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**Inflammation in COVID-19 – Exploration of Critical Aspects of Pathogenesis (ICECAP)**

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**Background:**

The current pandemic caused by SARS-CoV-2 is causing significant morbidity and mortality. Patients with severe coronavirus disease 2019 (COVID-19) develop acute respiratory distress syndrome (ARDS) leading to hypoxaemic respiratory failure that can require prolonged periods of mechanical ventilation.1 As this is a new disease the pathogenesis of lung injury is not understood which therefore limits the ability to identify targeted therapies. The key question is whether viral replication and cytotoxicity or the host immune response is primarily responsible for ARDS. Initial data and clinical experience implicate hyper-inflammation in the pathogenesis of at least a sub-set of severe COVID-19, with emerging evidence of macrophage activation syndrome and accumulation of CD8+ T cells2,3,4. There is an urgent need to investigate the pathogenesis of SARS-Cov-2 related ARDS by studying lung injury using lung tissue samples from patients who have died from COVID-19. Delineating the role of hyper-inflammation and viral load in the lung will directly inform clinical management and identify candidate targets for therapeutic interventions.

The related virus SARS-CoV-1 was found to replicate and cause pathology outwith the lungs, including in the intestine, kidneys and brain.4 The extent of extra-pulmonary viral replication and pathology is not well understood for SARS-CoV-2 but there is clinical evidence of myocardial damage indicating the heart may also be a target organ, consistent with the tissue distribution of the viral entry receptor ACE2.2 Understanding extra-pulmonary organ involvement is also an unmet need in understanding COVID-19 pathogenesis.

To date there is very limited published postmortem or lung tissue data describing histological or molecular changes in COVID-19 infection5,6. Due to this urgent, unmet clinical need we are co-ordinating a multi-disciplinary project drawing on local and national expertise.

**Hypothesis:** SARS-Cov-2 related ARDS is driven by a complex interplay between viral cytotoxicity and subsequent hyperinflammation and cytokine storm. Delineating these interactions will have direct, immediate implications for clinical management of COVID-19 by determining relevant, targeted therapeutic approaches.

**Aims:** In order to address this hypothesis, we will:

1. Conduct detailed post-mortem examinations on patients who have died from COVID-19 or non-COVID related illnesses.
2. Determine the range of tissues where viral replication/infection occurs in COVID-19 and map the spatial distribution of SARS-CoV-2 infection at a cellular and subcellular level within the lung and other organs.
3. Perform detailed tissue analyses to understand pathogenesis and biology of COVID-19, including comprehensive immunophenotyping and candidate target exploration for potential therapeutic interventions.
4. Establish a tissue bank of samples from deceased patients with COVID-19 that is available to local, national and international researchers for ethically-approved research.
5. Correlate biological features with detailed pre-mortem clinical, radiological and biochemical findings.

**Methods:**

1. *Post-mortem examinations*

Central to addressing our hypotheses has been esablishing a “warm” post-mortem service, to allow detailed tissue analysis in severe COVID-19 cases. We will aim to start post-mortem examinations 4-12 hours after death to ensure that key viral and cellular information that is vital to answering the most important clinical questions is not lost by tissue degradation and breakdown.

Suitable patients (PCR confirmed SARS-Cov-2 infection) with evidence of lower respiratory tract disease (pneumonia, pneumonitis or acute respiratory distress syndrome) or myocarditis (diagnosed biochemically using cardiac enzymes) will be referred to the research team by clinical teams within NHS Lothian.

Following death, a research nurse with significant experience in requesting post-mortem/organ donation authorisation will discuss the possibility of a hospital post-mortem with the nearest relative by telephone. If verbal agreement is given the request form will be sent electronically for the relative to sign and return electronically (approved by Caldicott Guardian). This process will be conducted following approved, published protocols established by the Edinburgh Brain Bank (REC reference 16/ES/0084). Control samples will be obtained from post-mortem examinations performed on patients who died of causes other than COVID-19 (following a similar authorisation process).

Post-mortem examinations will be conducted in the Category 3 High Risk Post-Mortem facility in the Royal Infirmary of Edinburgh by a Consultant Histopathologist. Representative tissues and fluids sampled from multiple organs namely lung, heart, liver, kidney, spleen, pancreas, adrenal, thyroid, stomach, small and large bowel, bone marrow and appropriate lymph nodes. All procedures will be conducted in compliance with NHS Scotland Standards7 and RCPath guidance on COVID-19 post-mortem examination8,9. No whole organs will be retained.

1. *Tissue Bank*

Patient tissue samples obtained at post-mortem will be fixed in formalin while other similar samples will be treated with an appropriate reagent (e.g. TRIzol) that inactivates virus10,11. All such tissue collected under the ICECAP study protocol will be stored in the ICECAP storage facility, Queen’s Medical Research Institute, Little France Edinburgh at room temperature or in dedicated -80oC freezers. This is a secure location requiring authorised card access. This facility also falls under NHS Lothian Tissue Governance Unit, in line with the Accreditation scheme for the collection and storage of NHS tissue in Scotland and is registered with the NHS Lothian SAHSC Bioresource and Tissue Governance Unit.

Snap frozen, potentially infected tissue obtained at post-mortem will be stored within in a BSL3 -80oC freezer in the University of St Andrews in accordance with biosafety regulations. Again, these are located within a secure location requiring authorised card access. Samples from the tissue bank will be available to local, national and international researchers, for ethically-approved research where appropriate, as determined by our data and materials access committee.

