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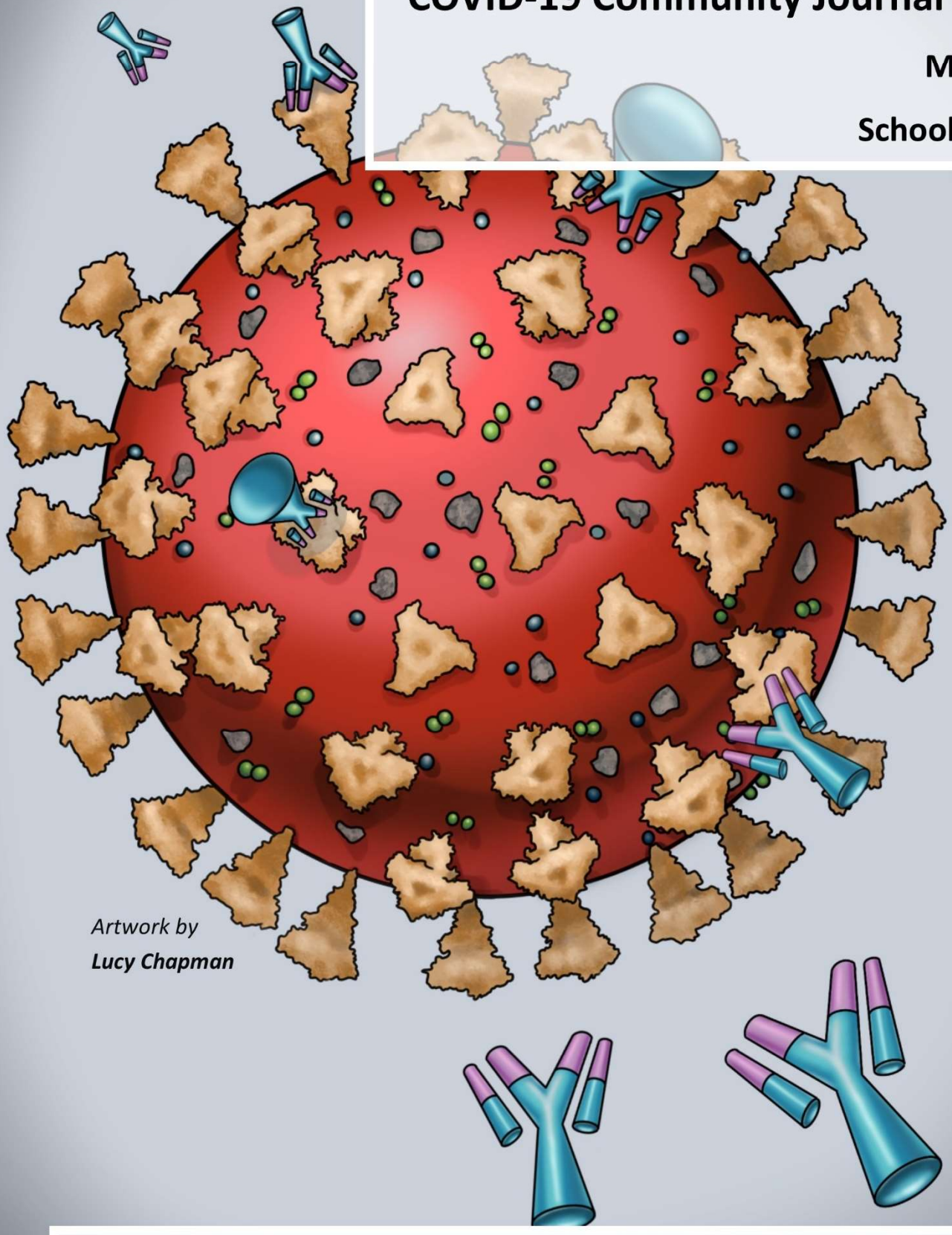

Artwork by
Lucy Chapman

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

NEW THIS WEEK:

ETHICAL CONSIDERATIONS FOR COVID-19 RESEARCH (PAGES 7 – 9)

BIOINFORMATIC RESOURCE (PAGE 10)

Thank you to:

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Oliver Scourfield for compiling the digest.

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

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Ethical Considerations for COVID-19 Research

Key criteria for the ethical acceptability of COVID-19 human challenge studies

WHO, 2020

Link: https://www.who.int/ethics/publications/key-criteria-ethical-acceptability-of-covid-19-human-challenge/en/

WHO working group compiled a document that aims to provide guidance to scientists, research ethics committees, funders, policy-makers, and regulators in deliberations regarding SARS-CoV-2 challenge studies. They set out eight ethical criteria that have to be satisfied in order for these studies to be ethically acceptable:

Criterion 1. Scientific justification: *SARS-CoV-2 challenge studies must have strong scientific justification*

The justification of challenge studies should situate the studies in an overall strategy that aims to improve the public health response to COVID-19, and should include specification of their role in vaccine development pathways, broader research programmes, and planning of public health responses. Investigators should aim to obtain the maximum amount of scientific knowledge per individual participant challenged without exposing them to undue risks.

Criterion 2. Assessment of risks and potential benefits: *It must be reasonable to expect that the potential benefits of SARS-CoV-2 challenge studies outweigh the risks*

Potential benefits and risks should be assessed for each of three key groups: participants, society and third-party contacts of participants. To the extent possible, these potential benefits and risks should be quantified and compared with other study designs. Expected benefits should be maximized and expected risks should be minimized.

Criterion 3. Consultation and engagement: *SARS-CoV-2 challenge research programmes should be informed by consultation and engagement with the public as well as relevant experts and policy-makers*

Public consultations should seek considered public views on proposed research plans, whilst focusing on transparently presenting relevant risks and potential benefits.

Criterion 4. Coordination of research: *SARS-CoV-2 challenge study research programmes should involve close coordination between researchers, funders, policy-makers and regulators*

All SARS-CoV-2 challenge studies must be pre-registered in appropriate repositories, and there should be a comprehensive list of all studies maintained at the international level. There should be coordination between researchers, policy-makers and regulators regarding vaccine development.

Criterion 5. Site selection: *SARS-CoV-2 challenge studies should be situated where the research can be conducted to the highest scientific and ethical standards*

SARS-CoV-2 challenge studies should only be conducted in centres with significant experience in designing, reviewing and conducting human challenge studies. These centres should have

access to appropriate facilities to prepare challenge strains and safe and comfortable isolation for patients.

Criterion 6. Participant selection: *SARS-CoV-2 challenge study researchers should ensure that participant selection criteria limit and minimize risk*
Initial studies should be limited to young healthy adults (18-30 years), with priority to those who face high background probability of infection.

Criterion 7. Expert review: *SARS-CoV-2 challenge studies should be reviewed by a specialized independent committee*
Review procedures should involve high levels of expertise and be conducted rapidly. A specialized review committee should include members with relevant scientific expertise and members with research ethics expertise specific to challenge studies.

Criterion 8. Informed consent: *SARS-CoV-2 challenge studies must involve rigorous informed consent*
Consent should be revisited throughout the study, as is often the case for other challenge studies.

Policy Forum —Ethics of controlled human infection to study COVID-19.

Shah *et al.* 2020. *Science*

Link: https://science.sciencemag.org/content/early/2020/05/06/science.abc1076.full

Summary:

Shah and colleagues provide a comprehensive ethical framework for controlled human infection (CHI) studies that emphasises '*high social value is fundamental to justifying these studies*'. Key aspects for consideration when designing a SARS-CoV-2 CHI study include:

- **Social Value:** CHI studies should design and regularly review the scientific rationale of the study. Only the most promising treatment and vaccine candidates should be selected for study. The CHI study should be designed to further identify and clarify infection dynamics, correlates of protection, disease mechanisms and in the case of vaccines, associated pathogenesis. Studies should ensure high quality data collection and disseminate results quickly and transparently to direct future research, clinical and/or public health practice.
- **Risk-Benefit Profile:** Risks should not exceed upper limit (comparable with living organ donation). Only young, healthy participants with no underlying co-morbidities should be recruited to CHI studies. During the study, participants should be closely monitored in isolation and the relevant public health authorities should be notified in case of participant withdrawal.
- **Engagement:** Engage both the public and researchers, sponsors, regulators and the relevant public health authorities about the CHI study.
- **Site Selection:** Participant recruitment, infrastructure, and risk to the local community and health care system should all be considered when selecting a CHI study site. Extra resources (such as PPE) should be acquired to ensure local pandemic resources are not compromised.
- **Participant Selection:** Participants should be young, healthy, with no co-morbidities.
- **Informed Consent:** Procedures should be implemented to test participants understanding of the key study information before enrolment. Consent should be ongoing as new data emerges.
- **Payment:** Financial incentives should not be offered as this risks potential participants hiding disqualifying information, compensation for costs can be offered.

Resource

https://covid19.edgebioinformatics.org/#/home

Los Alamos National Laboratory: COVID-19 Genome Analytics provides researchers with three separate tools related to COVID19.

The first tool, EDGE, allows users to upload their own raw viral sequence data (fastq files from Illumina or Nanopore) and performs sequential quality control, alignment, generation of a consensus sequence and identification of single nucleotide polymorphisms and variants. This can be done on the website, but users can also download the software and run it locally on their own machines. In addition to the provision of analysis, the aim is to provide a standardised framework for generation of 'a final SARS-CoV-2 genome ready for submission to GISAID and/or GenBank.' This follows on from previous work by this group aimed at unifying the various bioinformatics genomics tools into a single user friendly web suite (see Li et al, Nucleic Acids Research, Volume 45, Issue 1, January 2017, Pages 67–80).

