ISRCTN13450549 https://doi.org/10.1186/ISRCTN13450549

Investigating the inflammatory process of COVID-19

Submission date
30/12/2020

Recruitment status
No longer recruiting

Registration date
12/01/2021

Overall study status
Completed

Last Edited
24/02/2023

Condition category
Infections and Infestations

[_] Prospectively registered
[_] Protocol
[_] Statistical analysis plan
[X] Results
[_] Individual participant data

Plain English summary of protocol

Current plain English summary as of 14/02/2022:

Background and study aims
COVID-19 has caused widespread disruption to people's lives. Clinicians have, during the course of their encounters, recognised some key features of this disease. It is a viral respiratory illness, and as such, can present with catastrophic destruction of the lining (epithelium) of the lung, resulting in water in the lung. The disease can also precipitate the formation of clots in blood vessels, resulting in loss of blood supply to various organs, resulting in stroke, kidney failure, liver failure and cardiac arrest.
It is the hypothesis of this study that all these effects are due to the breakdown of the epithelium throughout the body. This would explain why water floods the lung in severe COVID-19, causing severe Acute Respiratory Distress Syndrome (ARDS), and why this disease predisposes to clot formation, since when the epithelium of blood vessels break down, clots naturally form.
Interleukin-18 (IL-18) has been found to play an important role in the breakdown of epithelium in some parts of the body, most notably, the gut. It is constitutively produced in all epithelial cells of the body and is the end-product of a process known as "inflammasome" activation, part of the body's normal, healthy response to fighting off an infection like COVID-19. When, however, IL-18 is produced in unregulated amounts, due to the failure of IL-18 Binding Protein (IL-18BP), which mops up excess IL-18 and regulates its production, then widespread epithelium breakdown throughout the body, is hypothesised to occur. This study therefore is investigating the levels of both IL-18 and its regulator, IL-18BP. The hope is that, if IL-18BP is not being produced in sufficient quantities, resulting in unregulated high "free" IL-18, perhaps this study could lay the groundwork for seeing an interventional study,
aimed at curbing "free" IL-18 by administration of IL-18BP in drug form, or other proteins, that may act in a similar way.

Who can participate?
Patients over the age of 18 years presenting with COVID-like symptoms

What does the study involve?
The study involves the sampling of excess blood from blood samples that are already taken for

clinical purposes from patients with and without COVID-19 disease. The study does not require participants to have further blood tests or additional interventions.

What are the possible benefits and risks of participating?
Since the study does not intervene in the patient's care, even to the extent of not taking further blood samples beyond what is already clinically necessary, there are no risks to participating in the study. The benefit is of course, in contributing to research. All patient data is pseudo-anonymised, and when published, will be presented in fully anonymised form.

Where is the study run from?
Redhill Hospital, Surrey and Sussex NHS Healthcare Trust (UK)

When is the study starting and how long is it expected to run for?
May 2020 to January 2021

Who is funding the study?
1. Cambridge University Hospitals Research Fund (UK)
2. Charitable donations from Mr Sulaiman Mubashir, Mr Sabahat Mubashir, Mr Elyas Nasser and Mrs Asma Rafi

Who is the main contact?
Dr Syed Muhammad Tahir Nasser
sash.isaacstudy@nhs.net

____
Previous plain English summary from 24/08/2021 to 14/02/2022:

Background and study aims
COVID-19 has caused widespread disruption to people's lives. Clinicians have, during the course of their encounters, recognised some key features of this disease. It is a viral respiratory illness, and as such, can present with catastrophic destruction of the lining (the epithelium) of the lung, resulting in water in the lung. The disease can also precipitate the formation of clots in blood vessels, resulting in loss of blood supply to various organs, resulting in stroke, kidney failure, liver failure and cardiac arrest.
It is the hypothesis of this study that all these effects are due to the breakdown of the epithelium throughout the body. This would explain why water floods the lung in severe COVID-19, causing severe Acute Respiratory Distress Syndrome (ARDS) and why this disease predisposes to clot formation, since when the epithelium of blood vessels break down, clots naturally form.
Interleukin-18 (IL-18) has been found to play an important role in the breakdown of epithelium in some parts of the body, most notably, the gut. It is constitutively produced in all epithelial cells of the body and is the end-product of a process known as "inflammasome" activation, part of the body's normal, healthy response to fighting off an infection like COVID-19. When, however, IL-18 is produced in unregulated amounts, due to the failure of IL-18 Binding Protein (IL-18BP), which mops up excess IL-18 and regulates its production, then widespread epithelium breakdown throughout the body, is hypothesised to occur.
This study therefore is investigating the levels of both IL-18 and its regulator, IL-18BP, in addition to Granzyme B, to see whether this hypothesis is borne out in the data. The hope is that, if IL-18BP is not being produced, resulting in unregulated high "free" IL-18, perhaps this study could lay the groundwork for seeing an interventional study, aimed at curbing "free" IL-18 by administering IL-18BP in drug form, or other proteins that may act in a similar way.