1. *Tissue analyses*

To address the urgent clinical questions outlined the following experimental approach will be taken to generate rapid, clinically relevant data:

1. Quantification of viral and host RNA as well as viral replication using viral culture, qPCR and RNA sequencing in lung, heart, liver, kidney, spleen, pancreas and gastrointestinal tract.
2. Analysis of parenchymal and immune cell phenotypes principally in the lung using techniques including flow cytometry, proteomics and single cell RNA sequencing and analysis (RNAScope). This will characterise inflammatory cell populations and phenotypes (including lymphocyte subsets, evidence of neutrophilic inflammation and macrophage activation syndrome) and determine the cellular replication niche(s) of SARS-CoV-2 in the context of severe disease.
3. Histological analysis of lung and the range of tissues described above will combine morphological assessment with multiplex immunofluorescence and integration of single cell phenotypic data to define the mechanisms of multi-organ injury in COVID-19.

The experimental protocols described are all already established in the lead investigators’ and collaborators’ laboratories with skilled scientific support to rapidly deliver on these objectives. Dissemination of research findings will be prioritised through appropriate clinical networks and publications. All published data generated will be accessible in online and open access formats, including deposition in the University of Edinburgh PURE archives.

**Ethical approval**

Ethical approval has been granted as an amendment to a current post-mortem tissue bank (MRC funded Edinburgh Brain Bank: PI Prof Colin Smith; REC 16/ES/0084 – amendment AM08 02/04/2020).

**Key risks**

1. Our key concern is ensuring that all the interactions with bereaved relatives and the process of requesting consent for a hospital postmortem is managed sensitively and appropriately. Drawing on the expertise of the Edinburgh Brain Bank as well as discussion with clinical colleagues in ICU and respiratory medicine in the development of the protocol will be central to this process. All protocols are being developed in accordance with the Human Tissue Act (Scotland) 2006 as well as NHS Scotland Standards on Hospital Postmortems7. Written information will be provided prior to the consent process being completed and a contact phone number will be provided to all relatives.
2. Principal risks within this project lie with the handling of bodies and tissues infected with SARS-Cov-2. All autopsies will be conducted in accordance with the Royal College of Pathologists guidance8.
3. Appropriate risk assessments and Safe Systems of work are currently pending approval for tissue handling within the Royal Infirmary Edinburgh and University of Edinburgh. All fresh, unfixed tissue will be handled within Category 3 laboratories.
4. Following processing in a Category 3 laboratory fresh samples will be put into appropriate fixative thereby inactivating SARS-Cov-2 and allowing analysis within Category 2 laboratory areas.
5. Due to the large volume of clinical data obtained there will be strict protocols adhered to with regards to de-identification of personal data and will mirror previously approved research methodology and data storage (REC reference 20/SS/0028).

**Potential of research for patient care**

As COVID-19 is a new disease the pathogenesis of lung and extra-pulmonary injury and ongoing viral replication is not understood. This therefore limits the ability to identify targeted patient therapies – specifically it is not known if strategies that target viral replication or those that target host hyperinflammation will be of most benefit pursuing. Our approaches will describe the pathogenesis of severe COVID-19 and directly inform clinical management and identify candidate targets for future therapeutic interventions.

**Funding**

At present this project is supported by pump prime funding from local funding bodies. Plans are currently being put in place for separate grant applications to fund tissue collection, the tissue bank and associated research.

**Study teams & key collaborations:**

Post-mortem pathology:

Dr David Dorward, Prof Colin Smith, Dr Chris Bellamy, Dr Ralph BouHaider, Dr Lorna MacIntosh, Dr Sam Pattle

Specialist Reporting Pathology:

Prof William Wallace & Dr David Dorward (Thoracic Pathology), Dr David Worrall (Gastrointestinal Pathology), Dr Wael Al-Qsous (Haematopathology), Dr Chris Bellamy (Liver & Kidney Pathology), Dr Mary Sheppard (Cardiac Pathology at St George’s Medical School, London), Dr David Proller (Endocrine Pathology at Queen Alexandra Hospital, Portsmouth)

Inflammation Biology:

Dr Chris Lucas, Dr David Dorward, Dr Clark Russell, Prof David Harrison, Dr Jill Stephens, Dr Asta Valanciute, Dr Jenna Gregory, Prof Adriano Rossi

Virology:

Dr Andrew Davidson & Dr David Matthews (University of Bristol)

Consent & liaison with relatives:

Tracey Millar, Chris Lerpiniere, Irene Young

COVID-19 Tissue Bank:

Naomi Gachanja, Emma Scholefield supported by Chris-Anne McKenzie (Edinburgh Brain Bank, UoE)

Local Management and Logistical Support:

Dr Ingo Johannessen (Director of Laboratory Medicine, NHS Lothian), Dr Marie Mathers (Clinical Lead, Pathology, NHS Lothian), Mike Gray (Laboratory Services General Manager, NHS Lothian), Amanda Malham (Lead Biomedical Scientist, NHS Lothian), Alison Anderson (Mortuary Services Manager, RIE), Dr Beth Henderson (UoE)

Data Management committee for ICECAP COVID-19 Tissue bank

Prof. Colin Smith; Dr Gareth Bryson, Lead Consultant Pathologist, Glasgow & Clyde NHS; Dr Ralph Bouhaider, Lothian NHS Crown Pathologist; Dr. Nik Hirani, NHS Lothian; Dr Richard Malham, Research Governance Officer University of St Andrews; Frances Rae (Tissue Governance Manager; Lothian NRS Bioresource)

International Severe Acute Respiratory Infection Consortium – lead by Dr Kenneth Baillie

*NHS Lothian/University of Edinburgh unless stated*

**References:**

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