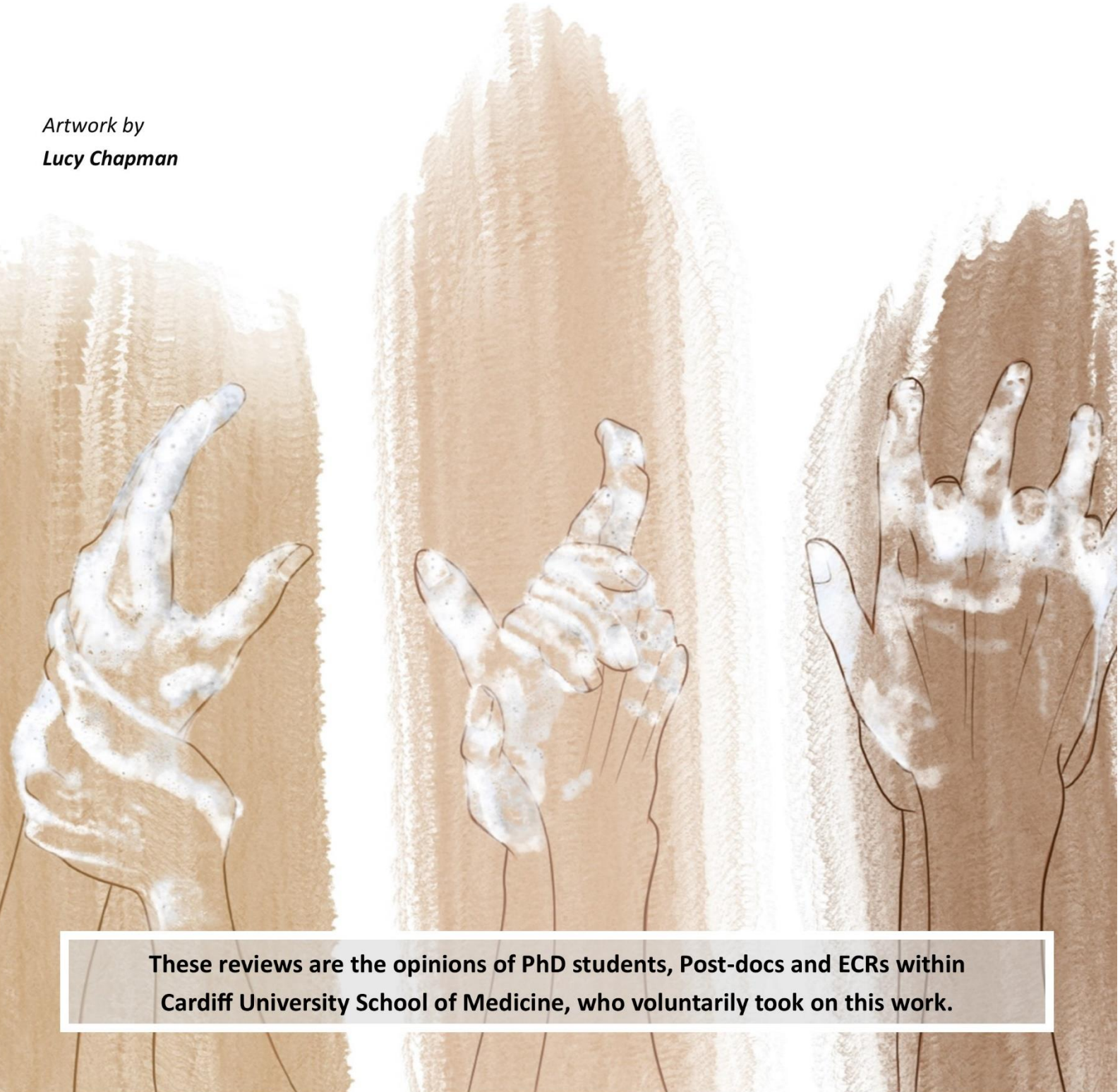
COVID-19 Community Journal Club No. 8

June 4th, 2020

School of Medicine

Artwork by
Lucy Chapman



These reviews are the opinions of PhD students, Post-docs and ECRs within Cardiff University School of Medicine, who voluntarily took on this work.

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Please direct any comments or queries to Awen at gallimoream@cardiff.ac.uk

Sources of Further Information

Statistical Reviews for Clinical Trials Testing Treatments for COVID-19
Conducted by the MRC-NIHR Trials Methodology Partnership
https://zenodo.org/communities/covid-19-tx-rct-stats-review/search?page=1&size=20

Coronavirus webinars from The British Society for Immunology

Controversy in immunity to COVID-19: do we want an immune response or not?
This session will be presented by former BSI President Professor Peter Openshaw and Dr Ryan Thwaites
Wednesday 27 May from 12:30 - 13:15 BST – *Recording available*
Learn more and register

Antiviral responses in COVID-19
This session will be presented by Professor Paul Klenerman
Wednesday 3 June from 12:30 - 13:15 BST – *Recording available*
Learn more and register

What can genomics tell us about SARS-CoV-2?
This session will be presented by Professor Judith Breuer
Wednesday 10 June from 15:30 - 16:15 BST
Learn more and register

To register or to access recording of previous webinars go to the following webpage.
https://www.immunology.org/coronavirus/connect-coronavirus-webinars

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News and Views

Adverse Consequences of Rushing a SARS-CoV-2 Vaccine

Trogen, B. *et al*. 2020. *JAMA*

Link: https://doi.org/10.1001/jama.2020.8917

The authors sound a warning against expediting a SARS-CoV-2 vaccine that could increase public mistrust in scientific researchers, clinicians and governments to the detriment of all future vaccination programs. In a climate of growing incidence of vaccine hesitancy and vaccine refusal (one of the top 10 global health threats listed by WHO in 2019[1]), it is important that convincing evidence of the safety and efficacy of new SARS-CoV-2 vaccines is both obtained and communicated to the public. There is compelling precedence (1955 Salk polio vaccine[2], 1976 swine flu program[3]) for significant harm caused by vaccines rushed into public use without adequate scientific rigor and scrupulous regulation of manufacture. Such errors, combined with fervid misinformation about vaccine side effects (such as autism), result in widespread public mistrust and low vaccine uptake, allowing resurgence of preventable diseases such as measles. This indirect impact is as critical as the direct and potentially life-threatening consequences of an ineffective or unsafe SARS-CoV-2 vaccine. The authors do suggest that with the current high levels of regulatory oversight of vaccine production (in the US), technological advances that support rapid communication of adverse effects and increasing genetic and immunological understanding, the 7 SARS-CoV-2 vaccines already in clinical trial have good prospects. However, it remains important to ensure scientific rigor and appropriate safeguards are not suspended with the growing pressure to roll out vaccination programs within months rather than years.

1. https://www.who.int/news-room/feature-stories/ten-threats-to-global-health-in-2019
2. Offit P. The Cutter Incident: How America's First Polio Vaccine Led to the Growing Vaccine Crisis. Yale University Press; 2005
3. Sencer DJ, Millar JD. Reflections on the 1976 swine flu vaccination program. Emerg Infect Dis. 2006;12(1):29-33. doi:10.3201/eid1201.051007

Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia

McGonagle, D. *et al*. 2020. *The Lancet Rheumatology*

Link: https://doi.org/10.1016/S2665-9913(20)30121-1

McGonagle *et al*., propose a model for the pathophysiology of diffuse pulmonary intravascular coagulopathy, a term coined by the authors for describing the lung pathology associated with COVID-19 pneumonia. Distinct from disseminated intravascular coagulation related to other pathologies, this paper describes the link between immunothrombosis and underlying risk factors associated with COVID-19 disease.

SARS-CoV-2 infection results in slowly evolving diffuse lung inflammation also involving the adjacent pulmonary vasculature. Immune activation similar to macrophage activation syndrome (MAS) causes microvascular thrombosis (immunothrombosis) and haemorrhage, pulmonary infarction, extensive alveolar inflammation deep in the pulmonary bed, interstitial inflammation and pulmonary hypertension induced by pulmonary intravascular coagulopathy. Pulmonary intravascular coagulopathy leads to right ventricular stress and the risk of a cardiac event, unmasking subclinical cardiovascular disease, especially in older patients. Immunothrombosis best explains the adverse effects of male sex, hypertension, obesity and diabetes on the prognosis of COVID-19 patients.

