Covid-19 Community Journal Club
April 23rd, 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.

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Further sources of Covid-19 literature can be found on Page 7.

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

Table of Contents Page

Clinical

Diagnostics and Therapeutics

Harvey B. Pollard *et al*. (Medrxiv), 2020
Link: https://www.biorxiv.org/content/10.1101/2020.04.09.034983#Sec3

A Trial of Lopinavir–Ritonavir in Adults Hospitalized with Severe Covid-19. **28**
Cao *et al*. *T*he New England Journal of Medicine. 2020
Link: https://www.nejm.org/doi/full/10.1056/NEJMoa2001282

Compassionate Use of Remdesivir for Patients with Severe Covid-19. **29**
Grein *et al*. The New England Journal of Medicine. April 10, 2020.
Link: https://www.nejm.org/doi/full/10.1056/NEJMoa2007016

COVID-19 and emerging viral infections: The case for interferon lambda **30**
Prokunina-Olsson *et al*. (Journal of experimental medicine), 2020
Link: https://rupress.org/jem/article/217/5/e20200653/151664/COVID-19-and-emerging-viral-infections-The-case

The establishment of reference sequence for SARS-CoV-2 and variation analysis **31**
Wang *et al*. / Journal of Medical Virology / 2020
Link: https://onlinelibrary.wiley.com/doi/full/10.1002/jmv.25762

Virological assessment of hospitalized patients with COVID-2019 **32**
Wölfel *et al*. Nature (2020)
Link: https://www.nature.com/articles/s41586-020-2196-x

Neutrophil extracellular traps (NETs) as markers of disease severity in COVID-19 **34**
Yu Zuo *et al.* (medRxiv preprint), 2020
Link: https://www.medrxiv.org/content/10.1101/2020.04.09.20059626v1.full.pdf+html

Estimating false-negative detection rate of SARS-CoV-2 by RT-PCR **35**
Wikramaratna *et al*. MedRxiV
Link: https://doi.org/10.1101/2020.04.05.20053355

Antibodies

Evaluation of Nucleocapsid and Spike Protein-based ELISAs for detecting antibodies against SARS-CoV-2 **37**
Liu *et al*. (Journal of Clinical Microbiology), 2020
Link: https://jcm.asm.org/content/jcm/early/2020/03/27/JCM.00461-20.full.pdf

Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019 **38**
Zhao J. *et al* (Clin Infect Dis), 2020
Link: https://www.ncbi.nlm.nih.gov/pubmed/?term=Antibody+responses+to+SARS-CoV-2+in+patients+of+novel+coronavirus+disease+2019

Distinct early IgA profile may determine severity of COVID-19 symptoms: an immunological case series 39
Dahlke C. MedRxiv preprint
Link: https://www.medrxiv.org/content/10.1101/2020.04.14.20059733v1.full.pdf

Potent human neutralizing antibodies elicited by SARS-CoV-2 infection 40
Ju *et al* bioRxiv preprint
Link: https://www.biorxiv.org/content/10.1101/2020.03.21.990770v2

The SARS-CoV-2 receptor-binding domain elicits a potent neutralizing response without antibody-dependent enhancement 41
Quinlan BD *et al*. (Unpublished BioRxiv). 2020.
Link: https://www.biorxiv.org/content/10.1101/2020.04.10.036418v1

Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications 42
Fan Wu *et al*. Medrxiv.2020
Link: https://www.medrxiv.org/content/10.1101/2020.03.30.20047365v2

Virology

Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. 44
Lan *et al*. (Nature), 2020
Link: https://doi.org/10.1038/s41586.020.2180-5

Structural basis of receptor recognition by SARS-CoV-2 45
Shang et al. (Nature), 2020
Link: https://www.nature.com/articles/s41586-020-2179-y

SARS-Cov-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells 46
Soeren Lukassen *et al*, The EMBO Journal, 2020
Link: https://stm.sciencemag.org/content/early/2020/04/03/scitranslmed.abb5883

SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion 47
Wang *et al.,* 2020. Nature Cellular and Molecular Immunology
Link : https://www.nature.com/articles/s41423-020-0424-9

Virus-host interactome and proteomic survey of PBMCs from COVID-19 patients reveal potential virulence factors influencing SARS-Cov-2 pathogenesis. 48
Liang et al., pre-print on BioRxiv.
Link: https://www.biorxiv.org/content/10.1101/2020.03.31.019216v1

