

# Investigating the inflammatory process of COVID-19

<b>Submission date</b> 30/12/2020	<b>Recruitment status</b> No longer recruiting	<input type="checkbox"/> Prospectively registered <input type="checkbox"/> Protocol
<b>Registration date</b> 12/01/2021	<b>Overall study status</b> Completed	<input type="checkbox"/> Statistical analysis plan <input checked="" type="checkbox"/> Results
<b>Last Edited</b> 24/02/2023	<b>Condition category</b> Infections and Infestations	<input type="checkbox"/> 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

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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.

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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

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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.

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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 (PaO<sub>2</sub>/FiO<sub>2</sub> 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.

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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 (PaO<sub>2</sub>/FiO<sub>2</sub> 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 IFN $\gamma$  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 IFN $\gamma$  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.

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#### 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: PaO<sub>2</sub>/FiO<sub>2</sub> ratio (PFR) obtainable through blood sampling analysis, concurrent to highest Free IL-18 value

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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 PaO<sub>2</sub>/FiO<sub>2</sub> 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

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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

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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

## 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?
<a href="#">Results article</a>		24/02/2023	24/02/2023	Yes	No
<a href="#">HRA research summary</a>			28/06/2023	No	No
<a href="#">Participant information sheet</a>	version v2	19/11/2020	04/02/2021	No	Yes
<a href="#">Participant information sheet</a>	Participant information sheet	11/11/2025	11/11/2025	No	Yes