The tropism of SARS-CoV-2 for the angiotensin-converting enzyme 2 (ACE2) receptor on type II pneumocytes in the lung and adjacent pulmonary network and the lack of SARS-CoV-2 virus detected in the blood in early disease, supports the theory that lung pathology is centred around pneumocytes and adjacent tissue pathology, rather than systemic viral infection. Downregulation of the ACE2 receptor in lung tissue using either knock-out mouse models or treatment of mice with SARS spike protein (thereby internalising the receptor), increases inflammation and may cause inflammation in cardiac cells.

Increased circulating levels of D-dimer associated with pulmonary bed thrombosis (indicator of coagulation) and fibrinolysis together with elevated levels of cardiac enzymes (e.g. troponin T) represent biomarkers of poor prognosis. D-dimer concentrations above 1 µg/mL are associated with an 18 times increased odds ratio for death.

Development of hypoxaemia, secondary to acute respiratory disease syndrome (ARDS) induced by COVID-19, might also activate the coagulation cascade and could be important in endothelial dysfunction beyond the capillary network. Importantly, mechanical ventilation forcing viral immune-stimulatory molecules into the microvasculature, may increase the likelihood of immunothrombosis.

Considerations for treatment of COVID-19 patients with anti-coagulants (e.g. heparin) and anti-cytokines (IL-1β) require investigation as it is not known whether these treatment strategies normally used in the context of arterial inflammation would be beneficial to COVID-19 patients with ongoing SARS-CoV-2 infection.

No Benefit for Lopinavir–Ritonavir in Severe COVID-19

Slomski, A. 2020. *JAMA*

Link: https://doi.org/10.1001/jama.2020.6793

Lopinavir-ritonavir did not accelerate recovery or improve mortality rates among hospitalized patients with severe coronavirus disease 2019 (COVID-19).

The study's 199 patients with confirmed severe acute respiratory syndrome coronavirus 2 infection were randomly assigned to receive standard care plus 400 mg of lopinavir and 100 mg of ritonavir twice a day for 14 days or standard care alone.

For both groups the time to clinical improvement—the primary end point—was a median of 16 days.

Reviews

Dysregulation of type I interferon responses in COVID-19

Acharya, D. *et al*. 2020. *Nature Reviews Immunology*
Link: https://doi.org/10.1038/s41577-020-0346-x

Introduction:

- SARS-CoV-2 infects nasal/airway epithelial cells via ACE2 and TMPRSS2 co-localisation, triggering pro-inflammatory cytokine production in alveolar type I/II epithelial cells and macrophages.
- Type I interferons (IFNs) are key to an early anti-viral response.

COVID-19/SARS-CoV-2 models/patients:

- Pro-inflammatory mediators can cause respiratory distress or multi-organ injury in COVID-19, indicative of Cytokine Release Syndrome.
- Systemic IFN is minimal in severe COVID-19 patients, despite some local lung production.
- In the lungs SARS-CoV-2 thought to avoid inducing IFNs during the incubation phase.
- Delayed IFN response may cause the observed:
    - Natural killer, $CD4^+$ and $CD8^+$ T-cells cells appear depleted/functionally exhausted.
    - Regulatory T-cells inversely correlate to disease severity.

SARS-CoV/MERS-CoV virus mouse models/patients:

- SARS-CoV models suggest lung IFN response comes after viral peak.
- Lack of an IFN response:
    - Prevented infiltration of monocyte-derived macrophages in lung tissue
    - Enhanced neutrophil recruitment to the lungs
    - Accumulation of natural killer cells
- Early IFN treatment (before viral peak) prevented lethal SARS-CoV/MERS-CoV but late IFN intervention slowed viral clearance and exaggerated immunopathology.
- IFNα detected in SARS-CoV patients “pre-crisis” phase and subsided after.
- Patients who died from SARS-CoV had a high number of pro-inflammatory macrophages, and low levels of pro-repair macrophages.
- Clinical trials of IFN with antivirals were inconclusive, attributed to timing of intervention and comorbidities.

IFN interventions in COVID-19:

- Prophylactic administration of IFNα nasal drops may prime anti-viral response in target cells (NCT04320238).
- Other IFN clinical trials underway though early administration is likely advantageous, and comorbidities need monitoring.
- Concerns that as IFN has been shown to induce ACE2 expression in human airway epithelial cells, therefore could enhance virus entry.

Highlights:

- In SARS-CoV-2 infection Anti-viral interferon (IFN) response appears detrimentally delayed in lungs.
- Prophylactic/early IFN interventions may be beneficial to treatment/vaccines but come with some risk.

Mass Spectrometry Resource

The COVID-19 MS Coalition—accelerating diagnostics, prognostics, and treatment

Struwe, W. *et al*. 2020. *The Lancet*

Link: https://doi.org/10.1016/S0140-6736(20)31211-3

A mass spectrometry coalition (https://covid19-msc.org/) has been set up involving 220 scientists from 18 countries, in addition to major manufacturers and pharmaceutical companies. The coalition can be joined by filling out the sign-up form on the website, resulting in the creation of a directory of experts so the work can be allocated as necessary.

Mass spectrometry has the ability to generate rapid, precise and reproducible diagnostic information and therefore accelerate understanding of COVID-19. This project will give information regarding the multi-omic profiling of the host response and correlate prognosis with disease severity, in addition to investigating spike protein glycosylation and plasticity, which will aid in vaccine design.

The aim of this coalition is to collect a large data set of molecular level information of SARS-CoV-2 in the human host by sharing optimised methods of sample collection and data generation so information can be combined, accessed and utilised by all. The aim is to use mass spectrometry to map the viral antigens and their interactions in blood and other bio-fluids (including urine, bronchial lavage and throat/nose swabs) and assay (QC) compounds to:

- Inform serological testing,
- Inform vaccine and therapeutic developments,
- Develop methods to determine disease prognosis,
- Determine the lifetime of infective particles in the environment.

Journal Reviews

Transmission and Social Distancing

Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients

Chia, P.Y. *et al*. 2020. *Nature Communications*

Link: https://doi.org/10.1038/s41467-020-16670-2

Summary:

Chia et al, screened surface and air samples from hospital rooms of COVID-19 patients for the presence of SARS-CoV-2 RNA. The hospital rooms sampled included airborne isolation rooms (AIIRs) in the ICU and in the general ward. Viral RNA was identified at higher levels in the first week of illness, 66.7% on high touch surfaces compared to 20% in the second week of illness. Air sampling detected viral particles between 1-4 µm but none below 1 µm.

Highlights:

1. Environmental contamination was associated with patient nasopharyngeal viral loads and peaked at day 4-5 of symptoms
2. Detected aerosol samples sized between >4 µm and between 1-4 µm but none below 1 µm

Clinical Impact:

- The finding that environmental contamination of high touch surfaces and the air peaked at days 4-5 of symptoms correlates with other SARS-CoV-2 studies and could help to inform public health strategies.

Important Methodologies:

- NIOSH bioaerosol samplers were used to collect air samples - authors state that this is the first study to use NIOSH samplers to capture coronaviruses
- SARS-CoV-2 infection was confirmed by a PCR-positive respiratory sample
- Surface sampling carried out using macrofoam sterile swabs

Limitations:

- Did not determine the ability of SARS-CoV-2 to be cultured from the environmental swabs so the infectiousness is unknown.
- Sampling in AIIRs is not representative of community settings
- Air and surface sampling was done at single timepoints and did not track the viral contamination over the course of the patients illnesses

Physical distancing, face masks, and eye protection to prevent person-to-person transmission of SARS-CoV-2 and COVID-19: a systematic review and meta-analysis

Chu, D.K. *et al*. 2020. *The Lancet*

Link: https://doi.org/10.1016/S0140-6736(20)31142-9

Summary:

This WHO-funded research conducted a systematic review of 172 observational studies to investigate the effectiveness of physical distancing and personal protective equipment on the risk of virus transmission in both health-care and community settings. Meta-analysis of 44 comparative studies revealed that physical distancing of 1 m or more greatly reduced transmission by over 10% compared to distances less than 1 m. Distancing of 2 m or more further reduced the risk. Protection of the face and eyes additionally reduce the risk virus transmission. Until a vaccine can be found, social distancing and personal protection is paramount to prevent COVID-19.