Who can participate?
Patients over the age of 18 years presenting with COVID-like symptoms

What does the study involve?
The study involves the sampling of excess blood from blood samples that are already taken for clinical purposes from patients with and without COVID-19 disease. The study does not require patient participants to have further blood tests or additional interventions.

What are the possible benefits and risks of participating?
Since the study does not intervene in the patient's care, even to the extent of not taking further blood samples beyond what is already clinically necessary, there are no risks to participating in the study. The benefit is of course, in contributing to research. All patient data is pseudo-anonymised, and when published, will be presented in fully anonymised form.

Where is the study run from?
Redhill Hospital, Surrey and Sussex NHS Healthcare Trust (UK)

When is the study starting and how long is it expected to run for?
May 2020 to January 2021

Who is funding the study?
Self-funded by CI Syed Nasser;
Cambridge Addenbrooke's Research Fund of Dr Shuaib Nasser;
Private funding by Mr Sulaiman Mubashir; Mr Sabahat Mubashir

Who is the main contact?
Dr Syed Muhammad Tahir Nasser
sash.isaacstudy@nhs.net

Previous plain English summary:

Background and Study Aims:
COVID-19 has caused widespread disruption to people's lives. Clinicians have, during the course of their encounters, recognised some key features of this disease. It is a viral respiratory illness, and as such, can present with catastrophic destruction of the lining (the epithelium) of the lung, resulting in water in the lung. The disease can also precipitate the formation of clots in blood vessels, resulting in loss of blood supply to various organs, resulting in stroke, kidney failure, liver failure and cardiac arrest.

It is the hypothesis of this study that all these effects are due to the breakdown of what is known as the "epithelium" throughout the body. This would explain why water floods the lung in severe COVID-19, causing severe Acute Respiratory Distress Syndrome (ARDS) and why this disease predisposes to clot formation, since when the epithelium of blood vessels break down, clots naturally form.

Interleukin-18 (IL-18) plays a fundamental role in the breakdown of epithelium in the body. It is the end-product of a process known as "inflammasome" activation, and is part of the body's normal, healthy response to fighting off an infection like COVID-19. When, however, IL-18 is

produced in unregulated amounts, due to the failure of IL-18 Binding Protein (IL-18BP), which mops up excess IL-18 and regulates its production, then widespread epithelium breakdown throughout the body, is the consequence.

This study therefore is investigating the levels of both IL-18 and its regulator, IL-18BP, in addition to Granzyme B, to see whether this hypothesis is borne out in the data. The hope is that, if IL-18BP is not being produced, resulting in unregulated high "free" IL-18, perhaps this study could lay the groundwork for seeing an interventional study, aimed at curbing "free" IL-18 by administering of IL-18BP in drug form, or other proteins that may act in a similar way.

Who can participate?
Patients over the age of 18 presenting with COVID-like symptoms.

What does the study involve?
The study involves the sampling of excess blood from blood samples that are already taken for clinical purposes from patients with and without COVID-19 disease. The study does not require patient participants to have further blood tests or additional interventions.

What are the possible benefits and risks of participating?
Since the study does not intervene in the patient's care, even to the extent of not taking further blood samples beyond what is already clinically necessary, there are no risks to participating in the study. The benefit is of course, in contributing to research. All patient data is pseudo-anonymised, and when published, will be presented in fully anonymised form.