Vaccines

Preliminary Identification of Potential Vaccine Targets for the COVID-19 Coronavirus (SARS-CoV-2) Based on SARS-CoV Immunological Studies **52**
Ahmed, S.F. et al. *Viruses,* 2020
Link: https://www.mdpi.com/1999-4915/12/3/254/htm

Sources of further information:

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/

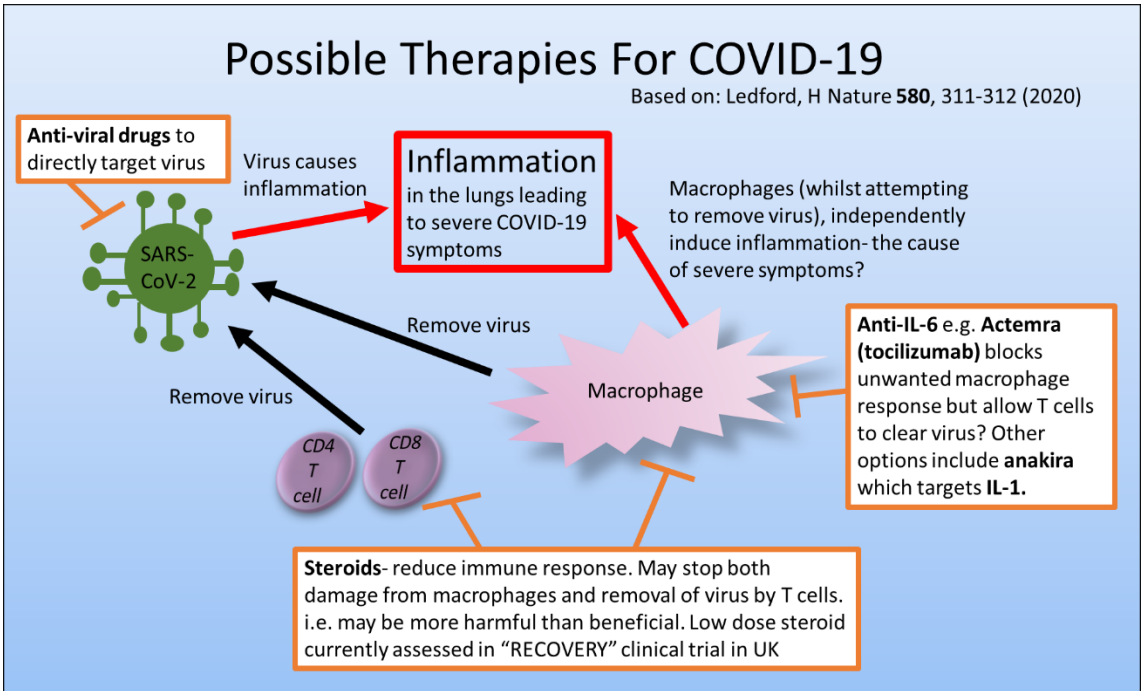
https://www.immunology.org/coronavirus/connect-coronavirus-webinars

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

News and Views

How does COVID-19 kill? Uncertainty is hampering doctors' ability to choose treatments

H. Ledford
https://www.nature.com/articles/d41586-020-01056-7



- This news article discusses possible therapies for COVID-19
- Inflammation in the lungs leads to severe COVID-19 symptoms
- The inflammation is caused by two things: the SARS-CoV-2 virus itself and the body's immune response to the virus
- In particular, macrophages (whilst attempting to remove virus), independently induce inflammation in the lungs- is this the cause of severe symptoms?
- **Anti-IL-6** e.g. **Actemra (tocilizumab)** could block the unwanted macrophage response but allow T cells to clear virus. Other therapeutic options include **anakira** which targets **IL-1.**
- **Steroids**- reduce immune response. May stop both damage from macrophages and removal of virus by T cells (i.e. may be more harmful than beneficial). Low dose steroid currently assessed in "RECOVERY" clinical trial in UK
- **Anti-viral drugs** to directly target virus would also be a useful treatment

Is the coronavirus airborne? Experts can't agree

Dyani Lewis, Nature News
https://www.nature.com/articles/d41586-020-00974-w

Summary

This article discusses evidence suggesting that coronavirus particles can be transmitted through aerosol, from breathing or talking, and not only from larger droplets expelled through coughing or sneezing. The article highlights the fact that evidence of aerosol transmission is conflicting and factors such as exposure time and viral density will increase the likelihood of transmission. They do concede that transmission by aerosol will be difficult to prove. The article states that some scientists are particularly keen to see precautionary measures implemented such as increasing indoor ventilation and the use of masks in public to reduce the potential risk of viral spread by aerosol.

