STUDY PROTOCOL

Investigating the effects of critical illness on the innate immune system

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TABLE OF CONTENTS

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INTRODUCTION

Infections acquired during a stay within an intensive care unit (ICU) are common, affecting approximately 20% of patients (1). Large-scale international studies have demonstrated that the risk of developing a hospital-acquired infection increases with the duration of ICU stay. Those individuals who develop an infection have twice the ICU mortality rate (25% vs .11%, $p<0.001$) and an overall longer duration of stay when compared to individuals who do not develop an infection (2, 3).

There are two reasons for this susceptibility to infection. Firstly, the use of necessary interventions such as mechanical ventilation and central venous lines increases the risk. Secondly, however there is mounting evidence that these patients have a dysfunction of their innate immune response. Dysfunction in several types of white blood cell within the ICU has previously been demonstrated and associated with an increased risk of developing an infection with the ICU. Complement mediated neutrophil dysfunction, a deactivation of monocytes (loss of HLA DR expression) and an increase in regulatory T cells that act to counter the inflammatory response have all be demonstrated in critically ill patients on the ICU. All were associated with an increased risk of developing a hospital-acquired infection (4).

Empirical antibiotics are often used in ICUs to ensure adequate coverage of critically ill patients and are often continued even after a specific pathogen is identified (5). Understandably critical care clinicians will often prioritise the health of their patient over the need to limit antibiotic use because of the rise of bacterial strains resistant to antibiotics (6). There is an urgent need to identify alternative treatments that are not antibiotics, but to do this the basic mechanisms underpinning the susceptibility of critically ill patients to infection need to be further understood.

Monocytes, one of the white blood cells involved in the innate immune response, are now divided into at least three subclasses that have different functional properties: classical, intermediate and non-classical. Classical are the most involved in bacterial killing, they display the most phagocytic activity, release the widest range of reactive oxygen species and the most cytokines. Intermediate monocytes have been shown to express the most HLA class II molecules whilst non-classical monocytes have been shown to 'patrol' the endothelium and may have a role in tissue repair (7-10).

Although classical monocytes are best suited to bacterial killing, previous work demonstrates that critically ill patients have a reduction in this subclass and instead have an expansion in the proportion of non-classical monocytes (11, 12). The cause of this change in monocyte subclass is not currently understood.

Human studies show that recombinant macrophage-colony stimulating factor (MCSF) can increase the proportion of non-classical monocytes in vivo. MCSF is present at high levels during sepsis and may explain the increase in the non-classical (13).

This study will investigate the mechanisms that lead to an increased susceptibility to infection in critically ill patients; with the aim of identifying therapeutic targets for improving their innate immune response and reducing the frequency of infections.

ORIGINAL HYPOTHESIS

We hypothesize that individuals who are critically ill have an increased susceptibility to infection due to a defect in their innate immunity. In particular the reduction in classical monocytes and concurrent predominance of circulating non-classical monocyte contributes to the impaired immune response. We believe that the increase in MCSF is the cause of the switch in monocyte subset and could potentially offer a novel therapeutic target.

STUDY DESIGN

Participant Enrolment and Selection

Individuals who have been admitted to the intensive care unit (ICU) and would be suitable for the study will be identified by their clinical care team or research nurse. The individual, or their next of kin, will be given a copy of the study's participant information sheet and consent form. If they wish to be included in the study they will be recruited following written informed consent.

Setting

The intensive care units (ICU) at South Tyneside and Sunderland NHS Foundation Trust (STSFT) and The Newcastle upon Tyne Hospitals NHS Foundation Trust (NUTH).

Inclusion criteria

- Expected to remain in the ICU for longer than 24 hours
- Expected to survive in the ICU for longer than 24 hours

- Provision of written informed consent (either from the participant or next of kin)
- Requires organ support (either inotropes, ventilator, non-invasive ventilator, haemofiltration)
- They or their relatives/legal representative will be provided written, informed consent
- Individuals with suspected or proven infection with Covid-19 may also be recruited providing the rest of the inclusion criteria are met

Exclusion criteria

- <16 years of age
- Pregnancy
- Known infection with human immunodeficiency virus
- Haematological malignancy
- Concurrent use of immunosuppressant medication other than corticosteroids (allowed up to prednisolone 10mg per day or equivalent)

Consent

Individuals eligible for the study will be identified by the clinical care team or research nurses and will be given verbal and written information regarding the study. All individuals who are approached will be given time to consider the information received prior to consent. All study subjects will be enrolled after giving signed informed consent. In those individuals where it is not possible to for them to give consent, the next of kin or appropriate legal representative will be approached. Wherever possible retrospective consent will be sought when an individual recovers. Consent will also include storage of blood for further testing, which will relate to this investigation.

Management of ineligible participants

For individuals found to be ineligible for the trial the reason for ineligibility or non-recruitment will be recorded. Only anonymised data will be entered on to the database and this will include gender, age, "ineligible" or "non-recruitment" and the associated reason.

Sampling

Study participation will involve two visits, each of less than 1 hour.