Main Findings:

- **Physical Distancing** – absolute risk reduced from 12.8% to 2.6% when distance was increased from less than 1 m to 1m or more.
- The strength of association increased with distance (2.02 change in relative risk per metre) and overall trends were seen irrespective of causative virus (SARS-CoV-2, SARS or MERS), health-care vs. community setting and by type of face mask worn.
- **Face Masks** – absolute risk increased from 3.1% to 17.4% when facial protection was not used.
- Adjusted analysis and within-study comparisons revealed that N95 respirators were more protective than disposable surgical or 12-16 layer cotton masks.
- The strength of protective association was higher in health-care settings than in the community – relative risk of transmission: 0.30 vs. 0.56. However, this is because more effective N95 respirators are more frequently used in health-care settings.
- **Eye Protection** – absolute risk reduced from 16.0% to 5.5% when eye protection (visors, face-shields, goggles etc.) used.
- Similar findings were seen when comparing unadjusted and adjusted studies, and when conducting subgroup and sensitivity analyses.

Highlights:

- First comprehensive review on the reduced risk of SARS-CoV-2 and related betacoronaviruses in both health-care and community settings by physical distancing, use of face masks and eye protection.
- Findings used to recommend WHO guidance.

Clinical Impact:

- Moderate

Important Methodologies:

- Systematic review of 172 observational studies across 16 countries including patients with WHO-defined confirmed or probable SARS-CoV-2, SARS or MERS infection and those in close contact with them. Study was not limited by language nor were there quantitative cut-offs for distance.
- Rated certainty of evidence according to Cochrane methods and GRADE approach.
- Meta-analysis of 44 comparative studies (25,695 patients) enabled risk ratios to be calculated.
- Conducted frequentist and Bayesian meta-analyses and random-effects meta-regressions.
- Analysed subgroup effects by virus type, intervention and setting.
- Findings were adjusted for many variables (sex, severity of case).

Limitations:

- No randomised controlled trials were available/included.
- Findings were of low to moderate reliability.

Changes in contact patterns shape the dynamics of the COVID-19 outbreak in China

Zhang, J. *et al*. 2020. *Science*

Link: https://doi.org/10.1126/science.abb8001

Summary:

The authors investigate changes in age-mixing patterns associated with COVID-19 interventions. Contact surveys were performed in Wuhan, the epicentre of the outbreak, and Shanghai. Children (0-14 years) were found to be less susceptible to SARS-CoV-2 infection than adults 15-64 years of age. Individuals over 65 years were found to be most susceptible to infection. It is suggested that while proactive school closures cannot interrupt transmission on their own, they could reduce peak incidence by 40-60% and consequently delay the epidemic. Provides evidence to support that social distancing alone, as implemented in China during the outbreak, is sufficient to control COVID-19.

Main Findings:

- Daily contacts were reduced 7-8-fold during the COVID-19 social distancing period, with most interactions restricted to the household.
- A transmission model was built to study the impact of social distancing and school closure on transmission is built using the available data.
- Susceptibility to SARS-CoV2 increased with age. Based on active testing of 7,375 contacts of 136 confirmed index cases.

- A baseline R value was estimated.
- Investigations into the pre-emptive mass school closure revealed that, assuming a baseline R0 of 2 - 3.5, a noticeable decrease in infection attack rate and peak incidence was achieved, and a delay in the epidemic, but transmission was not interrupted.
- Highlights that contact data are useful but seroepidemiology studies will be essential to fully resolve population susceptibility profiles to SARS-CoV-2 infection and disease.
- The patterns seen here may not be reflected in different locations across the globe where social distancing measures are implemented differently.

Highlights:

- The modelling exercise provides an indication of the possible impact of a nationwide pre-emptive strategy on the infection attack rate and peak incidence. However, the model is based entirely on figures from two locations in China; should this model be generalised to other contexts, location specific age mixing patterns and population structures should be considered.
- Overall, school-based closure policies are not sufficient to entirely prevent a COVID-19 outbreak, but they can impact disease dynamics, and hence hospital surge capacity. It is highlighted that individuals of school age (5-19 years) represent 9.5% of the population in Shanghai, which is markedly lower than in western countries.

Clinical Impact:

- Minimal

Important Methodologies:

- Disease model generated from contact tracing in two cities in China.

SARS-CoV-2 strategically mimics proteolytic activation of human ENaC

Anand, P. *et al*. 2020. *eLife*

Link: https://doi.org/10.7554/eLife.58603

Summary:

The authors suggest that the S1/S2 cleavage site of SARS-CoV-2 might has evolved to mimic a FURIN-cleavable peptide on the human epithelial sodium channel a-subunit (ENaC-α) which is essential for airway surface liquid homeostasis and needs to be cleaved for activation. In SARS-CoV-2 infected cells less cleavage of ENaC-α might occur, resulting in compromised fluid reabsorption driving lung pathology.

Main Findings:

- Comparing SARS-CoV-2 to SARS-CoV strains and related zoonotic viruses, SARS-CoV-2 uniquely expressed a tribasic 8-mer insertion at S1/S2 that is cleavable by furin
- This 8-mer peptide was uniquely expressed in the extracellular domain of human ENaC-α
- Co-expression of ENaC-α, ACE2 and furin or other proteases capable of target site cleavage were detected in cardiovascular, renal and pulmonary cell types linked to SARS-CoV-2 pathophysiology

Highlights

- SARS-CoV-2 pathogenicity might be linked to its mimicry of an essential homeostatic regulator of airway surface liquid

Clinical Impact:

- Low

Important Methodologies:

- Sequence alignment of viral S-protein sequences with Clustal-W and JalView
- Expression profiling of ACE2 and ENaC-a across human and mouse scRNA-seq datasets

Limitations:

- N/A

Clinical

Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study

Docherty, A.B. *et al*. 2020. *BMJ*

Link: https://doi.org/10.1136/bmj.m1985

Summary:

This study characterises the clinical features of patients admitted to hospital with COVID-19 in UK during the growth phase of the SARS-CoV-2 pandemic in the UK. From 208 acute care hospitals in England, Wales, and Scotland between 6 February and 19 April 2020, 20133 patients were enrolled. Increasing age, male sex, and comorbidities including chronic cardiac disease, non-asthmatic chronic pulmonary disease, chronic kidney disease, liver disease and obesity were associated with higher mortality in hospital. Docherty et al highlight the importance of pandemic preparedness and the need to maintain readiness to launch research studies in response to outbreaks.

Main Findings:

- 20133 patients suffering from COVID-19 were enrolled in the study, 34% of total hospital admissions for COVID-19 on 19 April 2020.
- Median time from onset of symptoms to presentation at hospital was 4 days.
- Median age of patients was 73 years (IQR 58-82); 1.5% were less than 18 years old and 1.0% were less than 5 years old.
- 59.9% of admissions were male, 40.1% were female
- Common symptoms were cough (68.9%), fever (71.6), and shortness of breath (71.2%). High overlap between these three most common symptoms was observed.
- 29% of all patients complained of enteric symptoms on admission, mostly in association with respiratory symptoms; however, 4% of all patients described enteric symptoms alone
- Common major comorbidities were chronic cardiac disease (30.9%), diabetes without complications (20.7%), chronic pulmonary excluding asthma (17.7%), kidney disease (16.2%), and disease chronic asthma (14.5%). 22.5% had no documented major comorbidity.
- Pregnancy was not associated with mortality
- 41% of patients were discharged, 34% continued to receive care at the date of reporting and 26% died. Median age of patients who died was 80 years, with only 11% of these patients having no documented major comorbidity.
- Increasing age was a strong predictor of mortality in hospital after adjusting for major comorbidity: 50-59 years, hazard ratio 2.63; 60-69 years, 4.99; 70-79 years, 8.51; ≥80 years, 11.09.

Highlights

- A prime example of the importance of putting plans in place for the study of epidemic and pandemic threats, and the need to investment in preparedness studies
- Increasing age, male sex, and various were associated with higher mortality in hospital.
- Interactive infographic is available at https://isaric4c.net/info

Clinical Impact:

- Significant

Important Methodologies:

- Prospective observational cohort study with rapid data gathering and near real time analysis.

Limitations:

- Capacity to enrol was limited by staff resources at times of high COVID-19 activity.
- Large amounts of data were missing, likely due to unprecedented numbers of patients at a time when many were seconded to clinical practice or themselves off sick.

Postmortem Examination of Patients With COVID-19

Schaller, T. *et al*. 2020. *JAMA*

Link: https://doi.org/10.1001/jama.2020.8907

Summary:

Schaller et al performed postmortem examinations on 10 patients with proven severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. They found that acute and organizing diffuse alveolar damage and SARS-CoV-2 persistence in the respiratory tract were the predominant histopathologic findings and constituted the leading cause of death in patients with and without invasive ventilation.