The second tool, addresses the very interesting problem of growing viral sequence diversity and corresponding efficacy of diagnostic PCR-based assays. Each available PCR-assay (at the time of writing, fourteen assays), is screened against each available viral genome (at time of writing, 17,638 sequences). A bar chart, table and customisable heatmap are provided detailing the number of viral genomes that are either a perfect match (to the PCR primer sequences), contain one or two mismatches, or result in failure (3+ mismatch or melting temperature >40 ˚C). Each assay is given a %Recall statistic, which ranged from 99.82% down to 79.97%. Furthermore the heatmap and phylogenetic tree allows researchers to inspect the geographic locations of the matching or mismatching strains.

The final tool is a simple case tracker visually packaged as a map where users can hover their mouse over World countries, or individual US counties (not just states) and look at the number of cases and deaths. This particular resource is easy to use, however corresponding sites such as Worldometer (very quick and easy to navigate: https://www.worldometers.info/coronavirus/) and Johns Hopkins (incredibly detailed, visually stunning, but slow: https://coronavirus.jhu.edu/) are far superior.

Journal Reviews

Highlighted Paper

Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals

Grifoni *et al.* 2020. *Cell*

Link: https://www.cell.com/cell/pdf/S0092-8674(20)30610-3.pdf

*** Detailed review on page 36 ***

Background:

- The adaptive immune system provides long lasting protection against viruses by generating memory B cells and T cells.
- B cells produce antibodies if they encounter the pathogen again, CD8+ T cells kill virus-infected cells and CD4+ T cells help B cells.
- Antibody responses to SARS-CoV-2 in infected individuals have been extensively reported in the literature, detection of an antibody response has been validated as a marker of individuals who have previously been infected. Vaccine development has predominantly focussed on approaches that will induce a memory B cell and antibody response.
- Relatively little is known about the role of T cells in the immune response to SARS-CoV-2.

Main findings:

- Grifoni *et al.* identified SARS-CoV-2-reactive CD4+ T cells in 100% of COVID-19 convalescent patients. The CD4+ T cells targeted the virus' spike protein and the M and N proteins, with lesser responses to other viral proteins. The strength of the CD4+ T cell response to the spike protein also correlated with the anti-SARS-CoV-2 IgG and IgA antibody titres in the plasma.
- 70% of convalescent patients had CD8+ T cells that recognised SARS-CoV-2, predominantly its spike and M proteins.
- Using blood samples from before SARS-CoV-2 emerged, they also found that ~ 50% of people who had not been exposed to SARS-CoV-2 had some reactive CD4+ T cells, which may have been generated in response to "common-cold", coronaviruses and could cross-react to recognise SARS-CoV-2
- These findings are in agreement with another study by Braun *et al.* who found spike-reactive CD4+ T cells in COVID-19 patients. They also found spike-reactive CD4+ T cells in healthy controls, but at a lower frequency than in patients, and targeting regions with high homology to "common-cold" coronaviruses

Implications:

- Memory T cells are generated in response to SARS-CoV-2, suggesting that the protective immune response may be long-lasting
- Assessment of T cell responses should be included in vaccine development
- Vaccines currently in development mainly target spike and may benefit from additionally targeting M and N proteins as these contain T cell targets.
- People who have been exposed to other coronaviruses may have CD4+ T cell responses that offer a degree of protection against SARS-CoV-2. This hypothesis would need further testing.

References:

- Grifoni *et al.* (2020) Cell. https://www.cell.com/cell/fulltext/S0092-8674(20)30610-3
- Braun *et al.* https://www.medrxiv.org/content/10.1101/2020.04.17.20061440v1 (not yet peer-reviewed)
- Commentary in Science https://www.sciencemag.org/news/2020/05/t-cells-found-covid-19-patients-bode-well-long-term-immunity#

Clinical

Back-to-back review:

Renin-Angiotensin-Aldosterone System Inhibitors and Risk of COVID-19

Reynolds, H.R. *et al*. 2020. *NEJM*
Link to paper: https://www.nejm.org/doi/full/10.1056/NEJMoa2008975

&

Renin-Angiotensin-Aldosterone System Blockers and the Risk of COVID-19

Mancia *et al*. 2020. *NEJM*
https://www.nejm.org/doi/full/10.1056/NEJMoa2006923

Summary:
SARS-CoV-2 infects host cells by binding ACE2 on the respiratory epithelium, ACE inhibitors and ARB are commonly used antihypertensives that increase ACE2 expression. These are commonly used antihypertensives and this has been a major of clinical concern during the COVID pandemic.
Reynolds and colleagues reviewed the association of 5 different classes of anithypertensives on 12500 patients in New York tested for COVID to assess likelihood of infection and severity of disease on ACEi and ARB.
Mancia et al conducted a case-control study in Lombardy comparing ACEi and ARB and likelihood on COVID infection and severity of disease.
There was no association between any medication and likelihood of a positive result and severity of disease.

Main findings:

- Patients with COVID are more likely to have hypertension associated cardiovascular co-morbidities and were older
- 25% of hypertensives in New York cohort required non-invasive ventilation, ICU care or died
- ½ of Italian cohort admitted and ~10% received intensive care or died
- Once risk was adjusted there were no significant difference in rate of COVID infection or severity of disease in either cohort.

Highlights
ACEi and ARB appear safe to continue use in spite of COVID pandemic in 2 large well characterised cohorts giving complementary results

Clinical Impact:
This is reassuring to continue current medications which are known to prevent significant sequalae of hypertension, cardiovascular disease and diabetes

Important Methodologies:

- Mancia 5 controls for each COVID case with propensity matching Jan – Mar 2020
- Reynolds Comparison of patients swabbed for SARS-CoV-2 in New York cohort. Bayesian methods propensity matched 1:1 for each medication class Mar-Apr 2020

Limitations:

- Retrospective studies
- Only patients with severe enough disease to seek medical attention are swabbed and included in both studies

An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study

Verdoni, L. *et al.* 2020. *The Lancet*

Link: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)31103-X/fulltext

Summary:

This study looks at Kawasaki (and Kawasaki-like) disease presentation in children during the COVID-19 pandemic. The study is based in the Italian region of Bergamo, the centre of the Italian SARS-CoV-2 outbreak. Two groups of children presenting with Kawasaki disease symptoms are compared, group 1 pre SARS-CoV-2 and group 2 post SARS-CoV-2 outbreak. There was a 30 times higher instance of Kawasaki disease in group 2, with children presenting with more severe illness (increased cardiac involvement, Kawasaki disease shock syndrome and macrophage activating syndrome) and with a higher mean age (7.5 years in group 2 compared to 3 years in group 1). More patients in group 2 required adjunctive steroid treatment to control their symptoms. The correlation between COVID-19 and Kawasaki disease should be expected and therefore monitored in other countries experiencing the SARS-CoV-2 epidemic.

Highlights

1. Demonstrated a strong association between SARS-CoV-2 epidemic and Kawasaki-like disease in children living in the Bergamo province of Italy.
2. The instance of Kawasaki-like disease was 30 times greater than the monthly incidence of the previous 5 years
3. 8/10 children presenting with Kawasaki-like disease tested positive for SARS-CoV-2 IgG or IgM
4. Patients presented with a severe form of Kawasaki-like disease (increased KDSS and MAS)
5. Patients presenting with Kawasaki-like disease during the SARS-CoV-2 epidemic were older (average 7.5 yrs) compared to before the epidemic (average 3 yrs)
6. Adjunctive steroids are a safe and effective treatment to control the symptoms of Kawasaki disease

Clinical Impact?
High – authors predict that there could be a similar outbreak of Kawasaki disease in other countries. Important to be aware when treating children presenting with symptoms during the SARS-CoV-2 epidemic. Authors state that using adjunctive steroids is a safe and effective form of treatment so should be considered by physicians treating patients with Kawasaki-like presentations during the COVID-19 pandemic.

Important Methodologies?
- Used the American Heart Association indications to diagnose Kawasaki disease
- Paediatric Rheumatology International Trials Organisation criteria was used to diagnose Kawasaki disease shock syndrome (KDSS) and macrophage activation syndrome (MAS)
- Current SARS-CoV-2 infection was determined using reverse-transcriptase quantitative PCR
- Previous SARS-CoV-2 infection was identified using serological qualitative test for IgM and IgG

Limitations?
- Small number of cases
- More detailed look into the genetic factors affecting risk of developing Kawasaki disease and COVID-19

Cardiovascular Disease, Drug Therapy, and Mortality in Covid-19

Mehra, M.R. *et al*. 2020. *NEJM*
Link: https://www.nejm.org/doi/full/10.1056/NEJMoa2007621

Summary:
Clinical and hospital outcomes data for 8910 Covid-19 patients from 169 hospitals in 11 countries in North America, Europe and Asia were analysed to determine the impact of underlying cardiovascular disease and treatments for mortality risk. Clinical concerns have been raised over the potential harm of ACE inhibitors and angiotensin receptor blockers (ARB) for cardiovascular patients testing positive for SARS-CoV-2 since the viral entry mechanism involves the ACE2 receptor that may be upregulated by these therapeutics. Reassuringly, the authors found no association between these treatments and Covid-19 mortality, although cardiovascular comorbidities remain major risk factors for poor survival outcomes.

Main findings:
- The authors analysed hospital and medical records for 8910 patients from 169 hospitals in 11 countries to address concerns of the disproportionate impact of Covid-19 on patients

with cardiovascular disease and the potential harm of ACE inhibitors and angiotensin receptor blockers (ARB).

