COVID-19 Community Journal Club No. 3

April 30th, 2020

School of Medicine, Cardiff University

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

Thank you to *Elise Moses, Pragati Sabberwal, Sophie Reed, Ruth Jones, Rebecca Bayliss, Emma Mitchell, Ruban Durairaj, Owen Moon, Sarah Carpanini, Stephanie Burnell, Camille Rabesahala De Meritens, Kate Liddiard, Ross Burton, Sarah Lauder, Patricia Dos Santos Rodrigues, Lorenzo Capitani, Ana Pires, Ellie Pring, Alicia Teijeira Crespo, Stephanie Hanna, Tom Pembroke, Valentina Bart, Sarah Curtis, Freya Shepherd, Stefan Milutinovic, Van Dien Nguyen, Yuen Chen, Amy Codd, Sarah Gallaway, Shayda Toyserkani, Lucy Chapman, Sandra Dimonte and Oliver Scourfield* for producing these reviews.

and to *Drs Ceri Fielding, Carmen Van den Berg, Luke Davies, Andrew Godkin, Kristin Ladell, Emma Jones and James Matthews* for paper selection.

Further sources of Covid-19 literature and webinars can be found on Page 6

NEW! Covid-19 in podcasts (pick of the week by Dr Emma Jones – see Page 7)

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

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Link: https://doi.org/10.1016/ S0140-6736(20)30937-5

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Zheng *et al*. the BMJ/2020
Link: https://www.bmj.com/content/369/bmj.m1443

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Muus, C., Luecken, M. D., Eraslan, G *et al*. bioRxiv April 21 2020
Link: https://doi.org/10.1101/2020.04.19.049254

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Jérôme Hadjadj *et al*. (medRxiv), 2020
Link: https://www.medrxiv.org/content/10.1101/2020.04.19.20068015v1

A single-cell atlas of the peripheral immune response to severe COVID-19 **28**
Aaron J. Wilk *et al*. *MedRxiv preprint server* (2020)
Link: https://doi.org/10.1101/2020.04.17.20069930

Antibodies and T Cells

A highly conserved cryptic epitope in the receptor-binding domains of SARS-CoV-2 and SARS-CoV **30**
M. Yuan *et al*., Science (2020)
Link: https://science.sciencemag.org/content/early/2020/04/02/science.abb7269

Potent neutralizing antibodies in the sera of convalescent COVID-19 patients are directed against conserved linear epitopes on the SARS-CoV-2 spike protein **31**
Chek Meng Poh **et al**., (Preprint article), 2020
Link: https://www.biorxiv.org/content/10.1101/2020.03.30.015461v1.full

Effectiveness of convalescent plasma therapy in severe COVID-19 patients **32**
Duan *et al*. (PNAS), 2020
Link: https://www.pnas.org/content/early/2020/04/02/2004168117

Vaccines

Epidemiology

Sources of further information:

Webinars – from the British Society for Immunology, hosted by Profs Danny Altmann and Arne Akbar

- "*these webinars hit the right tone between being informative and interesting without blinding you with detail*" …….Andy Godkin

https://www.immunology.org/coronavirus/connect-coronavirus-webinars/bsi-webinar-emerging-lessons-about-immunity-covid-19

https://www.immunology.org/coronavirus/connect-coronavirus-webinars/bsi-webinar-the-impact-altered-immunity-during-ageing-sars

https://www.immunology.org/coronavirus/connect-coronavirus-webinars/bsi-webinar-antibody-testing-neutralising-monoclonals-and

Other sources

NEWLY ADDED:
https://www.immunology.ox.ac.uk/covid-19/covid-19-immunology-literature-reviews

https://his.org.uk/covid-19-research-and-commentary/

https://observablehq.com/@ismms-himc/covid-19-sars-cov-2-preprints-from-medrxiv-and-biorxiv

https://www.rsb.org.uk/policy/policy-resources/policy-newsletter

https://reacting.inserm.fr/literature-review/

https://publons.com/publon/covid-19/?sort_by=date

Covid-19 in Podcasts

- *Selected by Dr Emma Jones*

https://www.microbe.tv/twiv/twiv-603/
This Week in Virology (TWiV) episode 603. Dr. Griffin is an MD PhD at Columbia University, USA. He talks about the clinical situation for Covid-19; disease progression and some of the more interesting symptoms that patients have presented with including neurological manifestations, cardiac problems and clotting issues. (116 mins).

https://www.microbe.tv/twiv/twiv-597/
This Week in Virology (TWiV) episode 597. Jon Yewdell, a very entertaining and illuminating immunologist from NIH, he discuss' immune responses in the context of infection with SARS-CoV-2. Lots of really interesting discussion about the antibody and T cell response plus lots of viral immunology that you may not already know. Very educational! (115 mins).

https://www.bbc.co.uk/programmes/m000hfqq
I really like the way More or Less on Radio 4 explain about when we reached the peak in COVID-19 deaths (8th April) and the peak in infection (18th March). Also, how we should make meaningful comparisons of COVID-19 cases and deaths in different countries. Plus do NHS staff have increased death rates due to COVID-19 (27 mins)

https://www.bbc.co.uk/programmes/w3cszl38
The Inquiry is an excellent BBC World Service program. In this episode they look at how we might come out of lockdown. The response to the 1918 Flu pandemic is discussed. Experts from Denmark and South Korea discuss the situation in their countries (23 mins).

https://www.bbc.co.uk/sounds/play/w3ct0sfg
Another great BBC World service programme. The Evidence is made in collaboration with the Wellcome Collection and in this episode looks at cases, models and responses in different countries including UK, China, USA and Kenya. Lots of other COVID-19 related issues are discussed but there is an interesting discussion on the flaws of testing and modelling – an ongoing discussion point at the moment! The importance of global solidarity is also made (53 mins).

News and Views

Editorial Concern—Possible Reporting of the Same Patients With COVID-19 in Different Reports

Bauchner *et al*. March 16, 2020.
https://jamanetwork.com/journals/jama/fullarticle/2763371

Summary (100 words max):
Howard Bauchner, editor in chief at JAMA, raises concerns in this editorial about the inclusion of the same patients between studies. First published online on the 16th March 2020, Bauchner reports that in some of the hundreds of manuscripts submitted to journals, often by the same authors, it is apparent that the same patients are reported in more than one article without clear indication that the patients are duplicated. Such reporting is not only misleading but may yield inaccurate data related to disease prevalence. Such data has the potential for serious harm if used to inform key decisions related to both SARS-CoV-2 clinical care and public health measures. Despite the speed at which both peer-reviewed and pre-print manuscripts are being published, Bauchner urges all researchers to maintain the highest level of ethical and scientific standards when publishing data related to SARS-CoV-2.

Coronavirus and Speed Science

See https://www.weforum.org/agenda/2020/03/speed-science-coronavirus-covid19-research-academic

and

bioRxiv is receiving many new papers on coronavirus 2019-nCoV. A reminder: these are preliminary reports that have not been peer-reviewed. They should not be regarded as conclusive, guide clinical practice/health-related behavior, or be reported in news media as established information.
—BioRxiv

The Promise and Peril of Antibody Testing for COVID-19

Jennifer Abbasi. JAMA. 2020.

https://jamanetwork.com/journals/jama/fullarticle/2764954

Summary

The presence of SARS-CoV-2 IgG and IgM antibodies in patient blood are indicative of an effective immune response to COVID-19. These antibodies are being utilised for both treatment of COVID-19 patients and diagnosis of a successfully cleared infection. Plasma from recovered patients containing neutralising antibodies can be infused via convalescent plasma or hyperimmune globulin into other patients to improve their immune response to the virus. However, it's possible that some survivors who undergo these passive immunity treatments won't develop their own immunity and are susceptible to reinfection. Multiple antibody tests such as ELISAs, lateral flow assays and chemiluminescence assays are currently in development to identify a test that can be effectively used by the public. Detecting these antibodies can determine those who have the lowest risk of contracting and spreading the disease, allowing people return to work. However, there is no clear evidence to suggest people cannot be re-infected by SARS-CoV-2. Additionally, an unreliable test result could be detrimental to public health.

Clinical

Care for critically ill patients with COVID-19

Murthy *et al*. JAMA 2020
Link: https://jamanetwork.com/journals/jama/fullarticle/2762996

Opinion by intensivists from Vancouver, Toronto and Hong Kong. Lists standard widely used strategies in the care of COVID19 patients and provides knowledge gap list namely

- Full risk factors remain unclear,
- safety of supportive strategies including non-invasive ventilation,
- risk of mortality
- risk-benefit of treatments such as steroids remains unclear

It is necessary to have regional surge preparation strategies

Main findings:
Factors associated with requiring ICU age (median = 60yrs) 40% have co-morbidities diabetes and cardiac disease. Median duration of symptoms to ICU = 9-10 days. Most patients admitted to ICU for respiratory support. Management is the based on standard treatment of viral pneumonia
-conservative IV fluids
- empirical antibiotics for possible bacterial pneumonia
- early invasive intubation
- periodic prone positioning during mechanical ventilation
- Consider extracorporeal membrane oxygenation
- ensure staff training and regional planning

Highlights
Overview of simple measures currently widely used.

Clinical Impact: Limited Description of current practices. Useful knowledge gap list.

Important Methodologies:
Opinion piece
Limitations:

- Does not push forward new strategies

Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China

Mao L *et* al. (2020). JAMA Neurology.
https://jamanetwork.com/journals/jamaneurology/fullarticle/2764549

This retrospective study is the first to report neurological manifestations among patients infected with SARS-CoV-2, which fall into 3 categories: CNS, PNS and skeletal muscle injury. Among 214 patients, 36.4% suffered with a neurological manifestation. Such symptoms were more prevalent in patients with severe COVID-19 (45.5% of total), who were statistically older and presented with fewer typical indicators of the infection, such as fever and a dry cough. Clinicians should be aware that patients who are admitted to hospitals with neurological manifestations could be severely infected with COVID-19 unknowingly.