Should scientists infect healthy people with the coronavirus to test vaccines?

Ewen Callaway
https://www.nature.com/articles/d41586-020-00927-3

Summary

This article is a Q&A of Nir Eyal, the director of the Centre for Population-Level Bioethics at Rutgers University, New Jersey, discussing the ethics, risks and potential benefits of 'human challenge' studies to test candidate coronavirus vaccines, over conventional phase III studies. Human challenge studies would require a group of healthy individuals, vaccinated or placebo, to be exposed to coronavirus. Allowing for monitoring of the efficacy of a candidate vaccine over a much shorter time frame. To reduce the risk to participants, Mr Eyal suggests only including young (20-45yr), healthy volunteers and monitoring them daily for any COVID-19 symptoms. He states that this method of trialling vaccines is ethical as long as appropriate consent is taken, participants are of sound-mind, are not enticed by large financial incentives or forced to participate. Finally he states that he believes that large government funders will participate as the 'net risks' do not outweigh the benefits to communities as a whole of a working vaccine.

Ending coronavirus lockdowns will be a dangerous process of trial and error

Kai Kupferschmidt
https://www.sciencemag.org/news/2020/04/ending-coronavirus-lockdowns-will-be-dangerous-process-trial-and-error#

Summary

This article discusses the lack of scientific consensus over coronavirus lockdown 'exit strategies'. It also points out the importance of the effective reproduction number (R). R is a measure of how many people the average infected person infects in turn, i.e. R below 1 indicates shrinking of a viral outbreak. The article suggests three possible strategies to regulate R; isolating patients and tracing their contacts, border restrictions and social distancing. Immunity is also key in dialling back control strategies, if virus exposure equals immunity, the article states that 30% population immunity would bring R down by the same amount. However, it does not advocate for herd immunity. The article suggests that the likely exit strategy could involve cycles of relaxing and enforcing social distancing, depending on the rate of infections. Finally, the article quotes Jeremy Farrar, the head of the Wellcome Trust, who states that in some way, whether it be due to treatments or vaccines, that 'science is the exit strategy'.

*For more information, see journal reviews in the **Transmission** section below.*

Journal Reviews

Transmission

Projecting the transmission dynamics of SARS-CoV-2 through the postpandemic period

Kissler *et al*. 2020 Science
Link: https://science.sciencemag.org/content/early/2020/04/14/science.abb5793

Kissler et al have modelled the the pandemic and post pandemic of SARS-CoV-2 transmission based on beta coronoaviruses OC43 and HK1 including seasonal effects and cross-immunity. Mathematical models combine viral environmental and immunological factors to predict the dynamics of SARS-CoV-2 from US data. Maintaining critical care demand below capacity was the key outcome metric. There are predicted recurrent winterout breaks after the initial most severe pandemic. Social distancing is the key intervention to prevent exceeding critical care capacity in the absence of other interventions. **Social distancing in some form may need to continue to 2022**

Main findings:

- SARS-CoV-2 can proliferate at any time of year
- If immunity is not permanent then annual, biennial or sporadic patterns will emerge
- High seasonal variation (ie summer reduction in R_0) will result in larger recurrent wintertime peaks
- If there is significant cross immunity then all beta conora viruses could decline and disappear
- Mild cross immunity from OC43 and KHKU1 will result in reduced transmission with resurgence in 3 years.
- Modelling of post social distancing surges with and without seasonality and with varying effect of intervention
- Size of initial peak inversely associated with post social distancing peak
- To remain under current critical care capacities epidemic could last to 2022 with social distancing between 25 and 75% of the time

Highlights
Robust modelling taking into account viral seasonal and immunological interactions.
Multiple outputs for social distancing effectiveness and length.