- Overall mortality was 5.8% and the mean age of the sample population was 49±16 years
- 5% all females in the cohort died, compared with 6.3% all males (40% of the total population sampled was female)
- In multivariate logistic regression analysis, increased Covid-19 mortality was associated with:
  - age >65 years (10% mortality for >65, 4.9% for <65)
  - coronary artery disease (10.2% mortality with disease, 5.2% for those without)
  - heart failure (15.3% mortality with disease, 5.6% those without)
  - cardiac arrhythmia (11.5% mortality with disease, 5.6% those without)
  - chronic obstructive pulmonary disease (14.2% mortality with disease, 5.6% those without)
  - current smoking (9.4% mortality with smoking, 5.6% among former smokers or non-smokers)
- There was no increased risk of Covid-19 in-hospital death linked to the use of ACE inhibitors (2.1% of those using, 6.1% not using) or ARB (6.8% of those using, 5.7% amongst non-users) or statins (4.2% with use, 6.0% without use). Hence ACE inhibitors and statins were more commonly used amongst Covid-19 survivors than non-survivors.
- Evaluations of therapeutic usage amongst relevant subgroups (ACE inhibitors and mortality only in patients with hypertension and statins and mortality only in patients with hyperlipidaemia) were consistent with observations reported for the overall cohort.
- There were no associations between other treatments (anti-platelet, beta-blockers, hypoglycaemia treatments) and in-hospital mortality from Covid-19.
- Ethnicity or pre-existing comorbidities of hyperlipidaemia, diabetes mellitus or immunosuppression were not independent indicators of mortality risk.

Highlights:

- ACE inhibitors and statins were more commonly used amongst Covid-19 survivors than non-survivors.
- Ethnicity or pre-existing immunosuppression, hyperlipidaemia or diabetes mellitus were not independent predictors of in-hospital death related to Covid-19.
- Increased Covid-19 mortality risk with age (>65 years), underlying cardiovascular disease, smoking and COPD was determined, corroborating observations from multiple other studies.

Clinical Impact:

Moderate. Randomised trials are required to fully investigate whether treatment with ACE inhibitors, angiotensin receptor blockers and statins is protective for patients with Covid-19, including those with no prior indication for these medications. This study provides preliminary evidence to suggest that these treatments should not be withdrawn following diagnosis with Covid-19, therefore guiding current clinical practice.

Important Methodologies:

- Description of the Surgical Outcomes Collaborative (Surgisphere) international registry of hospital outcomes data that collects inpatient and outpatient electronic health records, supply-chain databases, and financial records, combined with point-of-care data entry for procedures.
- Data are collected through automated data transfers at regular intervals on a prospective basis.
- A standardized Health Level Seven–compliant data dictionary supports the integration and exchange of electronic heath information.
- Data is verified using external databases and financial records, de-identified and stored in a cloud-based data warehouse, exempt from ethics.
- Records of all patients admitted into hospital between 20th December 2019 and 15th March 2020 and receiving a single positive laboratory finding confirming SARS-CoV-2 infection were extracted for multivariable logistic regression analysis of the effects of demographic characteristics, comorbidities and medications on the likelihood of in-hospital death.
- A tipping-point analysis assessed the potential impact of an unmeasured variable on the statistical conclusions.
- Specific subgroup analyses were employed to reduce the effects of missing data entry from the wider cohort.

Limitations:

- This was not a randomised trial and so it is not possible to formally exclude confounding variables, although the tipping point analysis determined that the impact of these would need to be large to alter the statistical confidence.
- The lack of *a priori* hypotheses for rigorous testing increases the likelihood of returning chance associations.
- This study cannot provide any information as to whether the therapeutics investigated would be beneficial for Covid-19 without pre-existing cardiovascular conditions.
- The dataset included only patients for whom complete records concerning SARS-CoV-2 laboratory testing and hospital death or discharge before 28th March 2020 could be confirmed. Patients remaining in hospital after this period or whose records were not registered were excluded.
- A single positive PCR test was deemed sufficient for inclusion, but the accuracy and sensitivity of these tests in the 11 countries has not been discussed. Data transparency and reliability remains an important consideration, although statistical breakdown by country did not reveal any notable discrepancies in the results.
- There were no codes to indicate missing or unknown data, so a lack of declaration on the patient records returned the assumption of absence. Hence the overall analyses may be confounded by undiagnosed underlying comorbidities, especially in countries where healthcare must be paid for upfront by the user?
- Despite recruiting medical records for more than 8000 Covid-19 patients in 11 countries, the ethnicity spread was highly skewed; 63.5% patients were white, 19.3% were Asian, 7.9% were black, and 6.3% Hispanic. There was no representation from southern hemisphere countries or the Indian subcontinent. Since the mortality risk in the black and minority ethnic (BAME) community is of current concern, this study provides limited opportunity to extricate associations based on ethnicity.

- Although the authors re-analysed the data according to income, this was at the country level, rather than relating to patient demographics, hence the coincidence of critical socioeconomic parameters with risk of death cannot be determined. It would have been interesting to compare the UK dataset (free healthcare) with similar countries where medications and hospital admissions must be paid for by the users. This would help establish whether patients taking ACE inhibitors, angiotensin receptor blockers and statins represent a wealthier subpopulation whose Covid-19 outcomes must be considered contextually.

Asymptomatic Seroconversion of Immunoglobulins to SARS-CoV-2 in a Pediatric Dialysis Unit

Hains, D.S. *et al*. 2020. *JAMA*

Link: https://jamanetwork.com/journals/jama/fullarticle/2766215

Summary

Hains *et al.*, describes how dialysis units are at especially high risk of infectious disease transmission, and concern exists about spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Dialysis units in Wuhan, China, reported high coronavirus disease 2019 (COVID-19) prevalence, due in part to unique exposure challenges that limit social distancing efforts, including open bay formats and rotating/multiple nursing assignments. This study describes SARS-CoV-2 seroconversion in patients and health care workers in a paediatric dialysis unit. Serial SARS-CoV-2 antibody levels were measured in 13 patients, 9 dialysis nurses, 2 nurse practitioners, 4 staff, and 10 physicians in a freestanding outpatient 5-bed/3–isolation room paediatric haemodialysis unit at Riley Hospital for Children, Indianapolis, Indiana. Haemodialysis occurs during 2 shifts on Monday, Wednesday, and Friday. All patients had temperature and symptoms of COVID-19 screened before entry. Patients wore surgical masks at all times, as did health care workers, who also had temperatures checked before and after shifts.

Main findings

- One week before this study began (day 0), a single patient presented with fever and generalised symptoms. An RT-PCR test result for SARS-CoV-2 on a nasopharyngeal swab was positive, and results on subsequent swabs remained positive on days 7 and 14 until day 19.
- The patient was dialysed in an isolation room on day 0 and thereafter.
- Serum IgM and IgG levels were measured on sera from whole blood samples from all study participants on days 7, 14, and 21 using SARS-CoV-2 ELISAs. Participants were considered to have seroconverted if positive for IgM or IgG.

- Between day 0 and day 7, 2 health care workers had negative PCR test results despite upper respiratory tract symptoms and fevers. One of these health care workers subsequently seroconverted on day 21 despite 3 negative PCR results.
- No other study participants had nasopharyngeal testing or symptomatology consistent with COVID-19 before day 7.
- By day 21, 11 of 25 health care workers and 3 of 13 patients had positive SARS-CoV-2 antibodies.
- No participants developed symptoms between days 7 and 21. No health care workers who directly cared for the PCR-positive patient seroconverted.
- Two of 11 health care workers who cared for 2 patients with subclinical seroconversion developed SARS-CoV-2 antibodies. Both health care workers remained asymptomatic, but one had a positive result on a nasopharyngeal PCR test obtained because of IgM seroconversion.

Clinical Impact

Minimal - Information on seroprevalence could allow strategically staffing the care of SARS-CoV-2–positive or patients suspected to be positive with seroconverted nurses and physicians. However, a greater understanding of the antibody response to SAR-CoV-2 is required.

Important Methodologies

- PCR to identify SARS-CoV-2 positive participants using nasopharyngeal swabs.
- Use of ELISA assays available for detecting SARS-CoV-2 antibodies.

Limitations

- Small sample size.
- Lack of large-scale sensitivity/specificity of ELISA.
- Lag of antibody positivity from PCR positivity.
- The setting of a single paediatric dialysis unit; replication in additional sites is needed to define the broad applicability of these findings.
- Short follow-up; longer-term follow up is needed to determine the persistence of the antibody response to SARS-CoV-2.

Severe Acute Respiratory Syndrome Coronavirus 2 Serology in Asymptomatic Healthcare Professionals: Preliminary Experience of a Tertiary Italian Academic Center.

Tosato, F. *et al*. 2020. *medRxiv*

Link: https://www.medrxiv.org/content/10.1101/2020.04.27.20073858v1

Summary:

A study of 133 healthcare professionals in an Italian tertiary centre revealed that, although asymptomatic, 5.25% had been infected with SARS-CoV-2. Nasopharyngeal swab analysis showed that 1 individual was currently infected, whilst serum analysis revealed that 6 (4.5%) had high IgG levels, indicative of a successful immune response. Serological analysis of both healthcare professionals and the general population will aid in epidemiological surveillance.

Main Findings

- Of 133 participants, mean age was 47 (± 10) and 117 were female.
- Among the asymptomatic healthcare professionals, only one (0.75%) was positive for SARS-CoV-2 through RT-PCR.
- 6 (4.5%) participants showed increased serum levels of IgG (>1.100 kAU/L).
- The majority of these 6 work at the phlebotomy centre, as nurses or nursing assistants.
- Hypothesise that 5.25% (4.5% + 0.75%) of total Italian population would be infected although asymptomatic or paucisymptomatic.

Highlights

- Identification of asymptomatic individuals for SARS-CoV-2 in the healthcare sector.

Clinical Impact?

Minimal.

Important Methodologies?

- IgM/IgG titres measured using chemiluminescence immunoassay (MAGLUMI, Snibe Diagnostic)
- RT-PCR to analyse nasopharyngeal swabs for active infection.