Main Findings

- 36.4% of patients had nervous system manifestations: CNS (24.8%), PNS (18.9%), and skeletal muscle injury (10.7%).
- Patients present with most neurological symptoms during early infection (1-2 days).
- Most common CNS symptoms: dizziness (16.8%) and headache (13.1%)
- Most common PNS symptoms: taste impairment (5.6%) and smell impairment (5.1%).
- Severely infected patients (45.5%) had fewer typical symptoms of COVID-19: fever (45.5% vs 73%) and dry cough (34.1% vs 61.1%).
- Neurological manifestations common among severe patients included impaired consciousness, acute cerebrovascular events and slight muscular injury.
- Severe patients had higher inflammatory responses in peripheral blood.
- Patients with CNS symptoms had lower lymphocyte levels, platelet counts, and higher blood urea nitrogen levels. Those severely infected with CNS symptoms had the lowest lymphocyte and platelet counts.
- Patients with muscle injury had higher neutrophil counts, lower lymphocyte counts, higher C-reactive protein levels, higher D-dimer levels and multi-organ damage. For the severe group, patients with skeletal muscle injury had decreased lymphocyte counts and more serious liver and kidney injury.
- There were no significant differences between those with and without PNS symptoms, irrespective of disease severity.

Highlights

- Identification of various neurological manifestations that may be caused by COVID-19 infection

Clinical Impact?

Moderate. Implications for diagnosis of COVID-19 in the clinic.

Important Methodologies

- Retrospective, observational study with data collected from electronic medical records, lab findings and radiologic exams.

- Neurological manifestations reviewed and confirmed by 2 trained neurologists – discrepancies confirmed by 3rd.

Limitations?

- Relatively small study limited to patients from 3 hospitals in Wuhan
- Mild neurological manifestations (impairment of taste and smell) may not have been identified during observations.
- The impact of manifestations on patient outcome could not be determined at the time.
- Neurological symptoms were determined through **subjective descriptions from the patient**.
- Whether neurologic manifestations were caused directly by the virus or could not be distinguished

Diagnostics and Therapeutics

Positive RT-PCR Test Results in Patients Recovered From COVID-19

Lan *et al*. *JAMA,* 2020
Link: https://jamanetwork.com/journals/jama/fullarticle/2762452

Lan, Lan *et al*. report of 4 patients treated at Zhongnan Hospital of Wuhan University, Wuhan, China between January 1st – February 15th 2020. All 4 patients tested positive during real-time reverse transcriptase-polymerase chain reaction (RT-PCR) tests for COVID-19 nucleic acid. Once criteria for discharge were met (including 2 consecutive negative RT-PCR test results) patients quarantined at home for at least 5 days, after which 3 repeat RT-PCR tests (and an additional test from a different manufacturer) were performed – all were positive. Despite this, patients were asymptomatic and reported no contact with individuals with respiratory symptoms.

Main findings:

- 4 patients (all medical personnel, 2 males and 2 females with an age range from 30 to 36 years old) with mild to moderate infection tested positive during RT-PCR tests for COVID-19 nucleic acid.
- Anti-viral treatment (75mg of oseltamivir taken orally every 12 hours) was provided to all 4 patients.
- Time from symptom onset to recovery of patients ranged from 12-32 days
- Patients were discharged when (i) fever was absent for longer than 3 days, (ii) respiratory symptoms resolved, (iii) substantial improvement was seen in acute exudative lesions on chest CT images and (iv) 2 negative RT-PCR test results were obtained separated by at least 1 day
- Between 5 to 13 days after discharge, all patients underwent 3 repeat RT-PCR tests and an additional RT-PCR test from a different manufacturer – all tests were positive
- Despite positive RT-PCR tests following discharge, patients were asymptomatic (according to clinical examination) and chest CT scan presented no changes. Patients did not report contact with individuals with respiratory symptoms.

Highlights

- 4 patients tested positive during RT-PCR tests for the presence of COVID-19 nucleic acid after having tested negative on 2 consecutive RT-PCR tests separated by at least 1 day

Clinical Impact:

- Moderate

Important Methodologies:

- Use of RT-PCR tests from two different manufacturers to assess presence of COVID-19 nucleic acid
- RT-PCR tests were all performed by the same technician with internal and negative controls being performed routinely with each batch of tests

Limitations:

- No further follow-up of patients to understand how long RT-PCR tests resulted positive (short-time scale of study)
- The extremely small sample size prevents an understanding of the extent at which positive RT-PCR tests occur following discharge

Detection of SARS-CoV-2 RNA by direct RT-qPCR on nasopharyngeal specimens without extraction of viralRNA

Hasan *et al*. medRxiv 202
Link: https://doi.org/10.1101/2020.04.18.20070755

The authors developed a protocol for direct qRT-PCR on SARS-CoV-2 in nasopharyngeal swabs that forgoes RNA extraction to offer a solution for shortages of RNA extraction kits and reagents. Their approach achieved a sensitivity, specificity and accuracy of 95%, 99% and 98.5% respectively. This could significantly reduce costs and improve turn-around time for SARS-CoV-2 tests.

Highlights

1. Several sample pre-treatment conditions and reagents were tested and compared to a standard approach that included viral RNA extraction. Optimal results were achieved by diluting 10µl of the specimen 4 fold in nuclease free water (diluting PCR inhibitors), incubating at 65 °C for 5 min (low heat to avoid RNA loss) and testing 8µl of the heat lysed specimen in a 20µl reaction using TaqPath™ 1-Step RT-qPCR Master Mix (higher sensitivity).

2. 132 previously tested specimens were evaluated by this method and only one positive sample could not be confirmed. On the other hand 1 previously negative tested sample was tested positive in the new direct approach.

3. PCR inhibition rate among the specimens that gave negative RT-qPCR results by the direct approach was 8% compared to 9% by the standard approach.

Clinical Impact? High

Important Methodologies? Direct qRT-PCR on SarsCov2 nasopharyngeal swabs without RNA extraction

Limitations? Not much was said about the 132 validated samples. It is unclear how many days after symptom onset they were obtained and what the viral load was. It was not tested how this direct method performs in samples with low viral load and for how many days after symptom onset sensitivity and specificity are comparable to tests with RNA extraction.

Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs

Wyllie *et al*., (medRxiv), 2020
Link: https://www.medrxiv.org/content/10.1101/2020.04.16.20067835v1.full.pdf

The current gold standard method of SARS-CoV-2 detection is real-time RT-PCR of nasopharyngeal swabs. In an effort to evaluate an alternative approach that increases assay sensitivity and minimises exposure to healthcare workers, Wyllie *et al*., tested paired saliva and nasopharyngeal swabs collected from 44 COVID-19 patients with severe disease and 98 asymptomatic healthcare workers. Using real-time RT-PCR of serial samples collected approximately every 2.9 days, they demonstrate saliva is a more sensitive alternative to nasopharyngeal swabs that could be developed for self-collection at home thereby enabling SARS-CoV-2 testing at the population level.

Main findings:

- SARS-CoV-2 viral titers from saliva were approximately 5 times higher than nasopharyngeal swabs ($p < 0.05$) for all positive samples tested (n=46 nasopharyngeal, n=37 saliva)
- For patient-matched saliva and nasopharyngeal samples (n=38 for each sample type), SARS-CoV-2 titers from saliva were significantly higher than nasopharyngeal swabs ($p = 0.0001$)
- SARS-CoV-2 RNA was detected in saliva but not nasopharyngeal swabs from eight matching samples (21%), while SARS-CoV-2 was detected only in nasopharyngeal swabs and not saliva from three matched samples (8%).
- SARS-CoV-2 RNA was detected from the saliva of two asymptomatic healthcare workers despite negative matched nasopharyngeal swabs

Highlights:

- This study suggests saliva may represent a viable alternative for identifying mild or subclinical infections. The data may validate other studies that have already gained FDA approval for a saliva SARS-CoV-2 detection assay (Rutgers Clinical Genomics Laboratory)

Clinical Impact:

- Moderate

Important Methodologies:

- Characterisation of SARS-CoV-2 RNA in saliva with comparable and sometimes superior sensitivity to current diagnostic methods using nasopharyngeal swabs
- Detection of SARS-CoV-2 RNA in saliva of asymptomatic healthcare workers

Limitations:

Study methods not described in detail, raising questions regarding assay design including: whether a human specimen extraction control was used, specimen volume (for accurate testing) and only N1 primer used in real-time RT-PCR. Diagnostic labs use N1, N2 and N3 primers for all 3 SARS-CoV-2 genes for increased accuracy. Study of small sample size.

Nelfinavir inhibits replication of severe acute respiratory syndrome coronavirus 2 *in vitro*

Yamamoto *et al.* BioRxiv 2020

Link: https://www.biorxiv.org/content/10.1101/2020.04.06.026476v1

Preliminary *in vitro* assays determining the efficiency (pre/post-entry qt-PCR for SARS-CoV-2 RNA) and cytotoxicity of 9 HIV-1 protease inhibitors in a SARS-CoV-2 infected monkey epithelial kidney line (VeroE6/TMPRSS2). Lopinavir and Nelfinavir both inhibited viral multiplication at low concentrations (EC_{50} = dose when antiviral effect is 50% of max), far lower than cytotoxic doses (CC_{50} = dose where 50% cells were dead), though Nelfinavir had the best Selectivity Index (CC_{50}/EC_{50}). Both these drugs were effective post-viral entry and C_{max}/EC_{50} and C_{trough}/EC_{50} clinical efficacy estimates suggesting Nelfinavir is a potential candidate for further pre-clinical tests.