Clinical Impact:
High - Likely to impact social distancing policy and critical care provision planning across the Western World over the short- medium term

Important Methodologies:

- Developed susceptible-exposed-infectious-recovered-susceptible (SEIRS) model based on HK and OC43 data
- Modelling of HK and OC43 corona viruses robust based on US data from 2014-2019

Limitations:

- Based on OC43 and HKU1 infections in the US over 5 years
- Based on measured not true incidence and hospitalisation from beta coronaviruses
- Model did not consider variation in natural history of betacorona viruses and impact of age, or geography
- Will need updating as immunity and cross immunity understood
- Critical admission is a poor indicator of health care and societal good
- Does not take into account therapeutics, vaccinations and contact tracing and isolation

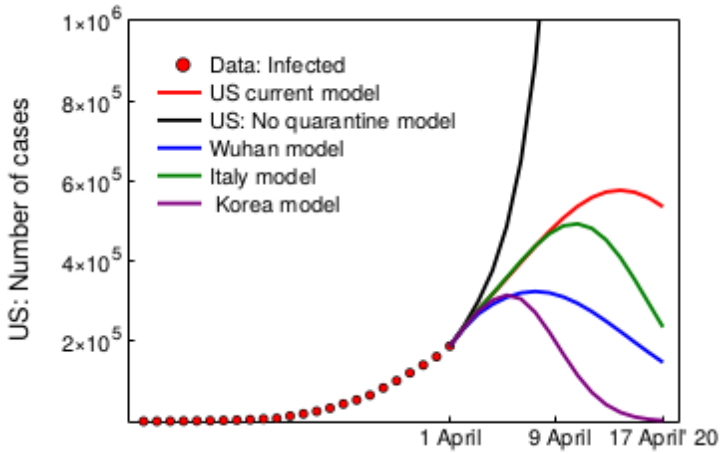
Quantifying the effect of quarantine control in Covid-19 infectious spread using machine learning

Raj Dandekar and George Barbastathis, MedRxiv, Apr 3rd 2020
Link: https://www.medrxiv.org/content/10.1101/2020.04.03.20052084v1

Dandekar and Barbastathis successfully demonstrate a novel approach to modelling infectious spread of SARS-Cov-2 infection that combines epidemiological equations derived from first principles with a shallow neural network trained on publicly available data from four regions/countries: Wuhan, Italy, South Korea, and the USA. They compare the impact of quarantine measures in each country in terms of effective reproduction number. Their findings unequivocally indicate that the countries which applied rapid interventions successfully halted the spread of infection. They demonstrate that quarantine measures in the USA could 'flatten' infective spread by April 20th whilst relaxing quarantine measures would lead to an exponential explosion in infected case count.

Main findings:

- Classical SEIR and SIR models, when applied to Wuhan data, fail to recover the stagnation seen in actual infected number, whereas the neural network model this with improved accuracy
- Neural network model that accounts for quarantine effects and trained on retrospective data from the current outbreak shows good agreement with actual infected number observed in South Korea and Italy
- The figure below shows the projected number of cases in the USA from April 1st for the current quarantine model (red) or gradual adjustment to one of the quarantine models implemented in other countries (blue, green, purple)



Highlights

- Demonstrates a data-driven epidemiological model trained on globally applicable data for the current outbreak
- Highlights the impact of quarantine measures on reproduction number and offers predictions that can inform quarantine policy

Clinical impact

- Minimal

Important methodologies

- SEIR and SIR epidemiological methodologies model the relationship between susceptible, infected, and recovered states as a system of non-linear ordinary differential equations. A limitation of this method is an inability to account for social distancing measures and mass quarantine effects.
- In this work they overcome this limitation by adding an additional 'quarantine strength' term the value of which is approximated using a neural network trained on large publicly available data from the region of interest
- The neural network, for each region, was trained on data from the date that the 500th infection was recorded, up until a month thereafter

Limitations

These models make assumptions regarding initial conditions, notably: the number of susceptible individuals is equal to the regions population and the number of recovered individuals starts with some small value (e.g. 10)

First-wave COVID-19 transmissibility and severity in China outside Hubei after control measures, and second-wave scenario planning: a modelling impact assessment

Leung *et al*. (The Lancet), 2020
Link: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30746-7/fulltext

This paper analysed the data outside epicentre of China (Hubei), and estimated the instantaneous reproduction number (Rt) and the confirmed case-fatality risk (cCFR) in several non-Hubei areas. Through a designed model, the authors predicted a potential 2nd wave of infection in these areas after relaxing containment. Thus, they suggested a close monitor of Rt and maintain it less than 1 is important.