Limitations?

- Limited to one centre in Italy – female skew.
- Vast assumption to get final conclusion (5.25%) – no modelling used.
- No methods section.
- No RT-PCR data shown.

Identification of Immune complement function as a determinant of adverse SARS-CoV-2 infection outcome

Ramlall, V. *et al*. 2020. *medRxiv*
Link: https://www.medrxiv.org/content/10.1101/2020.05.05.20092452v1

Summary:
Ramlall et al. performed a retrospective observational study of 11,116 patients suspected of SARS-CoV-2 infection and found that a history of complement and coagulation disorders are risl factors for morbidity and mortality in SARS-CoV-2 infected patients.

Main findings:

- History of macular degeneration or coagulatory dysfunctions predispose patients to poor clinical outcomes (including increased risk of mechanical ventilation and death) following SARS-CoV-2 infection.
- Complement deficiencies on the other hand, appear to be protective against poor clinical outcomes.

Highlights

- First study to identify complement and coagulation functions as an underlying risk-factors of SARS-CoV-2 disease outcome.
- Given the existing immune-modulatory therapies targeting complement and coagulation pathways, the discoveries made in this paper provide rationale to investigate these options for treatment of SARS-CoV-2 associated pathology.

Clinical Impact:

- Minimal

Important Methodologies:

- Data warehouse derived from electronic health records 316 (EHRs) from 11,116 patients treated at New York-Presbyterian/Columbia University Irving Medical 317 Center for suspected cases of SARS-CoV-2 infection.
- MySQL and python libraries (pymysql, pandas) to extract and prepare the data for modeling.
- Subjects were genotyped on the UK BiLEVE Array by Affymetrix or the Applied Biosystems UK Biobank Axiom Array.

Limitations:

- Retrospective studies of observational data have notable limitations in their data completeness, selection biases, and methods of data capture. As a result, claims on causality cannot be made.
- Interpretation of our results may be limited by sample size, site-specific biases in clinical care decisions, ancestral homogeneity in the biobank data, and socioeconomic status of affected populations.

Diagnostics & Therapeutics

ReScan, a Multiplex Diagnostic Pipeline, Pans Human Sera for SARS-CoV-2 Antigens

Zamecnik, C.R. *et al*. 2020. *medRxiv*
Link: https://www.medrxiv.org/content/10.1101/2020.05.11.20092528v1

Summary:
In this preprint, Zamecnik *et al* report the development of a human coronavirus (hCoV) proteome-wide programmable phage display assay named VirScan, which was used to profile coronavirus antigens enriched by COVID19 patient sera IgG. They also report the use of ReScan, a diagnostic workflow combining phage display with paper-based microarrays, and the identification of 9 SARS-CoV-2 antigens from a library of 534 peptides. The 9 antigen peptides were restricted to the spike (S), nucleocapsid (N) and accessory protein open reading frame (ORF) 3a.

Main findings:
- S and N were the most enriched proteins in COVID19 patient sera compared to pre-pandemic control sera in both HuCoV and Virscan libraries
- Significant antibody response to open reading frame (ORF) 3a accessory protein with HuCoV library
- ReScan produced plaque colonies of an average of 98% purity
- 9 SARS-CoV-2 antigens (all restricted to S, N or ORF3a) were found in more than 4 plaques and had >80% sequence clonality
- Dotblotr analysis of microarray found 95% of the COVID19 subjects significantly stained positive for at least one of the nine candidate antigens compared to controls, with one patient staining positive for all nine antigens
- Antigen peptides of the S protein were the most broadly reactive antigen targets
- PhIP-seq supported findings of Virscan and ReScan

Highlights
- Developed a pan-hCoV proteome-wide phage display assay capable of profiling CoV antigens enriched by COVID19 patient sera
- Identified 9 candidate antigens of the S and nucelocapsid N proteins, as well as ORF3a

Clinical Impact:
- Limited –provides basis for serological assays to be further developed with enhanced specificity, supports data suggesting S, N and ORF3a antigens are good targets, further work is needed

Important Methodologies:
- COVID19 diagnosis by RT-qPCR
- Virscan, ReScan and PhIP-seq

- Phage stocks acoustically printed by Labcyte ECHO acoustic array liquid handler
- Blots imaged on LICOR Odyssey CLX scanner and sequenced by NGS
- Images processed using a dotblotr algorithm

Limitations:
- Small sample size (serum from 20 COVID19 confirmed patients)
- Doesn't look at IgM targets (play a big role in early humoral immune responses)
- Post translational modifications and tertiary/quaternary structural epitopes not presented on T7 phage display library here

Potential antiviral options against SARS-CoV-2 infection

Ianevski, A. *et al*. 2020. *bioRxiv*
Link: https://www.biorxiv.org/content/10.1101/2020.05.12.091165v2

Summary:
Screening of 136 safe-in-man broad-spectrum antivirals (BSAA's) identified 6 candidates against SARS-CoV-2; nelfinavir, salinomycin, amodiaquine, obatoclax, emetine and homoharringtonine. Synergistic effects of nelfinavir were found with the above named 5 drugs, against SARS-CoV-2 *in vitro*. Convalescent plasma treatment from patients who have recovered from SARS-CoV-2 was effective at killing SARS-CoV-2 infected cells *in vitro*. Drug repurposing and convalescent plasma are valuable strategies as treatments in the SARS-CoV-2 pandemic, potentially reducing costs and time to clinically available therapeutics. An informative website has been created to disseminate knowledge on developing treatments and testing for COVID-19 infection.

Main findings:
- Five of seven serum samples from patients recovered from COVID-19 rescued >50% cells from virus-mediated death at 1 mg/mL. Sera from patients recovered from COVID-19 contains antibodies which neutralise SARS-CoV-2.
- Identification of *in vitro* killing of SARS-CoV-2 for safe laboratory work by virus incubation at 37^{o}C for 48 hours or UVC exposure for 10 seconds.
- Drug screening identified 9 compounds that rescued virus-infected cells from death *in vitro* (AUC >285).
- Further testing on these compounds for toxicity, efficacy and anti-viral activity validated only 6 drug candidates; nelfinavir, salinomycin, amodiaquine, obatoclax, emetine and homoharringtonine.
- Cells were treated with varying concentrations of two-drugs in combination and cell viability monitored for synergism testing. Nelfinavir with salinomycin, amodiaquine, obatoclax, emetine and homoharringtonine was found to be synergistic.
- Nelfinavir-amodiaquine combination is effective against multiple SARS-CoV-2 strains.

Highlights:

- Drug repurposing- identified potential safe-in-man anti-viral drugs for COVID-19.
- Development of a freely accessible website (*sars-coronavirus-2.info*) summarising current developments towards SARS-CoV-2 such as clinical trials and diagnostic tests.

Clinical Impact:

- Moderate.

Important Methodologies:

- Neutralisation assay using SARS-CoV-2 virus and Monkey Vero-E6 cells.

Limitations:

- All work is done *In vitro*. Testing of these compounds in COVID-19 patients needed.

Proteomics of SARS-CoV-2-infected host cells reveals therapy targets

Bojkova, D. *et al*. 2020. *Nature*
Link: https://www.nature.com/articles/s41586-020-2332-7

Summary:
The authors aimed to identify host cell pathways which are modulated by SARS-CoV-2 infection and to show that inhibition of these pathways may prevent viral replication in human cells. A human cell culture model was established. Quantitative translatome and proteome proteomics were used to obtain an unbiased profile of the cellular response to SARS-CoV-2 infection in human cells. Caco-2 cells were as a cell culture method of COVID-19. Different time points post infection were monitored and used to identify key determinants of the host cell response to infection. These findings revealed pathways relevant for SARS-CoV-2 infection. Several drugs targeting these pathways – translation, proteostasis, glycolysis, splicing and nucleotide synthesis – were tested. These drugs inhibited SARS-CoV-2 replication at non-toxic concentrations, indicating therapeutic strategies for COVID-19.

Main findings:

- Various proteomics analyses undertaken at various stages post infection.
- Translation inhibitors prevented replication of the virus in cells.
- Early time points (time points ranged from 2-24 hours) only showed minor proteome changes; the proteome underwent extensive modulation 24 hours post infection.
- Quantitative analyses of proteome changes upon infection with COVID-19 revealed host pathways changes upon infection and revealed spliceosome and glycolysis inhibitors, such as 2-deoxy-D-glucose, as potential therapeutic agents for COVID-19.
- Ribavirin, which inhibits inosine monophosphate dehydrogenase, the rate-limiting enzyme in de novo synthesis of guanosine nucleotides, inhibited SARS-CoV-2 replication at low micromolar and clinically achievable concentrations. This is

consistent with data from monkey cells. Considering this data and the clinical use of ribavirin to treat viruses such as hepatitis C – the author suggests that Ribavirin may be regarded as a treatment option for COVID-19 patients.
- A clinical trial for Ribavirin was recently initiated (NCT04356677).
- Proteomic analyses presented a profound increase in spliceosome, proteostasis and nucleotide biosynthesis pathway components. Revealing these pathways provided novel drug targets, which were based on the behaviour of SARS-CoV-2 in human cells and had not previously been tested with other coronaviruses.

Highlights:
- Successfully identifies drugs which inhibit translation, proteostasis, glycolysis, splicing and nucleotide synthesis of COVID-19; highlighting cellular pathways for therapeutic intervention.

Clinical Impact:
- Suggests various potential drug candidates for COVIS-19, therefore could have high clinical impact should these also be successful in human trials.

Important Methodologies:
- Caco-2 cells used as cell culture model for infection of COVID-19.
- For proteome analysis, a novel method described by the authors elsewhere is used; multiplexed enhanced protein dynamics (mePROD) proteomics.

Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein

Zost, S.J. *et al*. 2020. *bioRxiv*
Link: https://www.biorxiv.org/content/10.1101/2020.05.12.091462v1

Summary:
Zost et al., report the discovery of monoclonal antibodies (mAbs) directed against the SARS-CoV-2 spike (S) protein from four SARS-CoV-2 infected patients. Antigen-specificity to the S protein prefusion ectodomain (S2Pecto), S protein receptor binding domain (SRBD) and S protein N terminal domain (SNTD) was characterised by single-B-cell RNAseq methods. Sequences of mAbs were coupled with functional characterisation by high-throughput IgG micro-expression and real-time neutralization assays. The data was stratified into five classes of mAb based on the site of reactivity and cross-reactivity with SARS-Co-V.

Main findings:
- Isolated 386 recombinant SARS-CoV-2-reactive human mAbs
- ELISA and neutralization screening assays revealed groups of mAbs

- Class I mAbs bind to both S2Pecto and SRBD proteins and are SARS-CoV-2 specific; Class II mAbs also bind to both S2Pecto and SRBD proteins and cross-react with SARS-CoV; Class III mAbs bind to both S2Pecto and SNTD proteins and are mostly SARS-CoV-2 specific; Class IV mAbs bind only to S2Pecto protein and are SARS-CoV-2 specific; Class V mAbs bind only to S2Pecto protein and cross-react with SARS-CoV
- rapid cell-impedance-based SARS-CoV-2 neutralization test showed most neutralising mAbs mapped to the SARS-CoV-2 receptor binding domain (RBD)

Highlights:
Novel and rapid method for antibody discovery

Clinical impact:
Potentially high

Important methodologies:
- Approach 1: antigen-specific B-cells were either direct sequenced or expanded in cell culture producing lymphoblastoid cell lines (LCLs) secreting high amounts of antibody. Expanded LCLs were direct sequenced or selected by microfluidic device for an on-chip, fluorescence-based assay. Antibody heavy and light chain sequences for B-cells secreting antibodies with RBD-mFc- or S2Pecto-binding antibodies were sequenced and function verified by ELISA
- Approach 2: heavy and light chain antibody libraries from RBD-mFc- or S2Pecto protein-specific B-cells were profiled by Chromium Single Cell V(D)J workflow and sequenced
- Selected heavy and light chain pairs were synthesised and cloned to produce mAbs
- Neutralisation activity of recombinant mAbs assessed by real-time cell analysis assay (RTCA) (impedance assay)

Limitations:
- Samples collected from four SARS-CoV2 patients at different time points post-infection that may skew sequences of B-cell heavy and light chains throughout infection
- Ethnicity of patients not described. There may be differences in the genetic profiles of secreted antibodies from diverse ethnic groups

Vaccines

A strategic approach to COVID-19 vaccine R&D

Corey, L. *et al*. 2020. *Science*
Link: https://science.sciencemag.org/content/early/2020/05/15/science.abc5312.long

Summary:
Policy Forum: Evaluation of the ACTIV (Accelerating COVID-19 Therapeutic Interventions and Vaccines) partnership between the NIH and industrial partners which aims to generate safety/efficacy data from several candidate vaccines at once. The hope is that this harmonised approach will result in getting to the end goal, a global vaccine for SARS-CoV-2, faster.

Main findings:

- Question what level of immune response protects against re-infection.
- There are concerns vaccines could result in a more severe reaction to subsequent SARS-CoV-2 infection. Given that re-exposure is likely this is a major concern.
- Primary trial endpoints discussed:
    - Protection from infection (defined by seroconversion).
    - Prevention of clinically symptomatic disease.
- Given the number of asymptomatic cases a larger initial enrolment will be required, especially if testing in younger "low risk" adults.
- Would you see the pulmonary complications in a low-risk population?
- Hope is that identical serologic assays would be utilised between laboratories and samples shared between sites, if required.
- Would need to follow-up long term to see how long any protective effect may last.
- Companies are developing:
    - Nucleic acid-based vaccines. Based on viral sequence speeding up time to clinic but little known about the immunogenicity.
    - Recombinant expressing of spike protein (and adjuvant). Tried and tested, though novel vectors preferred to avoid pre-existing immunity.
- Candidates entering phase 1, need to start planning for phase 3 trials which are more labour intensive.
- This level of collaboration, including with the FDA and CDC means that a wider consensus can be reached on vaccine trials, rapid data sharing and hopefully efficient studies.
- Believe that common independent labs should be used and common "master" protocols for serologic assays to enable clear and comparative evaluation.
- Monitored by one common board looking at data which should be shared between partners to provide independent statistical analysis early on.
- Finally scaling up vaccine production may need to look at infrastructure and potential distribution to communities worldwide.

Highlights:
Discussion of ACTIV strategy to get a global SARS-CoV-2 vaccine by testing multiple vaccines in a large-scale collaboration.

Clinical Impact:
Low, outlined plan for ACTIV vaccine development.

Important Methodologies:
N/A.

Limitations:
N/A.

ChAdOx1 nCoV-19 vaccination prevents SARS-CoV-2 pneumonia in rhesus macaques

van Doremalen, N. *et al*. 2020. *bioRxiv*
Link: https://www.biorxiv.org/content/10.1101/2020.05.13.093195v1

Summary:
Doremalen and Lambe et al. evaluate the efficacy of ChAdOx1 nCoV-19 (adenovirus-vectored vaccine encoding SARS-CoV-2 spike protein) in mice and rhesus macaque monkeys. They demonstrate that ChAdOx1 nCoV-19 is immunogenic in both animals, eliciting a strong humoral & cellular immune response. Overall, results of trial in rhesus macaques suggests that the candidate vaccine may not be completely effective in preventing SARS-CoV-2 infection but could potentially reduce the severity of Covid-19. The results from this study enabled progression into human clinical trials with ChAdOx1 nCoV-19. As of 13th May 2020, 1000+ volunteers have participated in this phase I clinical trial in the UK.

Main findings:
- Virus-specific neutralising antibodies were detected in all vaccinated mice & rhesus macaque monkeys. No neutralisation was detected in serum of animals injected with control vaccine (ChAdOx1 GFP).
- The immune response was predominately Th1 post ChAdOx1 nCoV-19 vaccination in both animal models. Upregulation in IFN-γ (but no significant differences in TNF-α , IL-2, IL-4, IL-6, and IL-10) was observed between vaccinated and control rhesus macaques.
- All vaccinated rhesus macaque monkeys had spike-specific neutralising antibodies prior to SARS-CoV-2 challenge (vaccinated 28 days before).
- No difference in viral load in nasal swabs between vaccinated and control rhesus macaques.
- Viral load in bronchoalveolar lavage fluid and lung tissue of vaccinated rhesus macaques challenged with SARS-CoV-2 was significantly lower compared to control animals.

- Lungs of vaccinated animals were histologically normal, and no SARS-CoV-2 antigens were detected in lung tissue post vaccination.
- None of the vaccinated rhesus macaques developed pneumonia post viral challenge.

Highlights:

- A single dose of ChAdOx1 nCoV-19 (*2.5×10^{10} particles; half of the dose administered in ongoing human clinical trial*) induced a robust immune response and prevented lung damage following SARS-CoV-2 infection in rhesus macaques.
- None of the vaccinated animals showed immune-enhanced disease post SARS-CoV-2 infection.
- ChAdOx1 nCoV-19 vaccination did not prevent viral shedding in nasal secretions.

Clinical Impact:

High (some findings challenge safety, immunogenicity & efficacy of ChAdOx1 nCoV-19 against Covid-19 in humans).

Important methodologies:

- Generation and characterisation of SARS-CoV-2 candidate vaccine, ChAdOx1 nCoV-19.
- ChAdOx1 nCoV-19 trial in non-human primates against SARS-CoV-2 informed start of human clinical trials with candidate vaccine.
- IgG subclasses and cytokine expression profiling revealed strong Th1 response post ChAdOx1 nCoV-19 vaccination.

Limitations:

- Sample size was very small (BALB/c mice (N=5), outbred CD1 mice (N=8) and rhesus macaque monkeys (N=6) were vaccinated).
- Different vaccination regimes not explored (e.g. multiple doses, dose titrations etc.)
- Longitudinal/lengthier studies are required to assess long-term protective immunity from ChAdOx1 nCoV-19 and determine efficacy of candidate vaccine against SARS-CoV-2 reinfection.

Antibody repertoire induced by SARS-CoV-2 spike protein immunogens

Ravichandran, S. *et al*. 2020. *bioRxiv*

Link: https://www.biorxiv.org/content/10.1101/2020.05.12.091918v1

Summary:

Sera from rabbits immunized against several sections of the SARS-CoV-2 spike protein ectodomain was evaluated in-vitro. The sections were domain 1 (S1), domain 2 (S2), S1+S2 and the receptor binding domain (RBD; in S1). Each immunogen produced specific reactive antibodies. Rabbits immunized with S1+S2 had antibodies which mostly mapped to either the RBD or a specific region in S2. Rabbits immunized with either S1 or S2 mapped to those same respective areas. Antibodies to S1 were better able to neutralise pseudovirus whilst antibodies against S2 was less than half as effective but only RBD antibodies had a high affinity.

Main findings:

- Immunized rabbits generated antibodies specific to their immunogen by ELISA, SPR and phage panning based epitope mapping. By SPR, RBD produced the strongest binding antibodies to S1+S2, S1 and the RBD.
- By phage panning for the epitopes targeted in sera, the RBD, an adjacent region closer to the C termini and H2 region were immunodominant but the latter is unlikely to be exposed on virions or infected cells.
- S2 is less able to produce antibodies with a high affinity to full length spike.