Main findings:

- HIV-1 protease inhibitors tested were Nelfinavir, lopinavir, Ritonavir, Saquinavir, Atazanavir, Tipranavir, Amprenavir, Darunavir and Indinavir.
- Cytotoxicity (CC_{50}) was assessed using an MTS assay to asses VeroE6/TMPRSS2 cells treated with the inhibitors for 24 hours.
- 50% Effective Concentration (EC_{50}) was determined by adding inhibitors and SARS-CoV-2 virus (MOI of 0.01) VeroE6/TMPRSS2 cells for 2 hours. The media was then replaced with inhibitor-only media and incubated for a further 22 hours before supernatants were collected for qt-PCR analysis.
- Lopinavir and Nelfinavir had the highest Selectivity Indexes (CC_{50}/EC_{50}):

Inhibitor Name	EC_{50} [µM]	CC_{50} [µM]	SI
Nelfinavir	1.13	24.32	21.52
Lopinavir	5.73	74.44	12.99

- C_{max}/EC_{50} and C_{trough}/EC_{50} suggest both drugs have potential to achieve an EC_{50} (clinical efficacy) in humans.
- Lopinavir and Nelfinavir supressed viral RNA multiplication post-entry. Inhibitors were added to infected VeroE6/TMPRSS2 cells up to 3.5 hours post-infection (hpi) and RNA extracted at 6 hpi and SARS-CoV-2 measured via qt-PCR.

Highlights:

- *In vitro* comparison of 9 HIV protease inhibitors (EC_{50} and CC_{50}) suggested Nelfinavir as the best candidate for further testing.

Clinical Impact:

- Low, no tests in multiple cell lines/*in vivo.*

Important Methodologies:

- N/A.

Limitations:

- Not yet peer-reviewed.
- Methods lack details about viral production in VeroE6/TMPRSS2 cells.
- Is an MOI of 0.01 representative of clinical disease?
- Triplicate results but error margins not shown.

Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2

Brandy Williamson *et al*. (bioRxiv, 2020)
Link: https://www.biorxiv.org/content/10.1101/2020.04.15.043166v2

A study of the potential of the therapeutic remdesivir as a possible treatment for SARS-CoV-2. This study shows the clinical benefits of remdesivir in rhesus macaques as their data show how remdesivir prevents progression to severe pneumonia.

Main findings:

- Drug metabolities are delivered to the sites of SARS-CoV-2 replication in infected animals
- Lack of respiratory disease in rhesus macaques infected with SARS-CoV-2 and treated with remdesivir
- Reduced virus replication in the lower, but not upper respiratory tract after remdesivir treatment.
- Decreased viral loads in after remdesivir treatment
- Reduced pneumonia after treatment
- Absence of resistance mutations

Highlights

- Clinical benefits of remdesivir in early treated rhesus macaques infected with COVID-19

Clinical Impact:

- High

Important Methodologies:

- Rhesus macaques infection with strain nCoV-WA1-2020 via intranasal, oral, ocular and intracheal routes.
- Liquid chromatography mass spectrometry
- Quantitative PCR and next generation sequencing for RNA extracted from swabs and BAL
- Histopathology and immunohistochemistry performed on tissues (cervical lymph node, conjunctiva, nasal mucosa, oropharynx, tonsil, trachea, all lung lobes, mediastinal lymph node, right and left bronchus, heart, liver, spleen, kidney, stomach, duodenum, jejunum, ileum, cecum, colon and urinary bladder)

Limitations:

- Timing of the treatment. Hard to directly translate it to corresponding disease stages in humans.

Interleukin-6 blockade for severe COVID-19 patients

Roumier *et al*. (medRxiv), 2020

Link: https://www.medrxiv.org/content/10.1101/2020.04.20.20061861v1

Roumier et al. reports on short-term IL6 blockade with tocilizumab in 30 patients with >5 days of prior disease duration and severe rapidly deteriorating COVID-19 pneumonia in comparison with a matched control group of patients. In highly selected patients, IL6 blockade could not curb the 'cytokine storm', prevent ICU admission and the requirement for mechanical ventilation. However, those treated before ICU admission had a significantly reduced risk of subsequent ICU admission. This small sample-size study indicates early intervention, especially before patients require ICU admission may be more beneficial.

Main findings:

- Virus-induced pro-inflammatory cytokines (IL1β, IL6, TNFα and granulocyte colony stimulating factor) lead to hyperinflammatory and pro-coagulatory states at a late stage of disease.
- High levels of C-reactive protein, IL6 and D-dimer predictors of mortality.
- Patients with severe COVID-19 screened for hyperinflammation were given an off-label program of IV tocilizumab (8mg/kg renewable once in case of insufficient response to therapy).
- Patients all <80 years old, severe rapidly deteriorating pneumonia, high C reactive protein with ≥5 days of prior disease duration.
- The 7 patients in ICU had a significantly reduced requirement of subsequent mechanical ventilation compared to the control group but no significant change in mortality.
- 23/30 patients treated outside ICU with tocilizumab had a significantly reduced risk of subsequent ICU admission.
- Tocilizumab well tolerated yet mild hepatic cytolysis (n=2) and ventilator-acquired pneumonia were reported (n=1).
- As of April 4th, 3 (10%) patients in the study died and while 4/7 (57%) and 6/30 (20%) were discharged from the ICU and from hospital, respectively.

Highlights

- Tocilizumab treatment may reduce need for ICU beds in patients who have rapidly deteriorating pneumonia and high inflammatory parameters

Clinical Impact:

- Use of anti-inflammatory agents including tocilizumab may improve patient outcome if administered before ICU admission

Important Methodologies:

- Off-label use of tocilizumab in severe COVID-19 patients

Limitations:

- Small sample size
- 80% participants male
- 2 patients also received a 10-day course of hydroxychloroquine and azithromycin and 2 patients received high-dose methylprednisolone pulses.

Virology

SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes
Sungnak *et al*. (Nature Medicine), 2020
Link: https://www.nature.com/articles/s41591-020-0868-6

Sungnak et al. investigated the SARS-CoV-2 potential tropism by surveying expression of viral entry-associated genes (ACE2 and TMPRSS2) in single-cell RNA-sequencing data from multiple tissues from healthy human donors.

Main findings:

- viral entry-associated gene transcripts were detected in specific respiratory, corneal and intestinal epithelial cells, potentially explaining the high efficiency of SARS-CoV-2 transmission.
- These genes are co-expressed in nasal epithelial cells with genes involved in innate immunity, highlighting the cells' potential role in initial viral infection, spread and clearance.
- The results confirmed the expression of ACE2 in multiple tissues including some not previously investigated, including nasal epithelium and cornea and its co-expression with TMPRSS2.
- TMPRSS2 was highly expressed with a broader distribution (Fig. 1a; second column), suggesting that ACE2, rather than TMPRSS2, may be a limiting factor for viral entry at the initial infection stage

Highlights

- The study offers a useful resource for further lines of inquiry with valuable clinical samples from COVID-19 patients.

Clinical Impact:

- Minimal

Important Methodologies:

- The authors used datasets retrieved from published and unpublished datasets in multiple human tissues
- For each dataset where per-cell annotation was not available, the data was re-processed from a raw or normalized (whichever was deposited alongside the original publication) quantification matrix.
- The top 50 genes in each dataset were characterized based on gene ontology classes from the Gene Ontology database and associated pathways in PathCards were from the Pathway Unification database.

Limitations:

- The study was limited by the datasets available online.

- Studies lacked specific cell types due to their sparsity, the challenges associated with isolation or analysis methodology.
- Samples from the different datasets were processed differently and can't always be correlated.

Host, Viral and Environmental Transcriptome profiles of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

Butler *et al.* (BioRxiv Preprints) 2020

Link: https://www.biorxiv.org/content/10.1101/2020.04.20.048066v2

Butler et al. carry out an extensive amount of research including the development, optimisation and validation of a fast loop-mediated isothermal amplification (LAMP) test to identify SARS-CoV-2 and a large scale metatranscriptomic platform for nasopharyngeal swabs. They profile 338 clinical samples tested for SARS-CoV-2 and 86 NYC subway samples to provide a broad picture of the epidemic in NYC. In addition they explore the evolution of the strain predominant in NYC and go on to map host responses in patients identifying an upregulation of ACE pathways.

Main findings:

- Develop a 30 minute LAMP assay – 95.6% sensitivity and 99.4% specificity.
- Identify positive and negative SARS-CoV-2 clinical samples in line with previously run qPCR assays (golden standard).
- All NYC subways samples were found to be negative for SARS-CoV-2 when analysed by LAMP and RNA-Seq.
- Identify a novel NYC-enriched SARS-CoV-2 sub-clade, 82% associated with A2a – a western European derived clade.
- 9bp in-frame deletion (p141_143KSD) in NSP1 including removal of 3 amino acids (143Y>F) linked to host chemokine dysregulation and translational inhibition in SARS-CoV.
- 5,982 differentially expressed genes in COVID-19 samples – 2,942 up regulated genes and 3,040 down regulated genes.
- Increase in ACE2, IF127, IF16, IFIT1, SITFL, HERC6, CXCL10, CXCL11 and CCL8 expression.
- Down regulation of ALAS2 – iron regulation pathways.

Highlights

- Immediate application to SARS-CoV-2 diagnostics using the LAMP assay, public health monitoring in NYC and therapeutic development.

Clinical Impact:

- Rapid (30 min) colorimetric test with high sensitivity and specificity.
- Link ACEI used for hypertension with severe COVID-19 disease.

Important Methodologies:

- Analysis of clinical and environmental samples via LAMP compared to qPCR and RNA-seq data.
- Fragment based viral mapping and assembly.
- Human Transcriptome analysis.

Limitations:

- Concentrates on samples from NYC (one specific hospital).
- Only 86 environmental samples analysed from early in epidemic.

Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease

Dai *et al*, Science, 2020
Link: https://science.sciencemag.org/content/early/2020/04/21/science.abb4489

Dai et al report here the design and production of two novel compounds 11a and 11b targeted to the main protease of SARS-CoV-2, M^{pro}. To our knowledge, there is no human homolog of M^{pro} , making it a good target for antiviral drugs. Both compounds showed inhibitory activity against SARS-CoV-2 both *in vitro,* and 11a in particular showed good pharmokinetic values (PK) and low toxicity *in vivo*. X-ray crystal structures of SARS-CoV-2 M^{pro} in complex with compounds 11a or 11b were also obtained at a 1.5 Å resolution.