Main Findings:

- Postmortem conducted on 10 patients (7 males) median age of 79 years (range, 64-90 years)
- 6 patients had received invasive ventilation during hospital admission
- Infiltrations with ground-glass opacity predominantly in middle and lower lung fields were detected by chest x-ray
- Patients had a median of 4 known pre-existing comorbidities with cardiovascular disease being most frequent

- Pre-existing structural lung damage (eg, emphysema) was found in 2 patients
- Disseminated diffuse alveolar damage at different stages (the histopathological correlate of acute respiratory distress syndrome) was the major histologic finding
- Diffuse alveolar damage was detectable in all lobes but appeared unevenly distributed with pronounced manifestation in middle and lower lung fields
- Signs of exudative early-phase acute diffuse alveolar damage with hyaline membrane formation, intra-alveolar edema, and thickened alveolar septa with perivascular lymphocyte-plasmocytic infiltration were consistently found
- Organizing-stage diffuse alveolar damage with pronounced fibroblastic proliferation, partial fibrosis, pneumocyte hyperplasia leading to interstitial thickening and collapsed alveoles, and patchy lymphocyte infiltration was the predominant finding
- In areas of organizing diffuse alveolar damage, reactive osseous and squamous metaplasia were observed
- Fully established fibrosis was most prominent in patient 1, ultimately leading to almost complete destruction of pulmonary parenchyma
- In 5 patients, minor neutrophil infiltration was indicative of secondary infection and/or aspiration
- Mild lymphocytic myocarditis and signs of epicarditis were detectable in 4 and 2 cases
- Liver histology showed minimal periportal Lymphoplasmacellular infiltration and signs of fibrosis
- No morphologically detectable pathology in other organs
- No signs of encephalitis or central nervous system vasculitis were found
- At time of autopsy, SARS-CoV-2 was still detectable in the respiratory tracts of all patients. Polymerase chain reaction testing was positive in pleural effusion but negative in all CSF samples

Highlights:

- Pulmonary histologic characteristics of COVID-19 resembled those observed in diseases caused by other Betacoronavirus infections such as severe acute respiratory syndrome and Middle East respiratory syndrome

Clinical Impact:

- Limited

Important Methodologies:

- Specimens from lung, heart, liver, spleen, kidney, brain, pleural effusion, and cerebrospinal fluid (CSF) were assessed
- Postmortem nasopharyngeal, tracheal, bronchial swabs, pleural effusion, and CSF were tested for SARSCoV-2 by reverse transcriptase–polymerase chain reaction

Limitations:

- Small number of cases from only a single center
- Missing proof of direct viral organ infection

Description of COVID-19 in HIV-infected individuals: a single-centre, prospective cohort

Vizcarra, P. *et al.* 2020. *The Lancet HIV*

Link: https://doi.org/10.1016/S2352-3018(20)30164-8

Summary:

In this observational prospective study, Vizcarra et al. included all consecutive HIV-infected individuals who had suspected or confirmed COVID-19 as of April 30, 2020, at the Hospital Universitario Ramón y Cajal (Madrid, Spain). The authors compared the characteristics of HIV-infected individuals with COVID-19 with a sample of HIV-infected individuals assessed before the COVID-19 pandemic and described the outcomes of individuals with COVID-19. Of 2873 HIV-infected individuals regularly followed up in the clinics, 51 were diagnosed with COVID-19 as of April 30, 2020, resulting in a rate of infection of 1·8%. 35 (69%) had a laboratory-confirmed SARS-CoV-2 infection, establishing a confirmed COVID-19 rate of 1·2% (95% CI 0·8–1·7), whereas 16 (31%) were suspected cases. The authors found that higher BMI, comorbidities, and use of tenofovir before the COVID-19 pandemic were associated with diagnosis of COVID-19. This analysis was repeated with only laboratory-confirmed cases and the results were similar.

Main Findings:

- The authors describe the SARS-CoV-2 infection rate and clinical characteristics of COVID-19 among adults living with HIV
- 51 HIV-infected individuals were diagnosed with COVID-19 (incidence 1·8%, 95% CI 1·3–2·3). Mean age of patients was 53·3 years (SD 9·5); eight (16%) were women, and 43 (84%) men. 35 (69%) cases of co-infection had laboratory confirmed COVID-19, and 28 (55%) required hospital admission.
- Age and CD4 cell counts in 51 patients diagnosed with COVID-19 were similar to those in 1288 HIV-infected individuals without; however, 32 (63%) with COVID-19 had at least one comorbidity (mostly hypertension and diabetes) compared with 495 (38%) without COVID-19 (p=0·00059).
- 37 patients (73%) had received tenofovir before COVID-19 diagnosis compared with 487 (38%) of those without COVID-19 (p=0·0036); 11 (22%) in the COVID-19 group had previous protease inhibitor use (mostly darunavir) compared with 175 (14%; p=0·578).
- Clinical, analytical, and radiological presentation of COVID-19 in HIV-infected individuals was similar to that described in the general population.
- Six (12%) individuals were critically ill, two of whom had CD4 counts of less than 200 cells per µL, and two (4%) died. SARS-CoV-2 RT-PCR remained positive after a median of 40 days from symptoms onset in six (32%) individuals, four of whom had severe disease or low nadir CD4 cell counts.

Highlights:

- HIV-infected individuals should not be considered to be protected from SARS-CoV-2 infection or to have lower risk of severe disease. Generally, they should receive the same treatment approach applied to the general population.

Clinical Impact:

- Minimal

Important Methodologies:

- Observational prospective study

Limitations:

- Small number and bias in the rate of infection because local recommendations restricted confirmatory testing.
- Because of the observational design, individuals were managed from a clinical point of view and inflammatory markers were measured only in severe cases, biasing the effect of these variables on outcomes
- Time of undetectable HIV RNA level or treatment adherence were not adequately assessed.

Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area

Richardson, S. *et al*. 2020. *JAMA*

Link: https://doi.org/10.1001/jama.2020.6775

Summary:

Case series of 5700 patients presenting to New York hospitals with COVID-19 from a healthcare provider over 1 month. Detailing the characteristics interventions and outcomes. Of this selected group of patients at the extreme end of the healthcare system there was a very high rate of comorbidities. Approximately half had been discharged or died within the month period and their follow up was included- within the follow up period 21% died. For those who survived to the end of the study median time to censorship was 4.5 days. This report provides important information on very early poor outcome in New York but is very limited a clear picture on the longer term outcome in COVID19.

Main Findings:

- Median age 63 years IQR 52-75 39.7% female
- 56.6% hypertensive 41.7% obese, 33.8% diabetic

- At initial presentation 30.7% febrile, 17% raised respiratory rate 27.8% on oxygen
- It is interesting to note very low levels of asthma and viral hepatitis relatively in this cohort
- The primary outcome was death (21%) or (discharge 3.5%) – of these 14.2% received ICU care but only 3.2% received ventilation and 3.2% received renal replacement therapy- suggesting a group not for full escalation and possibly early appropriate decision for palliation/supportive therapy alone the majority were over 80 and had comorbidities
- 20.2% of the total study population received ventilatory support of these 72% were inpatients at the studies end

Highlights:

- Well characterised cohort of patients presenting with COVID19 in New York most usefully gives a snap shot of the presenting features

Clinical Impact:

- This will reinforce our perception of risk factors associated with poor outcome in COVID19 at presentation. The very early nature of the report indicates that this should only have limited impact on planning and management of COVID patients- at 3 month follow up study would be more useful

Important Methodologies:

- Retrospective review of healthcare system covering 11million people in New York city – chart review identifying those who are SARS-CoV-2 PCR+ve

Limitations:

- Too early, not all data complete
- Hard to distinguish the role that the American health care system has played in patient selection and treatment

Hyperinflammatory shock in children during COVID-19 pandemic

Riphagen, S. *et al*. 2020. *The Lancet*

Link: https://doi.org/10.1016/S0140-6736(20)31094-1

Summary:

This correspondence highlights an unprecedented cluster of Kawasaki disease shock syndrome-like symptoms among 8 children admitted to a paediatric intensive care unit in London over a 10 day period. During treatment, no child tested positive for SARS-CoV-2;

although half had known family exposure to the virus and all showed evidence of infection and inflammation. Despite no significant respiratory involvement, all but one required mechanical ventilation for cardiovascular stabilisation. 2 patients tested positive for COVID-19 following discharge or post-mortem examination. Multispecialty patient care is needed during this pandemic, particularly for asymptomatic children presenting with a hyper-inflammatory syndrome with multi-organ involvement.