Highlights

- 4 identified sera epitopes overlapped with in silico identified B-cell epitopes and have RBD epitopes have homology with SARS-CoV-1.
- This paper cites several studies correlating high affinity antibodies with beneficial clinical outcome. The RBD invokes the best affinity antibodies against spike protein S1+S2 indicating that targeting it in vaccine design is sensible.
- Sera from rabbits immunized with (HEK293T cell produced) S1 or RBD protein cross-react by ELISA with (insect cell produced) S1+S2 indicating differences between mammalian and insect post translational modification did not affect their results.

Clinical Impact:

- None, this paper is of interest to vaccine science.

Important Methodologies:

- The ectodomain portion of the SARS-CoV-2 spike protein (S, made up of S1 and S2) was produced in vitro, The S1 domain and the ACE2 receptor binding constituent (RBD) were both produced in HEK293T cells whilst the S2 and full ectodomain (S1+S2) was produced in insect cells
- Intramuscular immunization of Rabbits using the above proteins with a 14-day interval with Sera collection before, and after each immunization.
- Sera was tested by four in vitro parameters, ELISA, SPR, Pseudovirus neutralisation and epitope mapping via SARS-CoV-2 GFPDL phage panning.

Limitations:

- This paper would benefit table of abbreviations or inclusion of abbreviation definitions in text. Figure1, RBM and HR1 and 2 are not defined.
- The authors do not state if sera collection after immunization was immediate or after several days etc. I fail to see the point of taking sera immediately after the administration of the first vaccine. I assume the 14-day interval is considered part of the vaccination, and so sera is collected on day 14 and 28. However, it would be good to state this.
- After a literature search, Vero E6 cells (used in the pseudovirus neutralisation assay) do express the ACE2 receptor. This paper would benefit from referencing this fact or data showing this by Flow Cytometry.
- The number of rabbits immunised is not given.
- This study lacks any in vivo validation of their strategy of immunization.

Transmission

Investigation of a COVID-19 outbreak in Germany resulting from a single travel-associated primary case: a case series

Böhmer, M.M. *et al*. 2020. *The Lancet*

Link: https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(20)30314-5/fulltext

Summary:

Merle M Böhmer et al. report 16 subsequent cases emerged in 4 generations from a Chinese resident patient. Although patients presented with predominately mild, non-specific symptoms, infectiousness before or on the day of symptom onset was substantial. Additionally, the incubation period was often very short (4.0 (IQR: 2.3-4.3) days) and false-negative tests occurred. The secondary attack rate differs among contact categories. These results suggest that although the outbreak was controlled, successful long-term and global containment of COVID-19 could be difficult to achieve.

Main findings:

- A Chinese resident patient visited Germany. 16 subsequent cases, often with mild and non-specific symptoms, emerged in 4 transmission generations.
- Signature mutations in the viral genome occurred upon foundation of generation 2, and in one case pertain to generation 4.
- The median incubation period was 4.0 days (IQR: 2.3-4.3). The median serial interval was 4.0 days (IQR: 3.0-5.0).
- Transmission events were likely to have occurred presymptomatically for one case (possibly 5 more), at the day of symptom onset for 4 cases (possibly 5 more), remainders after the day of symptom onset or unknown. 1 or 2 cases resulted from contact with a case during the prodromal phase.
- Secondary attack rate:
  . 75.0% (95% CI: 19.0-99.0; 3 of 4 people) among members of a household cluster in common isolation.
  . 10.0% (1.2-32.0; 2 of 20 people) among household contacts only together until patient isolated.
  . 5.1% (2.6-8.9; 11 of 217) among non-household, high risk contacts.

Highlights

- Transmission could occur before or on the day of symptom onset in patients with mild and non-specific symptoms.
- Short incubation period and possibility of false negative test suggests that successful long term and global containment of COVID-19 could be difficult to achieve.

Clinical Impact:

- Substantial

Important Methodologies:

- Case series study
- Confirm infection by RT-PCR; use whole genome sequencing to support epidemiological links confirmation

Limitations:

- Case series study with small sized sample.

Modelling the evolution of COVID-19 in high-incidence European countries and regions: estimated number of infections and impact of past and future intervention measures

Fernandez-Recio, J. 2020. *medRxiv*
Link: https://www.medrxiv.org/content/10.1101/2020.05.09.20096735v2

Summary:
The study updated a previous Bayesian model of disease transmission to estimate the effects of 'major lockdown' on the reproduction number of SARS-CoV-2. This was carried out for countries with the highest numbers of totals cases in Europe (Spain, Italy, UK, France, and Germany) or countries with the highest number of cases in proportion to population (Iceland, Spanish region of La Rioja). Major lockdown had a similar impact on disease transmission in most countries. Modelled estimates of total infection numbers were significantly higher than currently reported infections. This model could be of use when future planning changes to intervention measures.

Main findings:

- Reproduction number following major lockdown intervention (R_t) was similar in analysed countries, with mean values ranging from 0.57 to 0.71
- R_t after intervention, as a percentage of R_0 before intervention, ranged from 12.0% (Spain) to 20.7% (Italy)
- Cumulative number of infections until May 5th 2020 were predicted, ranging from 3,350 (Iceland) to 3,800K (UK)
- The highest rates of infection detection were found in Iceland (54%) and German (21%), countries with the highest number of tests performed relative to their population, whilst estimated infection detection rates in other countries ranged from 5% (UK) to 10% (La Rioja)
- The reliability of the predictions depended on the stage of outbreak in each country, with increased predictive reliability observed if more time had passed from peak of outbreak (e.g. Spain, Italy), indicating that predictions made earlier in the outbreak are likely to be less reliable

- The potential effect on the reproduction number in Spain due to the gradual removal of lockdown measures was estimated using a range of scenarios without additional intervention measures, clearly predicting the possibility of a new epidemic outbreak in mid- September/October

Highlights

- Suggests significant impact of major lockdown intervention in all countries
- Generates realistic estimates of total infection numbers and active cases
- Provides a model that can aid planning future control measures

Clinical Impact:

- Minimal

Important Methodologies:

- Updated a Bayesian model of disease transmission inferred from reported deaths previously developed by researchers at Imperial College London to assess the impact of major lockdown interventions

Limitations:

- Model could benefit from the use of other available data distributions to not limit its predictive value
- Study modelled '*R*' as a constant value for long time periods (changing it only upon intervention) whilst '*R*' is a dynamic parameter
- Model did not consider geographical transmission

Antibodies and T-cells

A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2

Wu, Y. *et al*. 2020. *Science*
Link: https://science.sciencemag.org/content/early/2020/05/12/science.abc2241

Summary:
Four monoclonal antibodies isolated from a convalescent COVID-19 patient display neutralisation abilities *in vitro,* two of which (B38 and H4) block the interaction between ACE2 and the receptor-binding domain (RBD). B38 and H4 recognise different RBD epitopes and can reduce virus titres in infected mice – suggest using antibodies as a pair to avoid immune escape. Structural analysis to determine the binding of the antibodies to the RBD showed the specific residues responsible for this interaction and that 18 out of 21 amino acids on the COVID-19 RBD are identical in binding B38 and ACE2.

Main findings:
- Antibodies were tested for binding ability to COVID-19 RBD and neutralising activities:
  - B38 (Kd=70.1 nM, IC_{50} = 0.177 µg/ml)
  - H4 (Kd=4.48 nM, IC_{50} = 0.896 µg/ml)
  - B5 (Kd=305 nM, IC_{50} = 1.375 µg/ml)
  - H2 (Kd=14.3 nM, IC_{50} = 1.000 µg/ml)
- B38 and H4 showed complete competition with ACE2 for RBD binding, but recognise difference epitopes with partial overlapping. (B5 partial and H2 no competition)
- In hACE2 transgenic mice administered with 25 mg/kg of B38 or H4 12 hours after virus challenge, viral RNA copies were significantly lower with both treatments compared to PBS alone (32.8% and 26% respectively). Body weight of the B38 group recovered after 3 days compared to PBS and H4 groups
- Crystal structure of the B38 Fab with the viral RBD was determine at 1.9 Å resolution. Three complementarity-determining regions (CDRs) on the heavy chain (HCDR) and two CDRs on the light chain (LCDR) are involved in interaction with the viral RBD
- Authors detail the major interacting forces and specific residues responsible for the interactions of the binding interface between B38 and viral RBD and the similarities and differences between COVID-19 and SARS-CoV RBDs explaining why B38 is specific for COVID-19
- Binding of B38 results in steric hindrance to the RBD binding to ACE2 with no significant conformational changes, additionally, 18 out of 21 amino acids involved in the RBD binding to ACE2 and B38 are identical explaining why B38 prevents binding of COVID-19 to ACE2 receptor

Highlights
- Identification of two neutralising antibodies and elucidation of residues responsible for viral RBD binding to ACE2 receptor in COVID-19

Clinical Impact:

- Minimal

Important Methodologies:

- Bio-layer Interferometry (BLI) for antibody binding screening and to measure competitive binding of antibodies and hACE2
- Neutralisation assay
- Surface Plasmon Resonance (SPR) using BIAcore 8K system
- Crystal Screening and Structure Determination

Limitations:

- Preprint (has not been peer reviewed)
- Neutralisation assays do not use human cells (Vero), absence of control IgG1 in tests.
- Animal experiments - 3DPI termination seems a bit short for a virus that can take a week to 2 weeks to show symptoms. No control antibody shown in experiment. Administration only 12 hours after infection - would be nice to see if antibodies are effective if given after onset of symptoms, as this would be more relevant to the clinic. No data on half-life *in vivo* of antibody.

Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals

Grifoni, A. *et al*. 2020. *Cell*

Link: https://www.cell.com/cell/pdf/S0092-8674(20)30610-3.pdf

Summary:

Grifoni *et al.* examined the T cell response to SARS-CoV-2. All recovered patients had a CD4+ T cell response, predominantly targeting the spike, M and N proteins. The strength of the CD4+ T cell response to spike protein correlated with anti-SARS-CoV-2 IgG and IgA antibody titres. 70% of convalescent patients had CD8+ T cells that recognised SARS-CoV-2, predominantly its spike and M proteins. Using blood samples from before SARS-CoV-2 emerged, they found that ~ 50% of healthy donors had some SARS-CoV-2-reactive CD4+ T cells, which may have been generated in response to “common-cold”, coronaviruses and could cross-react to recognise SARS-CoV-2.

Main findings:

- SARS-CoV-2 spike protein-reactive CD4+ T cells were identified in 100% of COVID-19 convalescent patients based on expression of OX40+CD137+ in response to peptide pools. All patients also had a CD4+ T cell response to at least one other protein, mainly M and N proteins.

- The total CD4+ T cell response was comprised of spike:27%, M:21% and N:11% Most COVID-19 cases also had CD4+ T cells specific for SARS-CoV-2 nsp3, nsp4 and ORF8 (on average each accounting for ~5% of the total CD4+ T cell response).
- SARS-CoV-2-specific CD4+ T cells were functional, as the cells produced IL-2 in response to non-spike and spike MPs. Cells were TH1 type, with IFNγ production but little IL-4, Il-5, IL-13, or IL-17α.
- The strength of the CD4+ T cell response to the spike protein also correlated with the anti-SARS-CoV-2 IgG and IgA antibody titres in the plasma.
- 70% of convalescent patients had CD8+ T cells that recognised SARS-CoV-2, predominantly its spike and M proteins, based on expression of CD69+CD137+ in response to peptide pools
- Reactive CD8+ cells were mainly positive for IFNγ and granzyme B, many also produced TNF
- SARS-CoV-2 CD8+ T cell reactivity with spike protein accounted for ~26% of the reactivity, with N accounting for 12% , M;22%, nsp6;15%, ORF8;10% and ORF3a;7%
- Using blood samples from before SARS-CoV-2 emerged, ~ 50% healthy donors had some SARS-CoV2-reactive CD4+ T cells, hypothesised to be generated in response to "common-cold", coronaviruses and cross-react to SARS-CoV-2.
- All healthy controls were seroreactive to the "common-cold", coronaviruses HCoV-OC43 and HCoV-NL63
- In healthy donors with reactive CD4+ T cells, SARS-CoV-2-reactive CD4+ T cells targeted spike (23% of total reactive T cells), 2 nsp14 (25%), nsp4 (15%) and nsp6 (14%). However very low reactivity against N and M. Among donors with detectable CD4+ T cells, a shift in reactivity was observed towards SARS-CoV-2
- SARS-CoV-2-reactive CD8+ T cells were detected in 20% of healthy donors, but without clear targeting of specific SARS-CoV-2 proteins

Highlights:

- Memory CD4+ and CD8+ T cells are generated in response to a wide range of SARS-CoV-2 proteins, suggesting that the protective immune response may be long-lasting
- People who have been exposed to other coronaviruses may have CD4+ T cell responses that offer a degree of protection against SARS-CoV-2. This hypothesis would need further testing

Clinical Impact:

- Assessment of T cell responses should be included in vaccine development
- Vaccines currently in development mainly target spike and may benefit from additionally targeting M and N proteins as these contain T cell targets.

Important Methodologies:

- Megapool approach to peptide selection can predict dominant epitopes independently of ethnicity and HLA polymorphisms

Limitations:

- Studying the T cell response pre and post infection with SARS-CoV-2 would enable tracking of coronavirus cross-reactive cells and determine whether they can be protective.

Phenotype of SARS-CoV-2-specific T-cells in COVID-19 patients with acute respiratory distress syndrome

Weiskopf, D. *et al*. 2020. *medRxiv*

Link: https://www.medrxiv.org/content/10.1101/2020.04.11.20062349v1

Summary:

The phenotype of virus specific CD4+ and CD8+ T cells in COVID19 patients was measured 14 days after ICU admission by stimulating PBMC with three peptide pools (n=1095) covering the SARS-CoV-2 proteome. Activated T cells produced Th1 and Th2 cytokines and repeated measures during the ICU period showed an increase in Spike (S) protein epitope responsive CD4+ T cells.

Main findings

- Lymphopenia and an increased CD4:CD8 T cell ratio on day 14 in the ICU. There is a lack of consensus on the CD4:CD8 T cell ratio in COVID19 disease, which may be due different studies sampling at different time points. The authors speculate recruitment of CD8+ T cells to the lungs may alter the T cell ratio.
- COVID19 patients had a significantly higher frequency of CD69+ CD137+ (activated) CD4+ and CD8+ T cells in response to all of the SARS-CoV-2 peptide pools than healthy controls.
- PBMC stimulated with the S protein peptide pool produced high levels of Th1 cytokines INFγ TNFα and IL-2 and moderate Th2 cytokines IL-5, IL-13, IL-9, IL-10. Notably, there was no difference in IL-6 levels compared to healthy donors, suggesting T cells may not contribute to the IL-6-associated cytokine storm commonly observed in COVID19 patients.
- Viral load decreased over time, while virus specific serum IgG and activated CD4+ T cells (CD69+ CD137+) increased over time (Day 0- Day 21 in ICU).

Highlights

- SARS-CoV-2 responsive CD4+ T cells predominantly had a central memory (CD45RA-, CCR7+) phenotype. The activated CD8+ T cells phenotype was more varied, with large proportions of both central memory (CD45RA-, CCR7+) and terminally differentiated cells (CD45RA+, CCR7-). This seems to be the first study to investigate the effector/ memory phenotype of SARS-CoV-2 responsive T cells.

Clinical Impact

Minimal. The spike protein epitope data could be relevant for vaccine design.

Important Methodologies

- Peptide pools to stimulate CD4+ and CD8+ T cells respectively were designed from the SARS-CoV-2 proteome using epitope prediction. The CD4+ pool excluded predicted S protein epitopes as another peptide pool consisted of overlapping 15mer peptides from the S protein was produced to detect CD4 and CD8+ responses. PBMC were stimulated with 1µg/ml peptide pool or equimolar DMSO, PHA or 1µg/ml CMV peptide pool as controls.
- Virus positive status (RT-PCR), virus specific serum IgG (ELISA) and CD4+ T cell responses to the S protein peptide pool (flow cytometry) were measured at 0, 7, 14 and 21 days in ICU.
- Cytokines were measured from the supernatant of PBMC stimulation experiments by multiplex cytokine array.

Limitations

- Freeze-thawing the PBMC may have affected viability and impact cytokine production.
- The multiplex cytokine assay did not allow for determining which T cell phenotypes were producing the cytokines.
- There doesn't seem to be statistical analysis to support the claim that the S protein peptide pool induced greater CD4+ T cell responses in COVID19 patients than the non-S protein peptide pool. Additionally, the cytokine responses to the other peptide pools was not shown.
- Lack of statistical comparison to the baseline frequency of effector/ memory T cell phenotypes in healthy donors.
- Small sample size: n=8 patients, only n=2 of which were Day 21 in ICU or and n=8 controls.
- The T cell profiles were not correlated to the clinical course (possibly due to the small sample number).

Genomic Analysis

Emergence of genomic diversity and recurrent mutations in SARS-CoV-2

van Dorp, L. *et al*. 2020. *Infection, Genetics and Evolution*
Link: https://www.sciencedirect.com/science/article/pii/S1567134820301829

Summary:
van Dorp *et al.* analyse a data set of 7666 public genomic assemblies deposited since the beginning of the Pandemic to ascertain SARS-CoV-2 emergence of genomic diversity over time. All sequences appear to share a common ancestor towards the end of 2019 (Oct-Dec) when it jumped into its human host. They go on to identify multiple independent recurrent mutations at the protein level suggesting ongoing adaptation to the human host. They also provide links to an interactive user-friendly web application to query the alignment of the 7666 SARS-CoV-2 genomes. Such analyses enables monitoring patterns of genetic diversity to potentially inform targets for drugs and vaccine development and determine animal source.

Main findings:
- All 7666 sequences share a common ancestor (yet to be determined) which transmitted to human host between 6th Oct-Dec 2019.
- 9.6 SNPs between any 2 genomes supports a common ancestor.
- 198 multiple independent mutations were identified within SARS-CoV-2 genome.
- 80% of recurrent mutations resulted in non-synonymous changes at the protein level.
- 3 sites in Orf1ab in regions Nsp6, Nsp11, Nsp13 and one in the spike protein had a large number of recurrent mutations (>15 events) of particular interest in adaptation to the host.
- Strong recurrent mutation at nucleotide position 11,083 (codon 3606 Orf1a) encoding Nsp6, 2 further sites in Orf1ab encoding Nsp11 and 13 and the Spike protein (21,575, Codon 5, outside of N-terminal and receptor binding domain).
- Estimate mutation rate SARS-CoV-2 is approx. 6×10^{-4} nucleotides/genome/year (CI: 4×10^{-4} – 7×10^{-4}), which is modest for an RNA virus.
- Recurrent mutations at regions predicted to be linked to CD4+ T-cell epitope and CD4+ and CD8+ T-cells reactivity.

Highlights
- Local epidemics were seeded by a large number of independent introductions of the virus with the exception of China which displayed very little global diversity.
- Global diversity occurred extremely early on in the time line of the pandemic.
- 198 sites in SARS-CoV-2 appear to have recurrent mutations suggesting ongoing adaptation to human host.

Clinical Impact:
- None

Important Methodologies:

- Analyse 7710 SARS-CoV-2 assemblies (>29,000bp) from GISAID, after filtering, 7666 assemblies remained for analysis.
- Multi-sequence alignment against reference sequence Wuhan-Hu-1 and maximum likelihood tree assemblies.