Main findings:

- X-ray crystal structures showed the aldehyde groups of 11a and 11b covalently bound to Cys145 of SARS-CoV-2 M^{pro}
- 11a and 11b showed inhibitory action against SARS-Cov-2 infection in Vero 6 cells without significant cytotoxicity
- Pharmokinetic (PK) properties of 11a were more favourable than 11b, with 11b displaying a shorter half life (1.65h) and faster clearance rate (20.6mL/min/mg) so 11a was investigated further by intravenous administration in Sprague-Dawley (SD) rats and Beagle dogs
- 11a has a long half life in SD rats (7.6h) and Beagle dogs (5.5h) and a slow clearance rate in SD rats (4.01mL/min/kg) and Beagle dogs (5.8mL/min/kg)
- Dose range toxicity study in SD rats (2, 6 and 18mg/kg) and Beagle dogs (10-40mg/kg) by single daily dose via intravenous administration showed no obvious toxicity

Highlights

- Suggests compound 11a is a good candidate for further investigation

Clinical Impact:

- Limited – novel compounds synthesised exhibited anti-SARS-CoV-2 activity *in vitro* and good PK values *in vivo* were observed without obvious toxicities. Did not test anti-SARS-CoV-2 function *in vivo*

Important Methodologies:

- X ray crystallography of 11a and 11b in complex with SARS-CoV-2 M^{pro}
- Plaque reduction assay, immunofluorescence and qPCR to determine antiviral activity of 11a and 11b in vitro
- Single dose of 11a administered intravenously over course of 7 days, animals observed at least once daily for toxicity

Limitations:

- *In vivo* studies performed in SD rats and Beagle dogs, so results may not translate well in human clinical trials
- Anti-viral action of 11a against SARS-CoV-2 was only investigated *in vitro, in vivo* studies needed

Structure of the RNA-dependent RNA polymerase from COVID-19 virus

Gao *et al*. (Science), 2020

Link:

https://science.sciencemag.org/content/sci/early/2020/04/09/science.abb7498.full.pdf

Gao et al. report the Cryo-Electron Microscopy structure of COVID-19 virus full length RNA-dependent RNA polymerase (RdRp/nsp12) in complex with the cofactors nsp7 and nsp8 at 2.9-Å resolution, in an effort to aid in drug design. This complex had an overall similar architecture to that of SARS-CoV including a newly identified β hairpin domain at its N terminus which stabilizes the interaction between several nsp12 domains. Lastly the binding of remdesivir to nsp12 was modelled based on superposition with sofosbuvir (nucleotide analog) bound to HCV ns5b which shares several conserved motifs that form the RdRp active site.

Main findings:

- COVID-19 nsp12 in complex with one nsp7-nsp8 pair and a nsp8 monomer resolved by Cryo-EM at 2.9-Å resolution
- Nsp7-nsp8 pair conserved with SARS-CoV but nsp8 monomer has shifted orientation of N-terminal helix
- Nsp12 contains a nidovirus-unique N-terminal extension domain which adopts a nidovirus RdRp-associated nucleo-tidyltransferase (NiRAN) architecture
- The complete coronaviral NiRAN domain was elucidated and consists of the residues A4-T28 and Y69-R249
- Nsp-12 structure similar to SARS-CoV nsp 12
- Additional β-hairpin inserts in the groove clamped by the NiRAN domain and the palm subdomain in the RdRp domain and forms a set of close contacts to stabilize the overall structure
- Polymerase domain adopts same overall architecture of viral polymerase family and consists of three subdomains (fingers, palm and thumb subdomains)
- Motifs A-G in the palm domain are configured like other RNA polymerases
- Motif A (residues T616-M626) contains the classic divalent-cation-binding residue D618, which is conserved in most viral polymerases including HCV ns5b
- Motif C (residues F753-N767) contains the catalytic residues (759-SDD-761) in the turn between two β-strands also conserved in HCV ns5b
- Configurations of the template/primer entry paths, NTP entry channel, and the nascent strand exit path are similar to those described for SARS-CoV, HCV and PV polymerase
- Remdesivir diphosphate binding to COVID-19 virus nsp12 based on superposition with sofosbuvir bound to HCV ns5b
- Nsp12 of covid-19 virus has the highest similarity with the Apo state of ns5b.
- Catalytic residues D760, D761 and the classic D618 will undergo a conformational change to coordinate the divalent metal cations

- The latter will anchor the phosphate group of the incoming nucleotide or inhibitors together with the allosteric R555 in motif F
- T680 in COVID-19 virus nsp12 is also likely to form hydrogen bonds with the 2' hydroxyl of Remdesivir
- Hydrophobic side chain of V557 in motif F is likely to stack with and stabilize the +1 template RNA uridine base to base pair with the incoming triphosphate remdesivir

Highlights

- Resolved complex involved in synthesis of viral proteins which may aid in drug design
- Favipiravir (nucleoside analog) may share a similar binding mode and inhibition mechanism

Clinical Impact:

- Limited

Important Methodologies:

- Cryo-EM

Limitations:

- Nsp12 residues (S1-D3 and G897-D901) in NiRAN and thumb domain were not resolved
- Structure of RdRP in complex with primer-template RNA, NTPs and remdesivir not resolved

Mechanisms of Disease

Endothelial cell infection and endotheliitis in COVID-19

Varga Z *et al*. Lancet.2020
Link: https://doi.org/10.1016/ S0140-6736(20)30937-5

Varga et al. analysed whether vascular derangements in COVID-19 infection are due to endothelial cell involvement. For 1st patient, post-mortem analysis of the transplanted kidney by electron microscopy revealed viral inclusion structures in endothelial cells. Post-mortem histology of patient 2 showed lymphocytic endotheliitis in small intestine, lung, heart, kidney, and liver. The survived patient 3, histology of the small intestine resection revealed prominent endotheliitis of the submucosal vessels and apoptotic bodies. This histological study shows evidence of presence of viral elements within endothelial cells and an accumulation of inflammatory cells, with evidence of endothelial and inflammatory cell death. Induction of apoptosis and pyroptosis might have an important role in endothelial cell injury in COVID-19.

Main findings: Vascular derangements in COVID-19 are due to endothelial cell involvement by the virus

Highlights: SARS-CoV-2 infection facilitates the induction of endotheliitis in several organs as a direct consequence of viral involvement and of the host inflammatory response.

Clinical Impact: minimal

Important Methodologies: histology

Limitations: The study did not consider patients without co-morbidity

Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study

Zheng *et al*. the BMJ/2020
Link: https://www.bmj.com/content/369/bmj.m1443

Zheng et al. retrospectively analysed SARS-CoV-2 RNA viral load in respiratory, stool, serum and urine samples of patients with mild (22) and severe (74) disease suggesting a longer duration of viral RNA in stool samples compared to respiratory and serum samples as well as longer persistence of higher load in respiratory samples in patients with severe vs mild disease.

Main findings:

- SARS-CoV-2 RNA was detected in stool (59%) and serum (41%) of patients with confirmed disease
- Median duration of virus RNA in stool was significantly longer than in respiratory and serum samples
- RNA in urine was only detected in one patient
- There was no difference in viral duration between stool and serum samples from patients of different severities
- Virus duration was longer in males vs females as ell as patients over 60 years old
- Types and timeliness of antiviral treatment had no overall effect on duration of the virus and viral load
- Glucocorticoid treatment was positively correlated with duration in severe disease

Highlights

- Viral load was highest in respiratory sample, then stool, then serum
- Viral RNA could be detected longest in stool samples
- Duration of viral detection was longer in men vs women and longer in over 60s compared to younger individuals

Clinical Impact:

- Medium, highlights the necessity for stronger management of stool samples in control of the epidemic

Important Methodologies:

- qRTPR analysis of sputum, saliva, blood, urine and stool samples

Limitations:

- patients differed in treatments and treatment effects were not taken into account
- as recruitment depended on positive respiratory samples, it is unclear whether patients negative in those can test positive in stool and serum

Integrated analyses of single-cell atlases reveal age, gender, and smoking status associations with cell type-specific expression of mediators of SARS-CoV-2 viral entry and highlights inflammatory programs in putative target cells

Muus, C., Luecken, M. D., Eraslan, G *et al*. bioRxiv April 21 2020
Link: https://doi.org/10.1101/2020.04.19.049254

Muus *et al.* performed a meta-analysis on cell-specific RNA expression of *ACE2, TMPRSS2* and *CTSL* in 107 single-cell and single nucleus RNA-Seq studies, 22 from lung and airway data sets, and 85 from other organs. 1,176,683 cells were analysed from 282 samples from a range of patient ages. The study aimed to identify cell types liable to infection outside of the lung and airways. *TMPRSS2* and *CSTL* have been shown to be important in SARS-CoV infection, and the former has been studied in SARS-CoV-2 infection. This is the first single-cell meta-analysis with respect to COVID-19.