Main Findings:

- Of 8 children admitted, all were previously fit although 7 were above the 75th centile for weight. 5 were boys and 6 were of Afro-Caribbean descent.
- No child tested positive for SARS-CoV-2, using nasopharyngeal aspirates and broncho-alveolar lavage. However, 4 children had known family exposure to SARS-CoV-2.
- Despite evidence of fighting an infection (including increased C reactive protein), no pathological organism was identified in all but one patient.
- Common clinical presentations included unrelenting fever (38-40 °C), conjunctivitis, and significant gastrointestinal symptoms.
- All patients progressed to warm, vasoplegic shock and refractory to volume resuscitation, requiring haemodynamic support.
- Most had no significant respiratory involvement
- Cardiovascular system was highly implicated in most patients. 7 required mechanical ventilation for cardiovascular stabilisation and echo-bright coronary vessels were commonly seen on echocardiograms. In addition, elevated cardiac enzymes were seen.
- One child died from a large cerebrovascular infarct following development of arrhythmia with refractory shock. This child tested positive for SARS-CoV-2 post-mortem.
- Another child tested positive upon discharge from intensive care.

Highlights:

- Identification of Kawasaki shock syndrome cluster in children during COVID-19 pandemic.

Important Methodologies:

- N/A

Limitations:

- A small cohort. However since submission, over 20 children with similar clinical presentation have been admitted.

Therapeutics

Remdesivir for 5 or 10 Days in Patients with Severe Covid-19

Goldman, J.D. *et al*. 2020. *NEJM*

Link: https://doi.org/10.1056/NEJMoa2015301

Summary:

JD Goldman et al. report their results obtained for an ongoing phase 3 trial with remdesivir, a prodrug of adenosine analogue with antiviral activity against a range of RNA virus families. According to their study a total of 397 patients who were randomly assigned in a 1:1 ratio to receive intravenous treatment with remdesivir for 5 days or 10days. All these patients were confirmed with SARS-CoV-2 infection, oxygen saturation of 94% or less while they were breathing ambient air, and radiologic evidence of pneumonia.

Main Findings:

- More patients were discharged from the hospital in the 5-day group than in the 10-day group (60% vs. 52%), and mortality was numerically lower (8% vs 11%).
- The percentages of patient experiencing adverse events were similar in the two groups: 70% in the 5-day group and 74% in the 10-group.

Highlights

- Remdesivir efficacy might not be associated with a difference in time (5-day to 10-day treatment). Although patient who progress to mechanical ventilation may benefit from the 10 days of remdesivir treatment.

Clinical Impact:

- High

Important Methodologies:

- Treatment. All patients were to receive 200mg on day 1, followed by 100mg of remdesivir once daily for the subsequent 4 or 9 days.
- Clinical and laboratory monitoring. On trial days 1, 3, 5, 8, 10, and 14, blood samples were obtained for complete blood count and measurement of creatinine, glucose, total bilirubin, and liver aminotransferases.
- Statistical analysis. Using a two-sided significance level of 0.05. The difference in the proportion of patients with an event under evaluation between treatment groups and its 95% confidence interval were estimated from the Mantel–Haenszel proportions, with adjustment according to baseline clinical status.

Limitations:

- In this phase 3 trial lacked a placebo control. So it shouldn't be considered as a test of the efficacy of remdesivir.
- The groups were imbalance. According to the data, the 10-day group included a significantly higher percentage of patients in the most severe disease categories, also there were a higher proportion of men who are known to have a worse outcome with Covid-19.
- Patients were discharged from hospital as soon as medically indicated, regardless of whether they had completed the full assigned course of treatment with remdisivir.

Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2

Monteil, V. *et al*. 2020. *Cell*

Link: https://doi.org/10.1016/j.cell.2020.04.004

Summary:

Monteil et al. report that clinical-grade human recombinant soluble ACE2 (hrsACE2) can inhibit SARS-CoV-2 infection *in vitro*. Mouse recombinant soluble ACE2 (mrsACE2), which was made in the same way as hrsACE2, was used as a control and did not inhibit SARS-CoV-2 infection. It was shown that SARS-CoV-2 could establish infection in engineered human capillary blood vessel and kidney organoids. Addition of hrsACE2 significantly reduced SARS-CoV-2 infection in these models.

Main Findings:

- hrsACE2 (tested in phase 1 & 2 clinical trials a few years ago) was not toxic to cells or engineered human organoids.
- hrsACE2 inhibits SARS-CoV-2 growth in Vero E6 cells, by a factor of 1000-5000, and prevents infection of adjacent cells by newly produced viral particles.
- In contrast to mrsACE2, hrsACE2 inhibits SARS-CoV-2 infection in a dose-dependent manner; possibly by blocking SARS-CoV-2 attachment to the cells.
- SARS-CoV-2 could successfully infect human capillary & kidney organoids and generate infectious viral progeny; which could be inhibited by hrsACE2.

Highlights:

- hrsACE2 can significantly inhibit early stages of SARS-CoV-2 infection.
- Inhibition of SARS-CoV-2 infection by hrsACE2 was not complete but clearly dose-dependent.

Clinical Impact:

- Medium

Important Methodologies:

- Engineered human capillary blood vessels and human kidney organoids from stem cells to develop SARS-CoV-2 infection models.
- Single-cell RNA-seq analysis reveals expression of ACE2 in proximal tubule cells within human kidney organoids.

Limitations:

- Effect of hrsACE2 in later stages of SARS-CoV-2 infection was not explored.
- Inhibition of SARS-CoV-2 by hrsACE2 was not studied in human lung organoids (lung is the major target organ for SARS-CoV-2 infection & is severely affected in COVID-19).
- Additional *in vitro* & *in vivo* studies are required to understand short/long-term protective effects of hrsACE2 against SARS-CoV-2.
- Functional studies are required to determine whether active SARS-CoV-2 infection in blood vessels & kidneys can directly contribute to multi-organ damage observed in some COVID-19 patients.

Antibody Responses

A systematic review of antibody mediated immunity to coronaviruses: antibody kinetics, correlates of protection, and association of antibody responses with severity of disease

Huang, A.T. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.04.14.20065771

Summary:

Huang et al provides a broad scientific review of the literature of antibody (Ab) mediated immunity to the following human coronaviruses (HCoV): β-coronaviruses; endemic HCoV-229E & HCoV-NL63, and β-coronaviruses: Lineage A; endemic HCoV-OC43 & HCoV-HKU1, Lineage B; SARS-CoV-1 & SARS-CoV-2, and Lineage C; MERS-CoV. The review focuses on five key areas: Ab kinetics, correlates of protection, immunopathogenesis, antigenic diversity and cross-reactivity, and population seroprevalence. The review describes and digitalises data from 322 relevant papers on HCoV that may guide research into SARS-CoV-2 antibody mediated immunity. Limited data is included within this manuscript directly related to SARS-CoV-2.

Main Findings:

- **Ab kinetics post infection**; it is rare to detect any Ab during acute infection (1 – 7 days). Ab titres increase rapidly during the 2nd and 3rd week post-symptom onset. IgM wanes quickly in comparison to IgG. The few long term studies available suggest that IgG is detectable until at least 12 months, with some studies reporting detectable IgG up to 3 years post-symptom onset.
- **Correlation between severe disease and Ab rise**; the median time taken to detect Ab's following symptom onset was 11 - 12 days for SARS-CoV-2 and SARS-CoV-1 respectively whereas for MERS-CoV this was considerably longer (16 days). In cases of severe disease for all 3 viruses the time to detect Ab's was extended by 2 – 3 days.
- **Correlates of protection**; this review only details human challenge studies with mild HCoV's. However, these studies indicate that serum IgG and IgA, neutralising Ab titres, mucosal IgA provide possible correlates of protection from infection and disease. Rechallenge studies suggest individuals can be infected with the same HCoV strain but with a lower disease severity.
- **Antigenic diversity & cross-reactivity**; there is cross-reactivity within but minimal reactivity between α and β HCoV's. Endemic HCoV's rarely induce cross-reactive antibodies against novel emerging HCoV's, such as SARS-CoV-1 or MERS-CoV. However, these emerging strains can stimulate Ab's induced by previous infections with HCoV's.
- **Population Seroprevalence**; seroprevalence with four endemic HCoV strains rose rapidly during childhood and remained high during adulthood. There was no clear

trend in seroincidence with age and infection in elderly populations has been reported.