Limitations:

- Only look at data available at one point in time (up to April 19 2020)
- Reliant on quality of publicly available assemblies, some maybe artefactual.

Mechanisms of Disease

The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice

Bao, L. *et al*. 2020. *Nature*
https://www.nature.com/articles/s41586-020-2312-y

Summary:
Bao et al. demonstrate that transgenic expression of the human ACE2 receptor (hACE2) renders mice susceptible to infection with SARS-CoV2, describing a potential first animal model of the COVID19. Mice developed minor disease associated with weight loss, histological changes indicative of pneumonia and IgG antibodies against S protein of SARS-CoV-2.

Main findings:
- hACE2 was essential for SARS-CoV-2 infection and replication in mice
- infected lungs were infiltrated by macrophages, T and B cells and showed histological changes associated with pneumonia
- Viral antigen was detected in macrophages and lung epithelial cells but no other organs
- IgG antibodies against SARS-CoV-2 spike protein were identified 21 days post infection

Highlights
- First animal model for SARS-CoV-2

Clinical Impact:
Low, while a mouse model could be useful for testing of vaccines and antiviral therapies this model does not sufficiently represent human disease regarding severity and systemic spread of viral particles

Important Methodologies:
- Transgenic mice bearing human ACE2 were infected with SARS-CoV-2
- Mice were sacrificed at 1, 3, 5 and 7 days post infection to collect different tissues to screen virus for replication and histopathological changes
- Immunohistochemistry was used to identify lung infiltrating immune cell populations
- Viral loads in lungs were detected by qRT-PCR
- Specific IgG against SARS-CoV were determined by ELISA

Limitations:
- Very low number of mice, only 3 per time point
- The model does not represent human disease to a substantial extent

Meta-analysis of transcriptomes of SARS-Cov2 infected human lung epithelial cells identifies transmembrane serine proteases co-expressed with ACE2 and biological processes related to viral entry, immunity, inflammation and cellular stress.

Wruck, W. & Adjaye, J. 2020. *bioRxiv*

Link: https://www.biorxiv.org/content/10.1101/2020.05.12.091314v1

Summary:

Referring to previous knowledge about SARS-CoV using ACE2 for entry with serine protease TMPRSS2 for spike protein priming, the others used meta-analysis of transcriptomes to identify co-expressed proteins with ACE2 found with SARS-CoV-2. RNA-seq data analysis identified TMPRSS4 being the most prevalent transmembrane serine protease co-expressed with ACE2. TMPRSS4 can proteolytically cleave hemagglutinin of influenza viruses. TMPRSS11D and TMPRSS11E also alludes to co-expression with ACE2. The authors propose that inhibitors of TMPRSS family members must be studied further, where the most significantly co-expressed is TMPRSS4, suggesting a possible therapeutic target.

Main findings:

- TMPRSS4 (r=0.1942, $p=4.59^{-20}$) is one of the most significantly correlated gene with ACE2
- CXCL17 (r=0.9273, $p=1.1^{-21}$), ABCA12 (r=0.9256, $p=1.92^{-21}$) and ATP10B (r=0.9193, $p=1.14^{-20}$) also found with high correlation – involved in immune response
- Most significantly overrepresented pathways associated with the genes correlate with ACE 2 are *Bacterial invasion of epithelial cells* (q=4.4E-06), *Human papillomavirus infection* (q=0.0006), *Transcriptional misregulation in cancer* (q=0.0006) and *Endocytosis* (q=0.002).
- Most significant GO-BPs are *Interspecies interaction between organisms, Cytokine production* and *positive regulation of viral process.* These reflect activated mechanisms post-viral infection. As well as *regulation of coagulation.*

Highlights:

- TMPRSS4 (r=0.1942, $p=4.59^{-20}$) is one of the most significantly correlated gene with ACE2
- TMPRSS2 inhibitors have been proposed by Hoffmann et al.11 - and earlier for the SARS-CoV virus by Kawase *et al*. as working best together with cathepsin B/L inhibitors.
- *CALM1* inhibits shedding of the ectodomain of the virus receptor ACE2

Clinical Impact:

- Limited

Important Methodologies:

- Next-generation sequencing datasets measured in RNA-Seq experiments with lung cells (GSE147507:Illumina NextSeq 500; GSE146482: Illumina NovaSeq 6000; GSE85121: Illumina HiSeq 2500) were downloaded from NCBI GEO.
- The Pearson correlation of the normalized gene expression values for all samples was calculated between the gene *ACE2* and each other gene.

- The R package GOstats48 was employed for over-representation analysis of positively and negatively correlated genes with the CoV-2 receptor gene ACE2

Limitations:

- This is a meta-analysis based solely on transcriptome and not proteome data
- Further *in-vitro* and *in-vivo* studies needed

Neuroimmunology & Mental Health

Neurological manifestations of patients with COVID-19: potential routes of SARS-CoV-2 neuroinvasion from the periphery to the brain

Li, Z. *et al*. 2020. *Frontiers of Medicine*
Link: https://link.springer.com/article/10.1007/s11684-020-0786-5

Summary:
This review explores the potential routes of neuroinvasion of SARS-CoV-2 into the brain. The 'cytokine storm' induced by the immune system as well as systemic hypoxia following severe respiratory distress may facilitate entry, through various different proposed routes:

1. Olfactory/Trigeminal nerves: the virus adheres to nasal mucosa and directly infects olfactory sensory neurons, then is transported to the CNS via the olfactory or trigeminal nerve.
2. Blood: the virus causes widespread inflammatory damage, potentially including the blood-brain barrier, allowing infected circulating immune cells from the lungs into the brain.
3. Lymphatic system and CSF: the virus directly invades lymph nodes and damages lymphatic drainage pathway, meaning that the virus can enter CSF and therefore the brain.

Highlights
- It is likely that the olfactory nerve pathway may be the main way for the brain to enter in the early stages of infection
- In severe cases, the virus is likely to enter through a damaged blood-brain barrier and may case dysfunction of the cardiorespiratory centre in the brain stem

Clinical Impact?
Moderate: awareness of potential neuroinvasion will have significance for treatments plans, including long-term neurocognitive rehabilitation

Important Methodologies
N/A.

Limitations
N/A.

Brain MRI Findings in Patients in the Intensive Care Unit with COVID-19 Infection

Kandermirly, S.G. *et al.* 2020. *Radiology*
Link: https:/pubs.rsna.org/doi/10.1148/radiol.2020201697

Summary:
The paper presents a series of 10 COVID-19 patients with neurological symptoms, who had abnormalities found in brain MRI scans. 235 COVID-19 patients in Turkish hospitals were retrospectively analysed, 50 of these (21%) had clinically relevant neurological symptoms, 27 of which underwent MRI scans. In 12 of these (44%), abnormalities were found.

Main Findings
- The main findings (in 10 patients) were cortical FLAIR signal abnormalities.
- Affected areas were frontal lobe (n=4), parietal lobe (n=3), occipital lobe (n=4), temporal lobe (n=1), insular cortex (n=3) and cingulate gyrus (n=3) patients.
- Subcortical and deep white matter signal abnormalities were found in three patients each.

Clinical Impact
- High, a significant fraction of patients may develop CNS abnormalities, even in the absence of the virus in the CNS. This may contribute to long term consequences of infection.

Important Methodologies
- Retrospective analysis of clinical brain imaging

Limitations
- No previous imaging data existed for patients, the possibility that these were pre-existing undiagnosed conditions cannot be excluded.
- CSF was only available for 5 patients, it is unknown if any SARS-CoV-2 was present in the CNS of other patients.
- No attempts were made to identify the nature of the FLAIR abnormalities.

Does SARS-Cov-2 invade the brain? Translational lessons from animal models

Natoli, S. *et al.* 2020. *European Journal of Neurology*
Link: https://onlinelibrary.wiley.com/doi/abs/10.1111/ene.14277

Summary
This review considers animal models of previous coronavirus (CoV) infections, namely SARS and MERS and how they might inform our approach to understanding the current Covid-19 pandemic, caused by SARS-CoV-2. The common mechanism to all three viruses is the requirement for the presence of angiotensin-converting enzyme 2 (ACE2) as a cell entry receptor. Animal models have demonstrated the ability of coronaviruses to infiltrate the brain, but the mechanism of how this happens remains unresolved. This review highlights the need for SARS-CoV-2 research to focus on elucidating these mechanisms, particularly given the growing neurological impact of Covid-19.

Main Findings
- To produce a clinically relevant phenotype in an animal model of CoVs is challenging as it only occurs with manipulation of the animal or the virus.
- K18-hACE2 mice show neurons are susceptible to infection by SARS-CoV. The virus enters the brain via the olfactory bulb and spreads quickly transneuronally.
- In a MERS models model brain invasion occurs later than infection of the lungs and pathological changes were minimal and inconsistent.
- Analysis of WOM RNA seq data illustrated the lack of 2 key genes (ACE2 and TMPRSS2) in human olfactory sensory neurons. These are required for cell entry of SARS-CoV-2. However olfactory epithelial support cells do express these genes suggest a potential mechanism behind the anosmia some Covid-19 patients experience.

Highlights
- The pathogenicity of SARS-CoV-2 appears lower than SARS-CoV in mice demonstrating the need for the development of news tools to study SARS-CoV-2 infection that is translatable to humans.
- Challenging to produce a clinically relevant phenotype in animals

Clinical Impact
- Low

Important Methodologies
- Literature review of animal models of other coronaviruses.

Limitations
- Lack of neurological manifestations in SARS and MERS meant the corresponding research in animal models was not carried out.
- Does not highlight a common mechanism of how CoVs invade the nervous system, this may be via different routes and depend on disease stage