Study visit 1

This will occur following recruitment and include:

1. Data collection & preparation
    a. Details will be obtained from the subject through a medical interview and/or review of the medical notes; which will include but will not necessarily be limited to: age, sex, ethnicity, and cause for the ICU admission, co-morbidities and concurrent medications.
2. Blood sampling
    a. Blood will be sampled following initial recruitment. 20 mL of blood will be collected. The most likely risk is that blood sampling may contribute to the development of anaemia in the participant. This risk has been minimised by limiting the amount of blood taken to 20 mls. This is similar to what is taken in a routine clinical blood test.
    b. Blood sampling can be accompanied by discomfort or by vasovagal symptoms. To minimise these risks samples will be taken wherever possible from an indwelling line (an arterial line will be used preferentially if present, if not then a venous line). For the majority of this population the participant will be semi-recumbent in a hospital bed and sedated. In those few individuals who are not, samples will be taken with them in a bed or self-reclining chair. Participants who feel syncopal will be positioned supine and venepuncture will be discontinued.
    c. Plasma will be removed and frozen (at -80°C) for future testing
    d. The cell fraction will used to isolate white blood cells for future testing.
    e. Stored blood samples will be labelled with a unique anonymous identifier
3. Post procedure
    a. Subjects will be monitored by an ICU nurse

Study visit 2:

Where applicable this visit will occur within the next working day of discharge from the ICU between usual working hours. It will include:

1. Blood sampling – 20 ml of blood will be taken and processed as detailed above.
2. Post procedure - subjects will be monitored as following usual venepuncture with regular observations.

Adverse Events

Our research group has experience with taken blood samples from patients on the intensive care unit. Nevertheless, we feel that we must remain vigilant in detecting and recording any adverse events as a result of any procedures undertaken.

Whilst recognising that this current work is not a clinical trial, our groups' previous work (which included a clinical trial) benefited from classifying and monitoring adverse events in the manner described below, and we have elected to continue using this terminology in this research.

Definitions

An adverse event (AE) is any untoward medical occurrence in a study participant.

A serious adverse event (SAE) is any untoward medical occurrence in a study participant or effect that:

- results in death
- is life threatening (i.e. the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- results in persistent or significant disability or incapacity

If an adverse event is detected, a member of the research team will make an assessment of seriousness as defined by the above definitions. If the event is deemed to be serious (SAEs) a member of the research team will then consider if the event was:

- Related – that is it resulted from research procedures and/or
- Unexpected – that is a type of event that is not identified as an expected occurrence

Detecting and reporting AE and SAEs

All AEs and SAEs will be recorded from the time a participant consents to join the study and for up to an hour following venepuncture. Research nurses or members of clinical care team will inform the research team of any concerns that may constitute an AE or SAE. Information to be collected includes type of event, onset date, researcher assessment of implications, if any, for safety of participants and how these will be addressed, date of resolution as well as treatment required, investigations needed and outcome. All information will be recorded in the participants study file.

An AE/SAE may necessitate discontinuation of a given part of the study (but progression through the remainder of the study) or complete and immediate discontinuation of any further

participation. Such an event may include, but not be limited to, a vasovagal response to venepuncture. All participants will maintain the right to discontinue or completely withdraw from the study at any time for any reason, or without stating a reason. The reason and circumstances for premature discontinuation (where known) will be documented in the participant's study file.

If a SAE has occurred, the research team must report the information to the Newcastle upon Tyne Hospitals R&D within 24 hours. The SAE form must be completed as thoroughly as possible with all available details of the event, signed by the Investigator or designee. The SAE form should be transmitted by fax or by hand to the office.

NUTH R&D is responsible for reporting SAEs that are considered to be related and unexpected as described above to the Research Ethics Committee (REC) that approved the study (main REC) within 15 days of becoming aware of the event using the NRES Reporting of SAE Form. The Co-ordinator of the main REC should acknowledge receipt of related, unexpected safety report within 30 days.

Each AE should be clinically assessed for causality based on the information available, i.e. the relationship of the AE to the study should be established. All adverse events judged as having a reasonable suspected causal relationship to the study (i.e. definitely, probably or possibly related) are considered to be adverse reactions. If any doubt about the causality exists, the local Principal Investigator should consult the Chief Investigator. In the case of discrepant views on causality between the Principal Investigator and others, the main REC and other bodies will be informed of both points of view.

Data analysis

Statistical analysis

Measurements of biomarker blood levels and proportions of monocyte subclass will be expressed within 95% confidence limits. Comparisons will be made between the two dependent timepoints using a paired t test and statistical significance identified by a nominal *p value* of <0.05. Statistical analysis will be performed using standard University software. We intend to write a prospective statistical analysis plan ahead of embarking on experiments with statistical help from the university (Dr Joy Allen, Senior Research Associate-Methodologist, Newcastle University).

Power Calculations

This is an exploratory pilot analysis.

Outcome Variables

The primary outcome variable will be the measurement of monocyte subclasses during critical illness and on recovery. As well as the measurement of MCSF at both timepoints. Secondary outcomes will include the effects of critical illness on the function of immune cells and their interactions with the coagulation system and the endothelium (blood vessel lining).

Research team and project management

The proposed project will be conducted by Professor John Simpson, Dr Anthony Rostron, Dr Marie-Helene Ruchaud-Sparagano, Mr Jonathan Scott and Dr Kathryn Musgrave. Statistical work will be assisted by statisticians from the University.

EXPECTED VALUE OF RESULTS

To increase our understanding of the basic mechanisms under-pinning the susceptibility of critically ill individuals to infection.

To identify novel therapeutic targets to improve the innate immune response in the critically ill.

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