Main findings? **1)** Dual positive *ACE2*$^{+}$ *TMPRSS2*$^{+}$ expression in airways seen primarily in nasal goblet cells and multiciliated cells (proximal), and AT2 cells (distal). *ACE2*$^{+}$*TMPRSS*$^{+}$ expression seen in colonic enterocytes, pancreatic ductal cells, heart fibroblasts/pericytes and epithelial cells of the bladder, kidney, liver, prostate and testis. Although lymphopenia is seen in infected patients, *ACE2* mRNA was not detected in bone marrow or cord blood. Dual *ACE2*$^{+}$*CSTL*$^{+}$ cells enriched in olfactory epithelium, ventricular cardiomyocytes, heart macrophages and lung/heart/kidney pericytes.
2) Study found robust association of *ACE2* and *TMPRSS2* expression in a large number of lung cell types with age, sex and smoking status. *ACE2* expression increases with age (basal and multiciliated cells). Males show elevation in *ACE2* (airway secretory cells and AT2 cells). Past or current smokers have highly elevated levels of *ACE2* (multiciliated cells).
3) *ACE2* expression very low in healthy lungs of new-borns and is maintained at low levels until ~9yrs old. *CSTL* is expressed at high levels at the maternal-fetal interface in placenta, but otherwise dual positive cells are low across the tissue.
4) On a 'tissue level' the following genes were shown to be enriched: *CEACAM5/6, SLP1, CXCL17* and *PIGR*. At a 'cell level', genes related to TNF signalling were detected, suggesting *ACE2* and/or *TMPRSS2* expression could be affected by anti-TNF therapy.
5) *TMPRESS2* co-expressed with *ACE2* with members of the proprotein convertase subtilisin kexin (PCSK) family in a cell-specific expression pattern in multiple lung epithelial cell types. Co-expression of *ACE2* and *PCSK* family members seen in liver, ileum, kidney and nasal airways.

Highlights Enteric neurons and other brain cell types were show to express *ACE2*, which the authors suggest could be associated with the seizures and encephalopathy seen in some COVID-19 patients.

Clinical Impact? Moderate- study aims to understand the molecular and cellular basis of COVID-19 and therefore help in the search for therapies. Presence of dual positive cells in the lung, kidney and heart may account for the effect seen in these organs upon viral infection.

Important Methodologies? Single cell/single nucleus RNA-seq, Single-cell ATAC-Seq, Immunohistochemistry, proximity ligation in situ hybridisation (PLISH).

Limitations? N numbers in the analysis (i.e. donors) were low and sometimes as few as n=1; each cell was treated as an individual event, which could lead to 'inflated p-values' especially in groups where the donor numbers were low. A larger number of donors would make the data more robust. Further, one of the data sets analysed came from rejected donor lungs which may indicate that this data is not fully representative of healthy lung tissue in the general population. Finally, extensive modelling was carried out on these data to ensure comparability which the authors themselves say makes the data more complicated.

Impaired type I interferon activity and exacerbated inflammatory responses in severe Covid-19 patients.

Jérôme Hadjadj *et al*. (medRxiv), 2020
Link: https://www.medrxiv.org/content/10.1101/2020.04.19.20068015v1

The study aimed to test the hypothesis of a virally-driven hyperinflammation leading to severe disease, by using in-depth phenotypical analysis of immune cells, standardized whole-blood transcriptomic analysis and cytokine measurements on a group of fifty Covid-19 patients with variable severity from mild to critical. A phenotype in severe and critically ill patients was identified which showed an impaired interferon (IFN) type I response characterized by a low interferon production and activity. This the leads to downregulation of interferon- stimulated genes. The paper proposes that type-I IFN deficiency in the blood is a hallmark of severe Covid-19 and could identify and define a high-risk population.
Main findings:

- Expression of exhaustion-related genes, such as BATF, IRF4 and CD274, significantly increased with disease severity.
- The data presented suggests a severity grade-dependent increase in activation of innate and inflammatory pathways; in contrast, the IFN response is high in mild-to-moderate patients while it is reduced in more severe patients.
- Multiplex gene expression analysis showed an upregulation of genes involved in type I IFN signalling (such as *IFNAR1*, *JAK1*, *TYK2*) contrasting with a striking downregulation of interferon-stimulated genes (ISGs) (such as *MX1, IFITM1, IFIT2*) in critical patients.
- IFN activity in serum was significantly lower in severe and critical than in mild-to-moderate patients; the authors suggest that these data show that patients with severe and critical Covid-19 have an impaired type I IFN production and a lower viral clearance.
- The discrepancy between RNA quantification and protein measurement suggests that cellular sources of TNF-α and IL-6 originated more likely from the injured lungs and/or endothelial cells.
- Confirmed that IL-6 plays an important role in pathogenesis and severity of COVID-19.

Highlights

- Severe and critical Covid-19 patients could be potentially relieved from IFN deficiency by IFN administration and from exacerbated inflammation by adapted anti-inflammatory therapies targeting IL-6 or TNF- α.

Clinical Impact:

- Some - Type-I deficiency in the blood could be used as an indicator of severe COVID-19 and help to define a high-risk population.

Important Methodologies:

- Standardized whole-blood transcriptomic analysis

A single-cell atlas of the peripheral immune response to severe COVID-19

Aaron J. Wilk *et al*. *MedRxiv preprint server* (2020)
Link: https://doi.org/10.1101/2020.04.17.20069930

Wilk *et al* performed single-cell RNA sequencing of PBMCs from 7 patients with COVID-19 and 6 healthy controls. They identified altered peripheral immune cell phenotypes in COVID-19, including an interferon-stimulated gene signature, HLA class II downregulation, T cell and NK cell proliferation and NK cell exhaustion. Their findings suggest a highly novel B cell derived granulocyte population in patients with acute respiratory failure. Peripheral monocytes and lymphocytes from infected patients did not produce high levels of pro-inflammatory cytokines, suggesting that circulating leukocytes do not significantly contribute to the COVID-19 cytokine storm.

Main findings:

- Substantial phenotypic differences between COVID-19 cases and controls, predominantly in monocytes, T cells and NK cells
- Subsets depleted in COVID-19, included γδ T cells, dendritic cells, CD16+ monocytes, and NK cells
- No substantial expression of pro-inflammatory cytokines by monocytes, T cells or NK cells
- Increased plasmablast proportions in COVID-19 patients, which were further elevated in patients with ARDS, but no correlation between proportions of plasmablasts and days post-symptom onset, and did not appear to use particular Ig V genes
- CD14+ monocytes display downregulation of MHC class II and IFN-driven phenotypic reconfiguration. IFN signalling gene pathways were highly upregulated in CD14+ monocytes and NK cells. This correlated positively with age and negatively with disease duration, but did not correlate with disease severity.
- The numbers of proliferating T cells and NK cells were increased in most COVID-19 patients
- No clear change in CD4+ or CD8+ T cell exhaustion in COVID-19 patients but NK cells from patients expressed higher levels of exhaustion markers

- Activated granulocytes appeared to develop from class-switched B cells in patients with ARDS, with a spectrum of cellular phenotype between the two cell types. They lost expression of canonical plasmablast genes and instead acquired expression of genes encoding neutrophil granule proteins and other granulocytic markers

Highlights

- Tentative identification of a novel B cell-derived granulocyte population
- Characterisation of many changes in immune subset percentages and phenotypes in the periphery during COVID-19 infection.
- Downregulation of HLA class II molecules in COVID-19, particularly in severe disease with ARDS, may reflect immune dysregulation
- Illustrates the complex role of IFN signalling pathways in COVID-19
- Peripheral leukocytes did not produce proinflammatory cytokines

Clinical Impact: Limited, but suggests many avenues for future immunotherapies

Important Methodologies:

- Seq-Well-based13 massively parallel scRNA-seq.
- Whilst there have been other scRNAseqs of COVID-19 samples, this is a comprehensive study of relatively large size, with good controls, and focuses on peripheral leukocytes rather than samples from the lung.

Limitations:

- The B cell to granulocyte transition is based solely on the modelling of scRNAseq data. It is a very novel finding and will need to be validated and these cells studied using other methods
- Did not include B or T cell repertoire sequencing

Antibodies and T Cells

A highly conserved cryptic epitope in the receptor-binding domains of SARS-CoV-2 and SARS-CoV

M. Yuan *et al*., Science (2020)
Link: https://science.sciencemag.org/content/early/2020/04/02/science.abb7269
Yuan et al. map interaction of CR3022 (a neutralising antibody previously isolated from SARS patient) with SARS-CoV-2 at 3.1 Å resolution. CR3022 binds to receptor binding domain (RBD) of both SARS-CoV and SARS-CoV-2. It targets an epitope that is highly conserved, enabling cross-reactivity of CR3022 among SARS-CoVs.
Main findings:

- Location of CR3022 binding is highly specific and does not overlap with the binding site of ACE2 (a host receptor used by SARS-CoV and SARS-CoV-2 for entry into cells).
- CR3022 does not compete with ACE2 for binding to SARS-CoV-2 RBD.
- The CR3022 binding site is usually hidden inside the virus and can only be accessed by CR3022 when the virus undergoes structural changes (when '*at least two RBDs on the trimeric S-protein are in the "up" conformation and slightly rotated*').
- CR3022 binds to SARS-CoV-2 RBD with a significantly lower affinity than to SARS-CoV RBD (likely due to non-conserved residues in epitope).
- In contrast to interaction with SARS-CoV, CR3022 is unable to neutralise SARS-CoV-2 (even at highest concentration tested) *in vitro*.

Highlights:

- CR3022 binding site could be a functionally relevant cross-reactive epitope.

Clinical Impact: Low

Important methodologies:

- Implications to vaccine design and development: crystal structure of CR3022 in complex with SARS-CoV-2 RBD provides important insights into antibody-virus interactions.
- Identification of almost identical neutralising antibody binding site in SARS-CoV and SARS-CoV-2 by structural mapping. This finding lays the foundation for further research into antibodies that could effectively neutralise multiple species of SARS-CoVs (including novel species that may emerge in the future).

Limitations:

- No functional studies: CR3002 function is not fully defined.
- Crystal structure at 3.1 Å resolution is not completely accurate – many finer structural details and/or interactions could be missing/incorrect.
- The slight structural differences between SARS-CoV and SARS-CoV-2 could be functionally significant (reducing clinical implications of results).
- Other antibody binding sites/interactions not explored in detail.