Highlights:

- Provides both a description and digitalised figures from the data reviewed.

Clinical Impact:

- Minimal

Important Methodologies:

- Literature review methodology and data abstraction and digitization.

Limitations:

- The review includes very limited data from SARS-CoV-2 studies, and the data included was collected before 20th March 2020, relatively early in the pandemic.
- The review itself highlights the need to study SARS-CoV-2, at best the review provides a model based on immune responses to existing HCoV's that could be relevant to SARS-CoV-2

A human neutralizing antibody targets the receptor binding site of SARS-CoV-2

Shi, R. *et al*. 2020. *Nature*

Link: https://doi.org/10.1038/s41586-020-2381-y

Summary:

Shi et al isolated two SARS-CoV-2 specific human monoclonal antibodies (MAbs) from convalescent serum of a COVID-19 patient. Both antibodies (CA1 and CB6) showed potent neutralisation activity in vitro and CB6 also inhibited SARS-CoV-2 infection in vivo in a rhesus monkeys.

Analysing the structure of CB6 revealed that this antibody binds to an epitope that overlaps with the ACE2-binding side in the SARS-CoV-2 receptor binding domain (RBD) which leads to steric hindrance and direct interface-residue competition. The Authors state that the neutralising activity of CB6 is likely to be comparable to the one of MAb114 which has been a breakthrough therapy against Ebola and that CB6 deserves further translational development.

Highlights:

1. Two MAbs (CA1 and CB6) isolated from convalescent serum of a COVID-19 patient were able to block the binding of soluble SARS-CoV-2-RBD with hACE2 receptor transiently expressed on HEK293T cells. Flow cytometry showed that both MAbs were specific for the SARS-CoV-2 S protein, but not SARS-CoV or MERS-CoV. Assessment of binding kinetics suggest that both antibodies bind to overlapped epitopes on SARS-CoV-2-RBD.
   Both antibodies exhibit substantial neutralizing activities against SARS-CoV-2 infection in vitro, while CB6 exhibited superior neutralizing activity over CA1.

2. CB6 was further tested in a rhesus macaque SARS-CoV-2 infection model. To reduce the potential for antibody dependant enhancement, a LALA mutation was introduced to the Fc portion of CB6. CB6-LALA was able to inhibit SARS-CoV-2 viral titre and reduce lung damage if given as prophylaxis or as treatment.

3. Crystal screening showed that the blocking mechanism of CB6 is based on steric hindrance and direct interface-residue competition which hinders the binding of hACE2 to the SARS-CoV-2 RBD.

Clinical Impact:

- No immediate clinical impact, but possibly an effective treatment or prophylaxis in the future if further developed.

Important Methodologies:

- Surface plasmon resonance (SPR) to measure binding kinetics of antibodies.
- Bio-layer interferometry (BLI) to assess competitive binding of antibodies.

Limitations:

- The n number in the rhesus macaque study was low (only 3 per group) and only 1 monkey was from each group was used to assess lung damage.
- The authors address the potential of mutations in the RBD of SARS-CoV-2 and state that the so far observed mutations (2) are unlikely to influence CB6 binding.

Convergent Antibody Responses to SARS-CoV-2 Infection in Convalescent Individuals

Robbiani, D.F. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.05.13.092619

Summary:

Robbiani et al report on the neutralising antibody levels present in plasma of 68 COVID-19 convalescent individuals collected 30 days after the onset of symptoms. The majority of individuals that recovered from COVID-19 without hospitalisation did not possess high levels of neutralising antibodies. Rare recurring RBD specific antibodies were found in all individuals which displayed potent antiviral activity. Therefore a vaccine designed to induce such antibodies could be highly effective.

Main Findings:

- Plasma samples tested with anti-RBD = 88% IgG, 66% IgM (AUC)
- Plasma samples tested with anti-S protein = 40% IgG and 21% IgM (AUC)
- IgG binding to RBD and S protein were directly correlated to duration of symptoms.
- Antibody levels to S protein were similar between genders but females had lower titres to IgG-binding antibodies to RBD than males.
- Half maximal neutralising titre (NT50) was low – 18% undetectable, 78% below 1000. 2 samples >5,000.
- RBD and s-binding IgG correlated with NT50.

Highlights:

- Plasma from recovering COVID-19 individuals contains low levels of neutralising antibodies.
- Rare recurring receptor binding domain (RBD) specific antibodies with potential anti-viral activity were found all individuals tested.

Clinical Impact:

- Low – Understand immune response to SARS-CoV-2 in mild infections.

Important Methodologies:

- ELISA – Antibody to RBD and Spike protein
- Pseudo typed SARS-CoV-2 assay – HIV-1 virion carrying nanoluc luciferase reporter and SARS-CoV-2 spike.
- Virus neutralisation assay
- Flow cytometry – to isolate individual B lymphocytes with receptors bound to RBD in blood.

Limitations:

- Only tested individuals with mild disease.

Human neutralizing antibodies elicited by SARS-CoV-2 infection

Ju, B. *et al*. 2020. *Nature*

Link: https://doi.org/10.1038/s41586-020-2380-z

Summary:

SARS-CoV-2 entry into host cells requires binding of the receptor-binding domain (RBD) of the spike (S) protein to the ACE2 receptor. Antibody responses in 8 SARS-CoV-2 patients were characterised using 206 isolated mAbs specific to the SARS-CoV-2 RBD. The most potent antibodies were most competitive with ACE2, indicating that blocking the RBD and ACE2 interaction is a useful surrogate for neutralization. The crystal structure of 1 high-performing antibody indicated high competition with the S protein for the ACE2 receptor. Unexpectedly, there was no cross-reactivity with RBDs from SARS-CoV and MERS-CoV viruses. Potent antibodies have scope to be developed into therapies for SARS-CoV-2.

Main Findings:

- 8 SARS-CoV-2 patients antibody binding was quantified using serial plasma dilution on ELISA plates coated with either; SARS-CoV-2, SARS-CoV or MERS-CoV Trimeric S proteins, recombinant RBD or recombinant SARS-CoV-2 NP. Patient plasma had strongest binding to SARS-CoV-2 proteins.
- No cross-reactivity was found between SARS-CoV and MERS-CoV RBDs, but was found between their trimeric S proteins.
- 4 patients with high anti-SARS-CoV-2 RBD and S antibodies had high proportions of RBD-specific B-cells.
- Detailed characterisation of 1 patient's antibodies found the majority were scattered across branches, whereas 41% were clonally expanded into 3 major clusters.
- SPR was used to calculate dissociation constants between antibodies and the SARS-CoV-2 RBD. The Kd of representative clones from the three expanded clusters ranged from 4.65-8.91nM, suggesting expansion was driven by affinity maturation.
- Many antibodies with impressive Kd values minimally competed with ACE2, suggesting binding affinity does not predict ACE2 competing capacity.
- ACE2 competition correlated with neutralizing activity of pseudoviruses bearing SARS-CoV-2 S protein as well as live SARS-CoV-2.
- Pairwise SPR indicated antibodies recognised both overlapping and distinct epitopes.
- Crystal structure of 1 high-performing antibody indicated direct competition with the RBD of the S protein for the ACE2 receptor, primarily using heavy chain residues.

Highlights:

- Crystal structure of a potent SARS-CoV-2 antibody.

Clinical Impact:

- Moderate.

Important Methodologies:

- Surface plasmon resonance (SPR), Focus reduction neutralisation tests (FRNT), Crystallography.

Limitations:

- No conclusions between antibody response and disease progression.

Vaccines

Immunogenicity of a DNA vaccine candidate for COVID-19

Smith, T.R.F. *et al*. 2020. *Nature Communications*

Link: https://doi.org/10.1038/s41467-020-16505-0

Summary:

Smith et al. describes INO-4800, a DNA-based vaccine candidate targeting the SARS-CoV-2 S protein. The engineered construct results in robust expression of the S protein in vitro. Following immunisation of mice and guinea pigs with INO-4800 antigen-specific T cell responses, functional antibodies which neutralize the SARS-CoV-2 infection and block Spike protein binding to the ACE2 receptor as well as biodistribution of SARS-CoV-2 targeting antibodies to the lungs was measured. This preliminary dataset identifies INO-4800 as a potential COVID- 19 vaccine candidate, supporting further translational study.