Main findings:

- China used aggressive non-pharmaceutical interventions nationwide to prevent viral spread, however, herd immunity is absent.
- In areas outside Hubei, COVID-19 was mainly driven by imported cases from Hubei until late January. In most cities outside Hubei the Rt was less than 1, and gradually reduced since 23th Jan (Wuhan lockdown).
- From Feb 7 onward (end of Chinese New Year holiday and start of limited mobility), some magacities began to report imported cases from Hubei again.
- From March 4, China saw as surge in overseas imported cases.
- cCFR among cases outside Hubei was 0·98% (0·82–1·16), compared to Hubei's cCFR: 5·91% (5·73–6·09)
- Cumulative case count will increase after relaxation, according to their model. Once Rt > 1, simply tightening control interventions again to maintain Rt=1 would not reduce the burden back to its original baseline.

Highlights

- A 2nd wave of COVID-19 transmission is possible due to imported cases and restarted economic and social activities. This article gave advice on attentions should be paid when easing lockdown.
- **Early detection of cases is essential**. This article highlighted Guangdong, where more than 320 000 RT-PCR tests on those who had attended fever clinics and hospitals over 30 days between January and February, 2020. This test capacity can make sure possible reintroduction of infected cases to be detected, isolated, and their contacts traced and quarantined.
- **Always keeping Rt below 1** is the optimal strategy until effective vaccines become available.
- Closely monitoring real-time Rt is essential.

Clinical Impact:

- Mild

Important Methodologies:

- **Rt**: the average number of secondary cases generated by one primary case with symptom onset on day t. If Rt >1 the epidemic is expanding at time t, whereas Rt < 1 indicates that the epidemic size is shrinking at time t.
- **MATLAB, R** package **EpiEstim** was used to make **epidemic curve**.
- **cCFR**: confirmed case-fatality risk. The CI of cCFR was obtained using **boot.ci** function in the **R** package boot, version 1.3-24
- Simulating the potential effect of relaxing interventions was done in **MATLAB** 2019b

Limitations:

- It's an estimated result, based on data in non-epicentre in China. In majority of these areas, the Rt had been sustained less than 1 overtime, while herd immunity is absent. UK's situation could be different.
- Some important element, such as travel history or epidemiological links to Hubei, were unstable as the proportion of imported cases varied over time.
- The simulations of intervention relaxation were done for a limited number of illustrative scenarios regarding temporal changes in the reproduction number.

Temporal Dynamics in Viral Shedding and Transmissibility of COVID-19

He *et al*. (Nature Medicine), 2020
Link: https://www.nature.com/articles/s41591-020-0869-5.pdf

They reported on the patterns of viral shedding in 94 patients with laboratory confirmed COVID-19. In addition, modelling was performed on COVID-19 infectiousness profiles from another 77 infector-infectee transmission pairs. They concluded that viral shedding of patients with laboratory-confirmed COVID-19 peaked on or before symptom onset, and a substantial proportion of transmission probably occurred before first symptoms in the index case. Therefore, disease control measures should be adjusted to account for probable substantial presymptomatic transmission.

Main findings:

- The main demographics results from this study were: 47/94 (50%) were male, the median age was 47 years and 61/93 (66%) were moderately ill, but none were classed as severely ill.
- They detected high viral loads soon after symptom onset, which gradually decreased towards day 21.
- There were no differences in viral loads across sex, age and disease severity.
- Using the 77 pairs, they inferred that infectiousness started from 2.3 days before symptom onset and peaked at 0.7 days before symptom onset.

Highlights:

- Viral shedding may begin 2 to 3 days before the appearance of the first symptoms.

Clinical Impact:

- This study shows that COVID-19 has a greater similarity to the infection profile of influenza than of SARS.

Important Methodologies:

- The modelling of 77 infector-infectee transmission pairs.

Limitations:

- Symptom onset relied on patient recall after the confirmation of COVID-19 diagnosis.
- Shorter serial intervals were reported in other studies.
- The viral shedding dynamics were based on data for patients who received treatment according to nationally promulgated protocols, including, combinations of antivirals, antibiotics, corticosteroids, immunomodulatory agents and Chinese medicine preparations, which could have modified the shedding dynamical patterns.