Where is the study run from?
Redhill Hospital, Surrey and Sussex NHS Healthcare Trust (UK)

When is the study starting and how long is it expected to run for?
May 2020 to January 2021

Who is funding the study?
True Intelligence Limited (UK)

Who is the main contact?
Dr Syed Muhammad Tahir Nasser
sash.isaacstudy@nhs.net

Contact information

Type(s)
Scientific

Contact name
Dr Syed Muhammad Tahir Nasser

ORCID ID
https://orcid.org/0000-0002-4700-862X

Contact details
East Surrey Hospital
Canada Avenue

Redhill
United Kingdom
RH1 5RH
+44 (0)7875603852
syed.nasser1@nhs.net

Type(s)
Public

Contact name
Dr Syed Muhammad Tahir Nasser

ORCID ID
https://orcid.org/0000-0002-4700-862X

Contact details
East Surrey Hospital
Canada Avenue
Redhill
United Kingdom
RH1 5RH
+44 (0)7875603852
syed.nasser1@nhs.net

Additional identifiers

Clinical Trials Information System (CTIS)
Nil known

Integrated Research Application System (IRAS)
285842

ClinicalTrials.gov (NCT)
Nil known

Protocol serial number
IRAS 285842

Study information

Scientific Title
Levels of total IL-18, IL-18 binding protein and calculated free IL-18 according to disease severity and mortality in COVID-19

Acronym
ISAAC

Study objectives
Current study hypothesis as of 14/02/2022:
Interleukin-18 negative feedback dysfunction underpins COVID-19 severity and mortality.

Previous study hypothesis:
Levels of free Interleukin-18 (IL-18) increase, while IL-18 Binding Protein (IL-18BP) and Granzyme B decrease, with increasing severity of COVID-19 disease.

It is the hypothesis of this study that the widespread features of COVID-19 disease, such as acute respiratory distress syndrome (ARDS), the development of diarrhoea, and the formation of widespread clots, are due to a multi-system breakdown of epithelium throughout the body.

Interleukin-18 (IL-18) plays a fundamental role in the breakdown of epithelium in the body. It is the end-product of a process known as "inflammasome" activation, and is part of the body's normal, healthy response to fighting off an infection like COVID-19. When, however, IL-18 is produced in unregulated amounts, due to the failure of IL-18 Binding Protein (IL-18BP), which mops up excess IL-18 and regulates its production, then widespread epithelium breakdown throughout the body, is the consequence.

This study therefore aims to investigate the levels of both IL-18 and its regulator, IL-18BP, in addition to Granzyme B, to see whether this hypothesis is borne out in the data. The hope is that, if IL-18BP is not being produced, resulting in unregulated high "free" IL-18, perhaps this study could lay the groundwork for seeing an interventional study, aimed at curbing "free" IL-18 by administering of IL-18BP in drug form, or other proteins that may act in a similar way.

Ethics approval required
Old ethics approval format

Ethics approval(s)
Approved 16/09/2020, Berkshire Research and Ethics Committee (The Old Chapel, Royal Standard Place, Nottingham, NG1 6FS, UK; +44 (0)207 104 8224; berkshireb.rec@hra.nhs.uk), ref: 20/SC/0316

Study design
Prospective longitudinal cohort study

Primary study design
Observational

Study type(s)
Screening

Health condition(s) or problem(s) studied
Investigation of inflammatory process in patients with COVID-19

Interventions
Current interventions as of 14/02/2022:

The approach is of a cohort study, to accumulate data on total IL-18 and IL-18BP levels in hospitalised COVID-19 patients.

Cohort Analyses: By comparing IL-18 related parameters between COVID-19 positive patients and correlating it to primary (PaO2/FiO2 ratio) and secondary (mortality, oxygenation parameters/clinical features/disease course/biochemical parameters, neutrophil to lymphocyte

ratio (NLR) outcomes) we can see how Free-IL-18 levels correlate with disease severity at different timepoints throughout the disease course. High free IL-18 levels are seen in a condition known as macrophage activation syndrome (MAS), a condition with similarities to severe COVID-19, partly due to failure of release of IL-18BP.

The questions the study is asking, are as follows:
Q1: What is the profile of Total IL18, IL-18bp and Free IL-18 in COVID-19 patients?
Q2: What is the relationship between Free IL-18 levels and severity of COVID-19? (Primary Analysis)
Q3: What is the relationship between Free IL-18 levels and 60-day mortality in COVID-19?