Main Findings:

- Initial studies have already been performed which indicate SARS-CoV-2 interaction with its host receptor (ACE2) can be blocked by antibodies
- In vivo immunogenicity studies in both mouse and guinea pig models revealed levels of S protein- reactive IgG in the serum of INO-4800 immunized animals.
- INO-4800 immunization induced antibodies which bind to the receptor-binding domain, a target for nAbs from SARS-CoV convalescent patients
- Anti-SARS- CoV-2 binding antibodies were detected in lung washes of INO- 4800-immunized mice and guinea pigs. The presence of these antibodies in the lungs has the potential to protect against infection of these tissues and prevent LRD, which is associated with the severe cases of COVID-19.
- Induction of T cell responses against SARS-CoV-2 as early as day 7 post-vaccine delivery.
- The DNA plasmid manufacture process allows for scalable manufacture of drug product, which has the potential to circumvent the complexities of conventional vaccine production in eggs or cell culture.
- Observed seroconversion after a single intradermal administration of the INO-4800 in guinea pigs. Whether a single immunization will be sufficient in humans will be investigated in clinical trials.
- The majority of studies demonstrating CoV vaccine-induced immunopathology utilized the BALB/c mouse, a model known to preferentially develop Th2-type responses. The DNA vaccine platform induces Th1-type immune responses has demonstrated efficacy without immunopathology in models of respiratory infection, including SARS-CoV, MERS-CoV, and RSV.

Highlights:

- INO-4800 vaccine candidate induces measurable functional antibodies and T cell responses in multiple animal models.

Clinical Impact:

- The vaccine candidate could potentially prevent or improve outcome of patients infected with SARS-CoV-2

Important Methodologies:

- Western blot
- Immunofluorescence of transfected 293T cells.
- ELISA: Antigen binding ELISA. ACE2 competition ELISA
- SARS-CoV-2 Pseudovirus neutralization assay
- SARS-CoV-2 wildtype virus neutralization assays.
- IFN-γ ELISpot.
- Flow cytometry.
- Structural modelling.

Limitations:

- Need further translational study and clinical trials of the vaccine to determine effectiveness and safety.

Immune Evasion

The ORF8 Protein of SARS-CoV-2 Mediates Immune Evasion through Potently Downregulating MHC-I

Zhang, Y. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.05.24.111823

Summary:

Zhang and colleagues hypothesize SARS-CoV-2 is capable of downregulating MHC-I, enabling virus immune evasion, which could contribute to chronic clinical characteristics observed in COVID19 patients. They showed that HLA-A2 was downregulated when the protein encoded by SARS-CoV-2 open reading frame (ORF8) was expressed in HEK293T cells and other cell lines. T cell killing of peptide presenting ORF8-HEK293T cells was also impaired.

Main Findings:

- The SARS-CoV-2 ORF8 protein caused downregulation of HLA-A2 and b2M in HEK293T cells. ORF8 proteins from other SARS-CoV-1 strains, which had low homology with SARS-CoV-2, did not have this function. Introduction of ORF8 into colon and bronchial epithelial cell lines, and infection of ACE-2 expressing HEK293T cells, resulted in pan-HLA-I or HLA A2 downregulation, respectively.
- Inhibition of lysosomal degradation by bafilomycin A1 (Baf-A1) prevented HLA-A2 downregulation. HLA-A2 was enriched in the lysosome of ORF8 expressing cells.
- ORF8 was found to interact CALNEXIN and LAMP1, suggesting it may redirect trafficking from the ER to the lysosome. HLA-A2 degradation at the ER was excluded as an ER degradation inhibitor did not prevent HLA-A2 downregulation.
- A role for autophagy in the 're-routing' of HLA from the ER to the lysosome was proposed. This was based on the observation that autophagy inhibitors restored HLA-A2 expression, however knockdown of autophagy receptor and cargo proteins produced mixed results in the rescue of HLA-A2 expression, therefore the exact mechanism could not be conclusively determined.

Highlights:

- T cells from healthy donors activated against SSP-1 showed reduced killing of ORF8-transduced, SSP-1 presenting HEK293T cells. Similarly, T cells from a recovered COVID19 patient stimulated with a SARS-CoV-2 peptide pool had reduced killing of peptide loaded ORF8 transfected HEK293T cells.

Clinical Impact:

- Low- this needs to be validated in a clinically relevant model.

Important Methodologies:

- The endoplasmic reticulum, ubiquitin and lysosome protein degradation pathways were inhibited to determine which was involved in HLA-A2 downregulation.
- Cellular localisation (surface or cytosolic) of HLA-A2, and co-localisation with degradation organelles or ORF8, was analysed by immunofluorescence confocal microscopy.
- ORF8 interaction with degradation pathways was assessed by immunoprecipitation and mass spectrometry. Proteins of the top enriched pathways were knocked down using siRNA.
- CTLs were generated from healthy donor PBMC stimulated with an HLA-A2 predicted epitope from the SARS-CoV-1 spike protein, SSP-1. PBMC from recovered COVID19 patients (n=5) were stimulated with a SARS-CoV-2 peptide pool (mostly HLA-A2 peptides) and n=1 IFNγ responsive patient was tested in the cytotoxicity assay. Cytotoxicity was measured as a ratio of live antigen presenting HEK293T cells compared to HEK293T cells presenting an irrelevant peptide (HIV SL9).

Limitations:

- The data on ORF8 mediated MHC-I downregulation is relatively indirect; there was only one instance of SARS-CoV-2 infection (of ACE-2 transfected HEK293T cells). Human foetal colon and human bronchial epithelial cells were only included in one experiment.
- Downregulation of MHC-I was analysed by specific detection of HLA-A2, without concomitant analysis of other HLA alleles expressed by HEK293T cells. Pan-MHC-I downregulation was assessed only in the HBE and FHC cells.
- Despite many recent studies investigating potential epitopes of SARS-CoV-2, the healthy donors were activated against an HLA A2 epitope from SARS-CoV-1.
- T cell from the COVID19 patient were activated against a SARS-CoV-2 peptide pool and tested against ORF8- HEK293T cells without establishing the frequency of responsive cells or a peptide specific response. It was also not clear in the T cell assays whether the positive control (HIV SL9 peptide) was presented by both empty vector and ORF8 expressing HEK293T cells.

IL-6

Impaired immune cell cytotoxicity in severe COVID-19 is IL-6 dependent

Mazzoni, A. *et al*. 2020. *JCI*

Link: https://doi.org/10.1172/JCI138554

Summary:

Mazoni *et al.* analysed the peripheral blood of 30 COVID-19 patients using flow cytometry. Patients had decreased numbers of T, B and NK cells, with CD8+ T cells skewed towards a terminally differentiated/senescent phenotype. T and NK cells had reduced ability to produce anti-viral cytokines and reduced cytotoxicity. This was exacerbated in ICU patients, who also had higher levels of IL-6 (which inversely correlated to the frequency of granzyme+ NK cells). Treatment with anti-IL-6 (tocilizumab) restored the cytotoxicity of NK cells and reduced CRP. They hypothesise that blocking IL-6 could not only suppress harmful inflammation but also restore the anti-viral function of lymphocytes.

Main Findings:

In COVID-19 patients compared to healthy controls:

- Patients had lymphocytopenia, elevated CRP, ferritin, fibrinogen, d-dimer and lactate dehydrogenase
- Increased neutrophils and decreased lymphocytes, eosinophils and basophils absolute numbers in the peripheral blood
- T (both CD4+ and CD8+), B, NKT and NK cells absolute numbers were significantly reduced
- Frequency of transitional (IgMhighCD38high) B cells was significantly reduced
- Increase in the frequency of CD4+T central memory (Tcm, CD45RA-CCR7+) cells (i.e. since total count of CD4+ T cells is significantly lower in COVID-19 patients Tcm are relatively conserved)
- Reduction in CD8+ naïve (CD45RA+CCR7+) and Tcm (CD45RA-CCR7)
- Frequencies of TEMRA (CD45RA+CCR7-) and senescent (CD57+) CD8+ T cells increased
- Increased frequency of IL-2+ CD4+ T cells, but decrease IL-2+ CD8+ T lymphocytes
- Reduced frequency of IFN-γ+ CD4+ and CD8+ T cells and significantly reduced frequency of TNF-α+ NK cells
- Reduced frequencies of polyfunctional (IL-2+IFN-γ+TNF-α+ or IL-2+IFN-γ+TNF-α-) CD8+ T cells
- Reduced percentages of both perforin+ and granzyme A+ NK cells, reduced percentage of granzyme A+ CD4+ T cells.