Potent neutralizing antibodies in the sera of convalescent COVID-19 patients are directed against conserved linear epitopes on the SARS-CoV-2 spike protein

Chek Meng Poh **et al**., (Preprint article), 2020
Link: https://www.biorxiv.org/content/10.1101/2020.03.30.015461v1.full
The authors used sera from COVID-19 patients and screened for neutralising antibodies against a pseudo-typed lentivirus expressing a luciferase-tagged SARS-CoV-2 S glycoprotein. 6/25 patients that displayed neutralising activity were selected to assess the antigenic targets using a linear B-cell peptide library. Peptide pools spanned the S glycoprotein of both SARS-CoV and SARS-CoV-2. COVID-19 sera detected two distinct peptide pools (S14 and S21) from the SARS-CoV-2 library, leading to the discovery of individual peptides S14 P5 and S21 P2. Depletion assays validate that antibodies against these peptides possess neutralising roles against SARS-CoV-2 pseudo-typed lentivirus.

Main findings:

- Two distinct peptide pools from SARS-CoV-2 S library (S14 and S21) strongly detected by sera from COVID-19 patients, but not by recovered SARS patients. Sera from recovered SARS patients could neutralise SARS-CoV, but not SARS-CoV-2 pseudo-typed lentivirus.
- Sera from COVID-19 patients could detect SARS-CoV S library peptide pool S51, which overlaps with SARS-CoV-2 pool S21. The authors suggest a pan-coronavirus epitope at this location, as the region encompasses the fusion peptide, which is highly conserved between coronaviruses.
- Narrowed down the specific region of interest to S14 P5 and S21 P2. S14 P5 is localised in proximity to the receptor-binding domain, suggesting that antibodies binding to this region may sterically hinder binding to the ACE2 receptor or could be an allosteric effect on ACE2 binding. S21 P2 contains part of the fusion peptide sequence, which may affect virus fusion.
- Antibody depletion assays against S14 P5 and S21 P2 showed a significant reduction in the ability to neutralise the SARS-CoV-2 pseudo-virus infection.
- Low rate of mutations were found for S14 P5 and S21 P2, with low to moderate impact on the viral sequence

Highlights

- Guides the design and evaluation of efficient and specific serological assays and help prioritise vaccine target designs

Clinical Impact:

- Minimal

Important Methodologies:

- Generation of pseudo-typed viral particles expressing SARS-CoV-2 S glycoprotein
- Neutralization assay against SARS-CoV-2 S glycoprotein pseudo-typed lentiviruses
- Peptide-specific antibody depletion of pooled SARS-CoV-2 sera

Limitations:

- The article is a preprint and has not been peer reviewed
- Only 6 patient samples were analysed

Effectiveness of convalescent plasma therapy in severe COVID-19 patients

Duan *et al*. (PNAS), 2020
Link: https://www.pnas.org/content/early/2020/04/02/2004168117

While there are no specific antiviral agents available for COVID-19, Duan and colleagues have tested the use of convalescent plasma (CP) transfusion to rescue severe patients. They tested 10 severe patients and showed that one dose of CP could significantly increase or maintain neutralising antibodies at a high level, leading to viremia disappearance in 7 days. Clinical symptoms and paraclinical criteria quickly improved within 3 days, and radiological exams indicated different degrees of absorption of lung lesions. Since this therapy was well tolerated without adverse reactions, the authors suggest this is a promising rescue option for severe COVID-19.

Main findings:

- CP samples from 40 recovered COVID-19 patients were collected, from which 39 showed high antibody titers against SARS-CoV-2 (at least 1:160).
- 10 severe COVID-19 patients enrolled:
    - median age: 52.5 years;
    - all patients received antiviral treatments, antibacterial or antifungal treatments when patients presented coinfection, and six patients received corticosteroids treatment;
- Effects of CP transfusion:
    - All symptoms in all patients improved within 1 to 3 days post transfusion;
    - Patients showed different degrees of absorption of pulmonary lesions after transfusion;
    - Lymphocytopenia (index for prognosis) tended to improve, whilst parameters indicative of inflammation or liver dysfunction decreased;
    - Most patients showed an increase in oxygen saturation, which might indicate recovered lung function;
    - Neutralising antibody titers increases after transfusion in half of the patients;
    - SARS-CoV-2 RNA was decreased to undetectable levels in all patients;
    - Clinical outcome of CP treated group was significantly better than an historic matched control group.
- No serious adverse reactions.

Highlights

- One dose of 200 mL CP transfusion was well tolerated, while clinical symptoms significantly improved with increase of oxyhemoglobin saturation within 3 d, accompanied by rapid neutralization of viremia.
- All patients achieved serum SARS-CoV-2 RNA negativity after CP transfusion, accompanied by an increase of oxygen saturation and lymphocyte counts.

Clinical Impact:

- High

Important Methodologies:

- Virus inactivation using methylene blue photochemistry to maintain the activity of neutralizing antibodies

Limitations:

- Optimal transfusion time point still needs to be determined.
- Patients received antiviral treatment as well as CP transfusion. Antiviral agents could have contributed to the recovery of patients, or synergize with the therapeutic effect of CP.
- Corticosteroid therapy could have interfered with immune response.
- Need for robustly designed randomised controlled trials to show efficacy of this therapy.

Neutralization of SARS-CoV-2 spike pseudotyped virus by recombinant ACE2-Ig

Lei *et al.* (Nat Commun), 2020
Link to paper: https://www.nature.com/articles/s41467-020-16048-4

SARS-CoV-2 has been shown to bind to the ACE2 receptor using its spike (S) protein as a cell entry system. The authors synthesized a recombinant ACE2-Ig protein using the extracellular domain of ACE2 fused to the Fc segment of human IgG1. The data produced from neutralisation of pseudo-viruses, inhibition of cell fusion and *in-vivo* pharmacokinetic studies demonstrate promising preliminary data for the development of a therapeutic. Not only did the ACE2-Ig have neutralising effects on the pseudo-viruses and successfully inhibited cell fusion, it also demonstrated high stability, extending the plasma residence time (longer half-life). Moreover, studies with a mutant ACE2-Ig (mACE2-Ig) showed that the neutralising effects remained.

Main findings:

- Neutralisation assay IC50s using ACE2-Ig
  - 293T cells: 0.8 and 0.1ug/mL (SARS-CoV and SARS-CoV-2 respectively)
  - A549 cells: 1.4 and 0.1ug/mL (SARS-CoV and SARS-CoV-2 respectively)
- SARS-CoV S protein-mediated fusion IC50s
  - ACE2-Ig: 0.85 and 0.65ug/mL (SARS-CoV and SARS-CoV-2 respectively)
  - mACE2-Ig: 0.76 and 0.48ug/mL (SARS-CoV and SARS-CoV-2 respectively)
- Affinity of SARS-CoV-2 bound to ACE2-Ig is 16-fold higher than SARS-CoV which explains higher efficiency of neutralization

Highlights:

- **Evidence that neutralization of SARS-CoV-2 can be achieved with ACE2-Ig.**

- **Neutralizing effects of ACE2 persist despite modification of two active-site histidines (HH/NN)**

Clinical Impact:

- Limited

Important Methodologies:

- Generation of fusion proteins: DNA sequence of extracellular domain of ACE2 ligated to Fc segment of human IgG1 with the pcDNA3.4 vector and FreeStyle 293 expression system (Invitrogen)
- Reporter genes (luciferase and β-gal) activity used to calculate IC50s (defined as the concentration at which activity was reduced by 50%)

Limitations:

- **Use of pseudo-viruses containing the S glycoproteins of SARS-CoV and SARS-CoV-2**
- **Neutralisation studies limited to *in-vitro* experiments**
- Concerns for possible side effects of systemic administration due to prolonged half-life

Presence of SARS-CoV-2-reactive T cells in COVID-19 patients and healthy donors

Braun *et al.,* (unpublished; MedRxiv) 2020.
Link: https://doi.org/10.1101/2020.04.17.20061440.

Based on the rationale is that $CD4^+$ T cell responses correlated with positive outcomes in the previous SARS-CoV outbreak, this study investigates $CD4^+$ T cell responses to different regions of the SARS-CoV-2 spike glycoprotein. They used two overlapping peptide pools which span the S protein N terminus and C terminus, respectively to test responses in COVID-19 patients and healthy donors. CD4 T-cells from COVID-19 patients were reactive to peptide pools from the N and C-terminal regions of the spike protein, whilst CD4 T-cells from healthy donors had significantly stronger reactivity to the C-terminal peptide pool. A high proportion of COVID19 patients had reactive T cells, with a distinct activation phenotype, while surprisingly, some healthy donors also had reactive T cells, but lacked the same activation signature.

Main findings

1. CD4 T-cells reactive to spike protein from SARS-CoV-2 found in COVID-19 patients
2. CD4 T-cells reactive to the C-terminal portion of the spike protein from SARS-CoV-2 found in healthy donors (34% of those tested)
3. Pre-existing or cross-reactive immunity may be present 34% of seronegative healthy donors
4. SARS-CoV-2 reactive CD4 T-cells from COVID-19 patients had an activated phenotype (CD38+, HLA-DR+, Ki-67+)

5. SARS-CoV-2 reactive CD4 T-cells from healthy donors had low expression of CD38, HLA-DR and Ki-67- indicating a less activated phenotype

Highlights

Cross-reactivity in healthy donors to SARS-CoV-2: Reactive healthy donors had significantly more T cell responses to the C terminus peptide pool than the N terminus peptide pool. The C terminus shares more sequence homology with the S protein of other coronaviruses which cause the common cold. The authors speculate that cross-reactivity may contribute to asymptomatic cases in COVID19.

Days between onset of symptoms and sampling are given, an important consideration for the timing of the adaptive immune response which will become clearer as studies progress. It is used well in figure 3, g and h.

Clinical Impact

Low – but the identification of pre-existing SARS-CoV-2-cross-reactive CD4 T-cells in 34% of healthy donors may begin to explain the different manifestations of SARS-CoV-2 disease courses.
Identification of the reactive epitopes, particularly the cross-reactive ones, could impact future vaccine design.