(Secondary Analysis)
Patients enrolled on this study will have excess blood taken from clinically-requested samples, stored and analysed, for inflammasome-related parameters (Total IL-18 and IL-18 Binding Protein (BP)). Primary and secondary outcome measures related in time to each blood sampling event will then be correlated with the measured levels above, so as to build a picture of clinical disease progression, in tandem with changing levels of inflammasome parameters. Free IL-18 will be calculated by the standard law of mass action calculation, with an updated dissociation constant of 0.05 nM.

———

Previous interventions from 24/08/2021 to 14/02/2022:

The approach is of a case-control and a cohort study, to accumulate data on Total IL-18, IL-18BP, IL-18/BP-Complex levels and Granzyme B levels (as a marker for NK cell and CD8 T-lymphocyte cytotoxicity) in a variety of different patients with and without COVID-19.

Case-Control: By comparing COVID19 positive patients to healthy controls, we get information about how COVID-19 infection affects IL-18 related parameters generally as compared to those without COVID-19.

Cohort Analyses: By comparing IL-18 related parameters between COVID-19 positive patients and correlating it to primary (PaO2/FiO2 ratio) and secondary (mortality, oxygenation parameters /clinical features/disease course/biochemical parameters, neutrophil to lymphocyte ratio (NLR) outcomes) we can see how Free-IL-18 levels correlate with disease severity at different time points throughout the disease course. High free IL-18 levels are seen in a condition known as
macrophage activation syndrome (MAS), a condition with similarities to severe COVID-19, partly due to failure of release of IL-18BP. IL-18BP release is dependent on a pathway related to IFNgamma release from Natural Killer cells (innate immune system cells). So as to investigate why IL18BP may have impaired release in COVID-19, resulting in a high Free IL-18, the role of IFNgamma and Granzyme B (as a marker of NK cell function) will also be investigated.

The questions the study is asking, are as follows:
Q1: Is there an association between Free IL-18 and COVID-19 disease at time of presentation to hospital? (Case-Control)
Q2A: What is the relationship between free IL-18 levels and severity of COVID-19? (Cohort Study 1)
Q2B: How do free IL-18 levels change with change in disease severity? (Cohort Study 2)
Q3: What is the relationship between free IL-18 levels and activity of CD8+ T cells and NK cells, as measured by Granzyme B levels and IFN-gamma (Cohort Study 3)

Patients enrolled to this study will have excess blood taken from clinically-requested samples, stored and analysed, for inflammasome-related parameters (Total IL-18, IL-18 Binding Protein (BP) and IL-18/BP complex levels), IFN-gamma and Granzyme B levels. Primary and secondary outcome measures related in time to each blood sampling event will then be correlated with the measured levels above, so as to build a picture of clinical disease progression, in tandem with changing levels of inflammasome parameters.

_____

Previous interventions:

The approach is of a case-control and a cohort study, to accumulate data on Total IL-18, IL-18BP, IL-18/BP-Complex levels and Granzyme B levels (as a marker for NK cell and CD8 T-lymphocyte cytotoxicity) in a variety of different patients with and without COVID-19.

Case-Control: By comparing COVID19 positive and negative patients presenting with similar symptoms to hospital, we get information about how COVID-19 infection affects IL-18 related parameters generally as compared to other conditions.

Cohort Analyses: By comparing IL-18 related parameters between COVID-19 positive patients and correlating it to primary (degree of lymphopaenia) and secondary (oxygenation parameters /clinical features/disease course/biochemical parameters, neutrophil to lymphocyte ratio (NLR) outcomes, we can see whether IL-18 and Granzyme B levels correlate with disease severity. Given that high free IL-18 levels are seen in patients due to decreased IL-18BP levels in a condition known as macrophage activation syndrome (MAS), a condition with similarities to severe COVID-19, Granzyme B analysis may be useful in understanding the cause of high free IL-18.

The questions the study is asking, are as follows:
Q1: Is there an association between IL-18 parameters and COVID-19 diagnosis, at first presentation? (Case-Control)
Q2A: What is the relationship between free IL-18 levels and severity of COVID-19? (Cohort Study 1)
Q2B: How do free IL-18 levels change with change in disease severity? (Cohort Study 2)
Q3: What is the relationship between free IL-18 levels and activity of CD8+ T cells and NK cells, as measured by Granzyme B levels (Cohort Study 3)

Patients enrolled to this study have blood excess to clinical requirements, from samples taken solely for clinical purposes, stored and analysed for Inflammasome related parameters (Total IL-18, IL-18 Binding Protein (BP) and IL-18/BP complex levels) and Granzyme B levels. Primary and secondary outcome measures related in time to each blood sampling event are then correlated with the measured levels above, so as to build a picture of clinical disease progression, in tandem with changing levels of inflammasome parameters.

