack capacity; due to the requirement for a blood sample on the first day of admission, assent may be obtained from the patient's friend or relative, their personal consultee. Should the patient later withdraw their consent if they regain capacity, their sample and any data will be destroyed.

Samples will be collected from staff and students of the Department of Cardiovascular sciences. All participants will be free to withdraw their consent at any point in the study.

15. DATA HANDLING AND RECORD KEEPING

All study data will be entered on a Microsoft Excel Spreadsheet. Data analysis will be carried out in the Statistical Package for the Social Sciences (SPSS).

The participants will be identified by a study specific participants number and/or code in any database. The name and any other identifying detail will NOT be included in any study data electronic file.

Data will be stored in pseudoanonymised form in the central filestore at the University of Leicester. The imaging data will be stored on the Research File Store at the University of Leicester.

The Case ID code will be stored securely on the sampling log in paper form in the ISF.

16. STUDY GOVERNANCE

16.1 Trial Steering Committee (TSC)

N/A

16.2 Data Safety Monitoring Committee (DSMC)

N/A

17. FINANCING AND INSURANCE

This study is supported by grants from the BBSRC (approved) which covered the development and validation of the bioassay, and the use of samples from healthy volunteers. A further grant is pending from the Royal College of Anaesthetists to cover the application of this bioassay in septic patients.

Research costs - University

Description	Lab consumables – Tissue culture (6 months)	Amount	£300
Description	Lab consumables – Fluorescence	Amount	£188.24
Description	Lab consumables – Cell separation	Amount	£1492.00
Description	Use of Advanced Imaging Facility and Microscope	Amount	£400
Description	Consumables – Blood sampling	Amount	£42.39

NHS Treatment Costs
As this is a non interventional study, there are no NHS treatment costs

NHS Support Costs
As this is a non interventional study, the blood sampling is to be performed by the investigator under an honorary contract with the University hospitals of Leicester, there are no NHS Support costs.

18. PUBLICATION POLICY

Data collected as part of this study will be disseminated and published in appropriate scientific fora, including meetings, conferences, in journal publications on- and offline. Images taken using microscopy will be published as part of this. However, no participant identifiable information will be published as part of dissemination.

19. REFERENCES

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20. APPENDIX A: STUDY FLOWCHART

21. APPENDIX B: SCHEDULE OF PROCEDURES