ICU patients compared to non-ICU patients:

- ICU patients had increased neutrophil and decreased lymphocyte absolute numbers compared to non-ICU patients
- T (in particular CD4+), and NK cells absolute numbers were significantly reduced.
- Reduction in CD8+ Tem (CD45RA-CCR7-) and increase in TEMRA (CD45RA+CCR7) cells
- Frequencies of granzyme A+ NK cells reduced
- IL-6 serum levels were significantly higher in ICU as compared to non- ICU patients

Trial of anti-IL-6 (Tocilizumab):

- Tocilizumab treatment reduced CRP levels, increased lymphocyte count and granzyme A+ and perforin+ NK cells
- Mild improvement in PaO2/FiO2 ratio in four out of five patients

Highlights:

- Large, well conducted study with careful characterisation of lymphocyte subsets, suggesting an immunological phenotype in severe COVID-19 correlated to clinical outcomes
- Results suggest that, as well as driving excess inflammation, IL-6 can impair lymphocyte anti-viral function and blocking IL-6 can therefore restore lymphocyte function

Clinical Impact:

- Strengthens case for therapeutic use of tocilizumab

Limitations:

- Many patients were also on other therapies such as hydroxychloroquine which may confound interpretation of results
- Only 5 patients treated with tocilizumab, did not prove that tocilizumab has clinical efficacy (improvement in PaO2/FiO2 ratio was not statistically significant and lacked an untreated control group)

Neuroscience and Mental Health

Type I astrocytes and microglia induce a cytokine response in an encephalitic murine coronavirus infection

Lavi, E. and Cong, L. 2020. *Experimental and Molecular Pathology*

Link: https://doi.org/10.1016/j.yexmp.2020.104474

Summary:

This study demonstrates that infection with an encephalitic strain of the murine coronavirus (MHV-A59) produces a pro-inflammatory cytokine response via the innate immune system. The response was found to be mediated by microglia and type I astrocytes. Conversely this response is not observed when cells were infected with a non-encephalitic strain of coronavirus (MHV-2). In human brain samples, microglia and type I astrocytes are revealed to possess perivascular foot processes and are suggested to form part of the perivascular glymphatic system. The findings provide reason to consider anti-cytokine therapies in encephalitic coronavirus infections such as Covid-19.

Main Findings:

- Infection via MHV-A59 produces a pro-inflammatory cytokine response from type I astrocytes and microglia but not in type II or type III astrocytes.
- Infection with a non-encephalitic strain of coronavirus MHV-2 does not produce a pro-inflammatory response.
- Type I astrocytes and microglia have foot-like processes that wrap around blood vessels in the brain. They form part of the glymphatic system.

Highlights:

- Pathogenesis of encephalitic coronavirus MHV-A59 is mediated via induction of cytokines such as Il-6, similar to Covid-19.

Clinical Impact:

- Moderate, this suggest a similar process may take place during SARC-CoV-2 infection

Important Methodologies:

- A murine tissue culture system of CNS infection

Limitations:

- MHV-A59, whilst sharing similarities to SARS-CoV-2, is a different virus as such it may not produce the same response

Assessing the Real-Time Mental Health Challenges of COVID-19 in Individuals With Serious Mental Illnesses: Protocol for a Quantitative Study

Moore, R.C. *et al*. 2020. *JMIR Res Protoc*
Link: https://doi.org/10.2196/19203

Summary:
This paper suggests the mental well-being of people with high risk for poor mental health outcomes have been largely overlooked during the COVID-19 outbreak. This paper presents a protocol to assess the mental health impact of social distancing and COVID-19 in those with serious mental illnesses. Patients with schizophrenia and bipolar disorder will be compared to non-psychiatric controls using phone interviews between April and August 2020, and emotional functioning and measures taken to avoid COVID-19 will be ascertained. The first assessment occurred in April, the second will happen between June and August. The aim is to determine the psychological consequences of COVID-19 in vulnerable populations and identify targets for intervention.

Highlights:

1. Impact of COVID-19 on the mental well-being of most vulnerable individuals (such as those with serious mental illness) has been overlooked
2. This can be studied using telephone interviews
3. Results will provide opportunity to identify targets to reduce negative outcomes in these populations in the future

Clinical Impact:

- The psychological impact of dealing with COVID-19 in vulnerable individuals should be further investigated, and interventions aimed at reducing negative impact investigated.

Important Methodologies:

- Telephone-based interviews useful and safe in the current climate.

Limitations:

- It is not possible to obtain true baseline scores of emotional functioning as this is a retrospective study. Long-term impacts are more feasible, but will require extended study beyond the timelines proposed.

Multidisciplinary research priorities for the COVID-19 pandemic: a call for action for mental health science

Holmes, E.A. *et al*. 2020. *Lancet Psychiatry*

Link: https://doi.org/10.1016/S2215-0366(20)30168-1

Summary:

The psychological, social and neuroscientific effects of COVID-19 pandemic were expected to be significant based on the previous information gathered during the SARS epidemic in 2003 (eg. then, 30% increase was seen in suicide in those aged 65 and older). To prevent the numbers going much higher, the immediate actions and longer-term strategies were outlined for mental health science research based on surveys of the public and an expert panel. The broad areas covered include anxiety, depression and their possible outcomes (eg. self-harm, suicide), mitigation of consequences under pandemic conditions and effects of the virus on brain- and mental health.

Highlights – The suggested actions:

1. Immediate actions
    a. ***Improve monitoring and reporting*** of mental health issues in both the whole population and in more vulnerable groups
    b. Identify ***available psychological support to frontline staff*** and their families
    c. Find the most effective ways of ***signposting and delivering mental health services*** to vulnerable groups
    d. Investigate ***the role of*** traditional and social ***media in amplifying anxiety***
    e. Building ***clinically and geographically inclusive neuropsychological database*** of COVID-19 cases
    f. ***Expand facilities for handling SARS-CoV-2 infected tissues***
2. Longer-term strategies
    a. Determine mechanisms that effect the rate of mental health issues and ***predict potential long-term outcomes*** with and without intervention
    b. Develop ***novel, population level interventions*** in order to protect the mental wellbeing
    c. Create an ***evidence-based media policy*** around reporting information about the pandemic
    d. Promote ***mental and physical preparations*** for future pandemics tailored to specific groups.
    e. Understand ***how the virus might enter and propagate through the brain***
    f. Study ***how the immune response*** to the virus ***might cause mental health and neurological symptoms***
    g. Explore potential ***long-term relationship between infection and post-infective fatigue or depressive syndromes***
    h. ***Validate clinical biomarkers*** for identifying brain infection using MRI

Clinical Impact:

- The immediate actions have the potential to reduce the severity of the outcome of the pandemic in terms of mental health both short- and long-term. The longer-term programme suggestions could help with handling the aftermath of this pandemic and prepare the population for any future ones.

Important Methodologies:

- The conclusions were drawn from surveys of the public and an expert panel convened by the UK Academy of Medical Sciences and MQ: Transforming Mental Health (research charity).

Limitations:

- The financial needs and available funds have not been addressed, nor the potential geographic and technical limitations of the actions outlined.

Acute exacerbation of OCD symptoms precipitated by media reports of COVID-19

French, I. and Lyne, J. 2020. *Irish Journal of Psychological Medicine*

Link: https://doi.org/10.1017/ipm.2020.61

Summary:

The COVID-19 crisis has resulted in heightened awareness and promotion of methods to reduce the risk of contamination. This paper investigates the consequences of this message in patients with obsessive compulsive disorder (OCD). This patient group may be particularly affected by such messages as fear of contamination is already common in OCD patients. Exacerbation of OCD symptoms has been found following outbreaks of SARS, MERS and influenza. This paper highlights the case of one patient with well controlled OCD which became exacerbated in response to media reports of COVID-19. This highlights the impact which COVID-19 may have on psychologically vulnerable patients, and suggests extra support may be necessary in such situations.

Highlights:

1. COVID-19 has resulted in an increased awareness of potential sources of contamination
2. Patients with obsessive compulsive disorder, a group already sensitive to fear of contamination, are likely to be negatively affected by over-exposure to such messages
3. A case study demonstrates that these messages can exacerbate OCD symptoms

Clinical Impact:

- Those with mental illnesses may be particularly psychologically vulnerable to the COVID-19 crisis, and interventions aimed at reducing negative impact should be investigated.

Important Methodologies:

- N/A

Limitations:

- This is a single case study, but based on responses to previous outbreaks in OCD patients is likely to be clinically relevant and further study is warranted.