Important Methodologies

- T cell responses were analysed by the design of two overlapping peptide pools which span the SARS-CoV-2 spike protein and also included known HLA-II SARS-CoV epitopes (identified from the SARS 2002-2003 outbreak). PBMC were stimulated for 16 hr in the presence of the peptide pool and anti-CD28. DMSO was the negative control while HCMV peptide mix and bacterial toxins (acting as an adjuvant) was the positive control.
- Detection of SARS-CoV-2 RNA from nasopharyngeal swab samples.
- Healthy donors were assessed by SARS-CoV-2 IgG ELISA, those with reactive T cells were further tested by PCR of nasopharyngeal swab samples.
- T cells were identified and phenotyped by flow cytometry; surface markers CD4, CD8, CD69, CD38 and HLA-DR. Intracellular markers analysed were Ki-67, 4-1BB and CD40L.

Limitations

- Small-scale study (n=18 COVID19 patients and n=68 healthy donors).
- Explaining the relevance of cross-reactivity will require greater population wide detection of asymptomatic cases to recruit to studies.
- Flow cytometry control data lacking.
- The specific peptides within the pools that the T cells responded to were not identified; it would be useful to determine if reactivity overlapped with previously described SARS-CoV-2 HLA-II epitopes and identify the sequences that were cross reactive in healthy donors.
- No data to show that the epitopes in the overlapping peptide pools are real – i.e. processed and presented

Human leukocyte antigen susceptibility map for SARS-CoV-2

Nguyen *et al*. (medRxiv), 2020
Link: https://www.medrxiv.org/content/10.1101/2020.03.22.20040600v2

The authors carried out a comprehensive in silico analysis of viral peptide-MHC class I binding affinity across 145 HLA -A, -B, and -C genotypes for all COVID-19 peptides. They also explore the potential for cross-protective immunity. They found COVID-19 is presented by a diversity of HLA alleles. The author recommend integrating HLA testing into clinical trials and pairing HLA typing with COVID-19 testing where feasible to more rapidly develop and deploy predictor of viral severity in the population, and potentially to guide future vaccination strategies to genotypically at-risk populations.

Main findings:

- HLA-B*46:01 was predicted to present the fewest SARS-CoV2 peptides, keeping with previous clinical data associating this allele with severe disease.
- **Potentially cross-protective peptides**: After analysing all known human coronaviruses, 48 highly conserved amino acid sequence spans had been identified. Among conserved sequences, 44 COVID-19 sequences including linear peptide epitopes (564 in total) were anticipated to present within at least one other common human coronavirus.
- HLA-A*02:02, HLA-B*15:03, and HLA-C*12:03 were the top presenters of conserved peptides, while 56 different HLA alleles (including A*25:01, B*46:01, C*01:02) demonstrated minimal or no appreciable binding affinity (<500nM) to any of the conserved peptides.
- There is no correlation between the HLA allelic frequency in the population and allelic capacity to bind SARS-CoV or SARS-CoV-2 peptides.
- HLA-A alleles exhibited the relative largest capacity to present SARS-CoV-2 antigens.
- As all individuals have two HLA-A/B/C haplotypes or six HLA alleles, the lack of presentation from a single poorly-presenting allele may be potentially buffered.

Highlights

- Understanding how variation in HLA may affect the course of COVID-19 could help identify individuals at higher risk from the disease, and guide vaccine development: individuals with high-risk HLA types could be prioritized for vaccination.

Clinical Impact:

- Mild

Important Methodologies:

- Coronavirus protein/proteomes sequence data was retrieved from the National Center of Biotechnology Information (NCBI)
- Sequences were aligned using the Clustal Omega v1.2.4 multisequence aligner tool.
- MHC class I binding affinity predictions were performed using netMHCpan v4.0, MHCnuggets 2.3.2 and MHCflurry

- Global HLA-A, -B, and -C allele and haplotype frequency data were obtained from the Allele Frequency Net Database

Limitations:

- It is an entirely in silico study.
- It's unable to assess the relative importance of HLA type compared to known disease-modifying risk factors such as age and clinical comorbidities.
- Binding affinity is not the only factor to determine T cell response.
- They explored only a limited set of 145 well-studied HLA alleles.
- They did not assess genotypic heterogeneity or in vivo evolution of COVID-19, which could modify the repertoire of viral epitopes presented.
- The author did not address the potential for individual-level genetic variation in other proteins, e.g. ACE2, to modulate the host-pathogen interface.

Vaccines

Safety and immunogenicity of a modified vaccinia virus Ankara vector vaccine candidate for Middle East respiratory syndrome: an open-label, phase 1 trial.

Till Koch *et al*., Lancet Infect Dis, April 20, 2020.
Link: https://doi.org/10.1016/S1473-3099(20)30248-6

This study uses the recombinant modified vaccinia virus Ankara (rMVA) vaccine vector platform, which has been shown to be safe and immunogenic in humans. MVA vaccine candidates have demonstrated a favourable safety profile in various populations and disease settings. The MERS-CoV spike glycoprotein consists of S1 and S2 subunits and mediates viral attachment to host cells, entry, and membrane fusion. It is a target for neutralising antibodies and, therefore, vaccine development. The MERS vaccine candidate MVA-MERS-S investigated in this study encodes the full MERS-CoV spike glycoprotein. This study aimed to assess the safety, tolerability and immunogenicity data for a novel viral-vectored MERS candidate vaccine in healthy adults.

Highlights

- 26 participants (14 in the low-dose group and 12 in the high-dose group) were enrolled in this study.
- For the prime immunisation, participants received doses of 1×10^7 plaque-forming unit (PFU; low-dose group) or 1×10^8 PFU (high-dose group) MVA-MERS-S intramuscularly.
- A second identical dose was administered intramuscularly as a booster immunisation 28 days after first injection.
- Homologous prime–boost immunisation with MVA-MERS-S revealed a benign safety profile with only transient mild-to-moderate reactogenicity.
- Solicited local reactions were the most common adverse events; pain, swelling, headaches and fatigue or malaise. All adverse events resolved swiftly (within 1–3 days) and without sequelae.
- Following booster immunisation, nine (75%) of 12 participants in the low-dose group and 11 (100%) participants in the high-dose group showed seroconversion using a MERS-CoV S1 ELISA at any timepoint during the study.
- Binding antibody titres correlated with MERS-CoV-specific neutralising antibodies.
- A single injection of MVA-MERS-S showed no induction of neutralising antibodies. The virus neutralisation test revealed detectable neutralising capacity only after boosting, but mainly in participants in the low-dose group.
- T-cell responses against MERS-CoV spike emerged after a single vaccination with MVA-MERS-S in some of the participants and were enhanced after boost immunisation.

Clinical Impact

Moderate. The favourable profile of MVA-MERS-S might also make useful contributions for the development of future vaccine strategies against other coronavirus pathogens.

Important Methodologies

- MERS-CoV spike-specific T-cell responses were evaluated by ELISpot.
- MERS-CoV S1 ELISA assay.
- Sera were tested for neutralisation capacity with a plaque reduction neutralisation test.
- CD8 IFNγ-expressing T cells were analysed using flowcytometry.

Limitations

- The restricted number of study participants in this phase 1 trial limits the generalisability of results and necessitates follow-up studies in larger cohorts to advance MERS vaccine development.
- The study did not include an additional late boost, which induced strong and increased antibody responses in a previous MVA-H5-sfMR trial.
- This early study format also did not allow for data generation on antibody dependent enhancement in the context of MERS-CoV infections, which has been previously discussed for severe acute respiratory syndrome (SARS) coronavirus.

Rapid development of an inactivated vaccine for SARS-CoV-2

Qiang Gao/bioRxiv preprint/2020

Link: https://www.biorxiv.org/content/10.1101/2020.04.17.046375v1

The development and testing of new PiCoVacc vaccine generated using an inactivated SARS-Cov-2 strain isolated from a hospitalised Chinese patient is described. This strain remained relatively genetically stable over 10 passages and stimulated production of predominantly Spike (S) and Receptor Binding Domain (RBD) immunoglobulin in rodents and macaques. Importantly, the elicited antibodies exhibited neutralisation of 9 other SARS-Cov-2 strains in global circulation. Immunised macaques exhibited no lymphocyte, cytokine, histopathological or physiological evidence of toxicity and were significantly protected against intra-tracheal challenge with a related SARS-Cov-2 strain with no antibody-dependent enhancement (ADE) observed and only minor changes in lung histopathology.

Main findings:

- The authors isolated SARS-CoV-2 strains from the bronchoalveolar lavage fluid (BALF) samples of 11 hospitalized patients; 4 from China, 1 from Italy, 1 from Switzerland, 1 from UK and 1 from Spain (notably none from the US or Southern hemisphere).
- Chinese strain CN2 is used to generate a purified inactivated vaccine, PiCoVacc. The other strains are used as pre-clinical challenge strains.
- Culture of this viral strain over 10 passages resulted in 2 amino acid substitutions that the authors consider an indicator of genetic stability since these mutations are not in the S regions that would impact production of effective neutralising antibodies – several other studies have indicated the S and RBD regions as critical for anti-viral targeting.
- Immunisation of rodents with PiCoVacc resulted in robust production of S and RBD immunoglobulins representative of isolates from recovered Covid-19 patients within 6 weeks.

- Rodent sera effectively neutralised the 10 other SARS-CoV-2 viral strains tested.
- Vaccine safety evaluations in macaque primates revealed effective production of S protein-specific neutralising antibodies (with a degree of individual variation in titres) following 3 consecutive intra-muscular injections. No gross physiological or pathological changes were reported; there was no evidence of fever, weight loss, alterations in T lymphocyte proportions or selected inflammatory cytokines. The authors suggest this indicates the vaccine would not produce the cytokine storm effects associated with enhanced viral pathogenesis (although this was not clearly assessed in challenged animals).
- Intra-tracheal challenge with 10^6 50% tissue culture infective doses ($TCID_{50}$) resulted in protection of all vaccinated macaques, with complete abrogation or 95% reductions (compared with controls) in viral loads detected in animals immunised with high (6μg) and medium (3μg) doses, respectively. Control animals suffered severe pneumonia, whereas vaccinated animals had minimal lung histopathological effects and no reported ADE.