Intervention Type
Other

Primary outcome(s)
Current primary outcome measure as of 14/02/2022:

No case-control analysis (due to loss of obtained controls due to equipment malfunction)

Cohort Primary Outcome Measure: PaO2/FiO2 ratio (PFR) obtainable through blood sampling analysis, concurrent to highest Free IL-18 value

_____

Previous primary outcome measure from 24/08/2021 to 14/02/2022:

Case-Control:
High free IL-18 measured at baseline (admission), obtainable through blood sampling analysis

Cohort Study 1:
Lowest PaO2/FiO2 ratio (PFR) obtainable through blood sampling analysis

Cohort Study 2:
PFR measured from baseline to the lowest level during the inpatient stay using patient records

Cohort Study 3:
Granzyme B and IFN-gamma levels measured by blood sampling throughout inpatient stay

_____

Previous primary outcome measure:

Case-Control:
High free IL-18 measured at baseline (admission), obtainable through blood sampling analysis

Cohort Study 1
Lowest lymphopaenia level during attendance at hospital, obtainable through blood sampling analysis

Cohort Study 2:
Lymphopaenia percentage measured from baseline to the lowest level during the inpatient stay using patient records

Cohort Study 3:
Granzyme B levels measured by blood sampling when attending tertiary care centre for routine appointments

Key secondary outcome(s))
Current secondary outcome measure as of 14/02/2022:

60-day mortality with hypoxaemic respiratory failure

_____

Previous secondary outcome measures:

Case-Control
Measured at baseline using blood sample analysis:
1. High total IL-18
2. Low IL-18/BP Complex

3. Low IL-18 Binding Protein
4. Low Granzyme B levels

Cohort Study 1
Measured at the end of hospital stay using patient records:
1. Hospital length of stay
2. ITU admission
3. Mortality
4. Highest CRP level
5. Highest ferritin level
6. Oxygenation parameters throughout admission
7. Neutrophil to lymphocyte ratio (NLR)

Cohort Study 2
Measured at the end of hospital stay using patient records:
1. Hospital length of stay
2. ITU admission
3. Mortality
4. Highest CRP level
5. Highest ferritin level
6. Oxygenation parameters
7. Neutrophil to lymphocyte ratio (NLR)

Cohort Study 3
There are no secondary outcome measures

Completion date
14/01/2021

Eligibility

Key inclusion criteria
Added 14/02/2022: Note: no case-control portion of study as of 10/12/2021

Criteria for Case/Control portion of study:
1. Above 18 years of age
2. Tested for COVID-19, positive or negative

Criteria for Cohort portions of study:
1. Above 18 years of age
2. COVID-19 positive swab test

Participant type(s)
Patient

Healthy volunteers allowed
No

Age group
Adult

Lower age limit
18 years

Sex
All

Total final enrolment
272

Key exclusion criteria
Added 14/02/2022: Note: no case-control portion of study as of 10/12/2021

Case-Control and Cohort:
1. Does not meet inclusion criteria

Cohort study only:
1. Not admitted to hospital after presentation to A&E (discharged without admission)

Date of first enrolment
08/11/2020

Date of final enrolment
14/01/2021

Locations

Countries of recruitment
United Kingdom

England

Study participating centre
Redhill Hospital
Surrey and Sussex Healthcare Trust
Canada Ave
Redhill
United Kingdom
RH1 5RH

Sponsor information

Organisation
Surrey and Sussex Healthcare NHS Trust

ROR

https://ror.org/0480vrj36

Funder(s)

Funder type
University/education

Funder Name
Cambridge University Hospitals Research Fund

Funder Name
Charitable donations from Mr Sulaiman Mubashir, Mr Sabahat Mubashir, Mr Elyas Nasser and Mrs Asma Rafi

Results and Publications

Individual participant data (IPD) sharing plan
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

IPD sharing plan summary
Available on request

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Results article		24/02/2023	24/02/2023	Yes	No
HRA research summary			28/06/2023	No	No
Participant information sheet	version v2	19/11/2020	04/02/2021	No	Yes