Highlights:

- Production of an inactivated SARS-CoV-2 virus vaccine with no detected toxicity or ADE effect in non-human primate model.
- Immunisation schedule of 3 intra-muscular injections resulted in viral S-protein-specific neutralising antibodies with titres similar to those in recovered human Covid-19 patients.
- Neutralising antibodies were effective against 10 SARS-CoV-2 strains, so might provide practical immunity against diverse SARS-CoV-2 global strains.
- Immunisation with medium (3μg) and high (6μg) doses of vaccine resulted in significant reductions (95-100%) in viral load within one week of infection and minimal evidence of histopathological changes in the lung.
- Whilst levels of neutralising antibodies dipped by 30% at day 3 post-infection, these had recovered to pre-infection levels by day 7, supporting continued anti-viral defence.

Clinical Impact:

High: the authors state that Phase I, II and III trials of the PiCoVacc vaccine described in this article are expected to commence later in the year. Sinovac Biotech is currently collaborating with the US Dynavax company to boost the immune response to PiCoVacc by combining it with the CpG 1018 adjuvant used in the US HEPLISAV-B vaccine.

Important Methodologies:

Description of the production of inactivated Covid-19 vaccine: strain used (although sequence not given GenBank accession number in this pre-print), production, inactivation and purification details and viral titres.
Macaque challenge model regime and vaccine safety evaluation protocols.
Expression of purified viral proteins, ELISA detection of anti-viral antibodies, RT-PCR detection of viral load, CryoEM visualisation of virus.

Limitations:

- Most of the authors are affiliated with Sinovac Biotech Ltd., thus have a vested interest in reporting favourable toxicity and antibody responses to the PiCoVacc vaccine. The fact that they have subsequently partnered with Dynavax to employ the Dynavax

adjuvant suggests the authors may have doubted the immunogenicity of PiCoVacc in its current state.

- The report is in preprint format, so the text and figures are not presented as clearly as would be necessary to properly evaluate the data. Figure legends are almost illegible and there are errors in the text (including which strains used) that could result in data misinterpretation.
- There is limited information provided about the 11 SARS-Cov-2 strains employed; the sequences have not yet been published/GenBank accession numbers awarded and arbitrary nomenclature rather than viral clade names is used. Since none of the strains was isolated from US or Southern hemisphere countries, it is not clear whether the vaccine is likely to be effective against all global strains.
- The viral strain used is declared genetically stable after 10 passages, but the number of passages used/required in full-scale vaccine production is not detailed. Long-term stability is uncertain and drifts in S or RBD protein-encoding domains would have severe impact for vaccine efficacy.
- The virus is inactivated with β-propiolactone, but the large-scale supply-line security of this reagent is not discussed (currently unavailable with some suppliers).
- The challenge model involves only a single viral dose at 10^6 $TCID_{50}$ that may not be representative of natural infection? ADE resulting from recurrent or related coronavirus infection is not discussed, either.
- Some experimental details are omitted – e.g. the assays employed to measure inflammation in the immunised rodents, the precise immunisation protocols (intra-muscular vs intra-peritoneal), the source of the human data used for comparison in Figure 2, the means of assessing ADE. This makes it difficult to rigorously critique the work.
- Importantly, incomplete information is provided about the vaccine safety and challenge assays in macaques, so it is unclear as to whether the experimental groups overlap and/or are comparable. The ages and genders of all animals used in each assay are also not reported. The macaques used in the challenge assay were young (3-4 years) and so potential discrepancies in efficacy in young and old humans cannot be inferred.
- Some valuable tests are missing, including T lymphocyte counts after the first (of 3 in the schedule) immunisation in the macaques, whereas data post-2^{nd} and 3^{rd} immunisations are reported. It would also be of value to report actual lymphocyte counts, rather than just proportions that might mask gross numerical abnormalities.
- Only selected cytokines were measured, whereas a broader spectrum of factors involved in cytokine storms in Covid-19 patients would have been useful (IL-7, GCSF, IP10, MCP1). There is also no report of ferritin levels even though hyperferritinaemia has been suggested as a predictor of fatality in Covid-19 patients.
- The study was limited to 29 days' post-vaccination in the macaques, so cannot reveal any potential lasting immunity that might result from immunisation with this vaccine.

Epidemiology

Connecting Clusters of COVID-19: An Epidemiological and Serological Investigation.

Yong *et al* (Lancet Infectious Disease), 2020
Link: https://doi.org/10.1016/S1473-3099(20)30273-5

Summary : To achieve this, they used active case finding using RT-qPCR and contact tracing. Moreover, serological tests for the presence of a previous COVID-19 infection were also used. In summary, they found three clusters of COVID-19 comprised of 28 locally transmitted cases, there were 2 churches (Church A and Church B) and a family gathering. The clusters in Church A (A2) and Church B were linked by an individual from Church A, who transmitted SARS-CoV-2 infection to the primary case from Church B at the family gathering attended by both. However, A2 was negative for active disease, but eventually identified as having had the infection by serological testing.

Main findings:

- The clusters were linked to two travellers (W1 and W2) from Wuhan, China, who attended a church service at Church A.
- 17 locally transmitted cases were linked to Church B. Thorough review of activity maps found that F1, an induvial infected from the family gathering, continued to work at Church B while ill. Therefore, becoming the primary case of the Church B cluster.

Highlights:

- For much of the people in the three clusters reported here, transmission of infection was accounted for by close contact with a symptomatic case.
- Therefore, COVID-19 is largely transmitted by close contact, particularly when contact occurs over a prolonged period and in close congregation.

Clinical Impact:

- They have highlighted the importance of serological testing for epidemiological investigation of COVID-19 cases, and they ask for further development of serological testing capabilities.

Important Methodologies:

- Both RT-qPCR and serological testing was used to diagnose cases of COVID-19. RT-qPCR testing alone is limited by its ability to detect convalescent cases of COVID-19, because RT-qPCR can only detect SARS-CoV-2 during the period of viral shedding.
- Serological testing can be useful in detecting previous infection in people with suspected infection who have recovered, assisting in epidemiological investigation and containment efforts.

Limitations:

- Information could be inaccurate as a result of recall and other biases.

Supporting pandemic response using genomics and bioinformatics: a case study on the emergent SARS-CoV-2 outbreak

Denis C. Bauer, Transboundary and Emerging Diseases, 19 Apr 2020
Link: https://onlinelibrary.wiley.com/doi/10.1111/tbed.13588

At present selection of the best viral isolates for animal models that inform on diagnostics, vaccines, and experimental treatments are derived from practical considerations rather than a detailed analysis of the characteristics of the virus strain chosen. In this work they suggest a methodology for choosing appropriate viral strains that is driven by both phylogeny as well as a novel alignment-free bioinformatics approach that assess genome-wide-co-developing functionalities. In this work they demonstrate a concerning lack of coverage in terms of genetic variants offered by the currently selected viral strains for animal models and provide an approach for choosing more appropriate models.

Main findings:

- Phylogenetic clusters reveal three major groups separated by distinct mutations but also evidence that other additional clusters may be emerging. This differs to prior analysis with fewer included isolates and different rooting that postulates only two distinct clusters.
- An alignment-free methodology that takes into account genomic variation and deletions was applied and successfully separated all SARS-CoV2 strains against 17 SARS and 6 MERS isolates
    - Genetic distance between different isolates of the same coronavirus strain is relatively small whilst substantial differences exist between different coronavirus strains
    - MERS isolates separated into two subclusters reflecting host origins and prior findings
- Alignment-free analysis shows Australian isolates were closest to Wuhan-Hu-1 (consistent with phylogenetic results) reflecting only mutational changes in their core sequences.
- Isolates with deletions were placed further away from Wuhan-Hu-1 in K-mer analysis than compared with phylogenetic analysis, showing the alignment-free methods ability to represent deletions accurately
- The alignment-free analysis is not as suggestive as the phylogenetic trees for visualising transmission route but more accurately reflects the ‘cloud of variants’ and captures recombination events
- Alignment-free analysis suggests that current animal models do not cover the evolutionary space of circulating viral strains
- USA/WA1 (available through BEI) could also be a good choice for models due to its central location (in terms of genetic distance to other strains), likely ability to represent especially the newly emerging clusters, and comparability of animal models with non-human primate studies in the US which have chosen this on the basis of it being readily available

Highlights

- The work here highlights a novel approach for the visual interpretation of viral variants when considering strains for animal models; traditional phylogenetic trees are generally based on the presence of shared mutations whereas an alignment-free method as shown here is more concerned with 'global' similarities and differences
- According to phylogenetic analysis, the main clusters are fairly represented by current animal models, but the alignment-free method suggests this could be misleading and in-fact current animal models do not cover the evolutionary space of actively circulating viral strains

Clinical impact

- Minimal clinical impact/Moderate impact on vaccine development

Important methodologies

- Two Australian isolated were sequenced using the MiSeq platform and combined with all available sequences on GISAID (157 genomes in total)
  - Low quality sequences removed leaving 178 strains in total
  - 181 sequences aligned against each other using Muscle (v3.8.31)
- Maximum likelihood phylogenetic trees generated from alignments using RaxML-NG. GTR+G+I evolutionary model used because it is the most general model. Visualised with iTOL.
- Alignment-free model that captures the 'genomic signature' of strains counted all possible k-length (10 base) sequences and generated a conceptual distance between isolates using PCA applied over all N-dimensional K-mer frequency vectors, reducing signatures to a two-dimensional space for visualisation

Limitations

- The aetiology of SARS-CoV2 is not fully understood, especially any intermediate vector, and so artificially rooting phylogenetic tree by introducing a distant relative may bias the results
- Given that the pandemic is in its early stages still, the number of circulating strains and the virulence cannot be assumed with the currently available epidemiological and clinical data provided, therefore the methods presented here should be re-examined as more datasets become available