Spread of SARS-CoV-2 in the Icelandic Population

Gudbjartsson *et al.* NEJM, 2020
Link: https://www.nejm.org/doi/full/10.1056/NEJMoa2006100

The results of targeted testing and random population screening for SARS-CoV-2 in the Icelandic population from 31st January-4th April 2020 are presented. Targeted testing of symptomatic individuals was conducted both prior to and post implementation of social and travel restrictions, whereas two independent approaches to general population sampling (online open application versus random telephone text invitation) were performed only for the latter period. Early infections linked to foreign travel were succeeded by community transmission, with reduced incidence amongst children and females. Virion sequencing permitted tracking of haplotype diversity and assessment of the origins and spread of infection amongst the population.

Main findings:

- Overall infection rates following the implementation of travel and social restrictions on 16th March 2020 have remained stable (<100 per day) and the total number of deaths has reached only 9 in a population of 364,000 inhabitants, suggesting testing and contact tracing in Iceland has resulted in successful disease management.
- Targeted qRT-PCR testing and Illumina sequencing of nasopharyngeal and oropharyngeal samples from symptomatic individuals commenced 28 days prior to the detection of the first infection in an individual returning from Italy. 13.3% people undergoing targeted testing were found to be SARS-CoV-2 positive. Early cases were linked to foreign travel (65% positive samples) and predominantly (143/157 virions) consisted of A2 clades associated with European infections.
- General population screens of asymptomatic individuals commenced on 13th March, with 0.8% samples obtained by open online application and 0.6% those obtained following random invitation testing positive for SARS-CoV-2. Only 23% individuals testing positive had undergone recent foreign travel, with 60% these being to the UK, not then deemed to be a high risk destination. Accordingly, 39% sequenced virion haplotypes were clade A1a, associated with UK infections. The authors suggest this evidences earlier viral circulation in the UK than previously suspected.
- Amongst all groups tested, incidence in children <10 years and females was lower than in adults and males.
- 409 sequence variants were detected in 643 virions sequenced, revealing mutation rate and haplotype tracing as an effective means of clustering cases and estimating numbers and sources of original introductions. Clades A2a2a and A2a3a are the most common amongst the Icelandic population.

Highlights:

- **Testing, quarantine and contact tracing are effective means of stabilising infection and limiting death rates from SARS-CoV-2.**

- Population-wide screening associated with virion sequencing provides information concerning the country and timeline of original infections, as well as a means of tracing disease spread and virus mutation rates.
- Infection rates amongst children <10 are low: 6.7% children underdoing targeted testing and 0% children from the general population screen were SARS-CoV-2 positive.
- Incidences of infection were 1.5-fold higher for males than females amongst both targeted and general screening populations despite higher numbers of females being sampled.

Clinical Impact:
High.
Methodologies and findings provide important comparison with datasets from larger countries.
In particular, the virion sequence data will provide useful means of assessing infection spread across the world, as well as potentially highlighting vulnerabilities for vaccine and other treatment strategies.

Important Methodologies:
Details of methods employed for:

1. Contact tracing and quarantine
2. SARS-CoV-2 testing by qRT-PCR; including RNA extraction details, primers, probes and reagents
3. Illumina MiSeq sequencing of overlapping tiles of virion amplicons generated in multiplex PCR
4. Sequence data analysis and network analysis of haplotypes for infection tracking

Limitations:

- Authors are affiliated with deCODE Genetics, conducting ~75% all Icelandic tests since 15th March 2020 – no external validation of data.
- The deCODE qRT-PCR assay has a detection limit of 6 copies/reaction, which is less sensitive than the WHO recommended pipeline (originally used at the National University Hospital of Iceland) that has a sensitivity of 5.2 RNA copies/reaction for the non-specific E-gene and 3.8 RNA copies/reaction for the 2019- nCoV specific confirmatory RdRp gene. This difference may explain why 2 samples previously identified as positive could not be confirmed using the deCODE methodology. Reduced sensitivity may mean cases with low viral load in the general population and/or infections with more than one haplotype may be missed.
- The sequencing methodology using overlapping amplicon tiles generated in multiplex PCR with imputation for missing nucleotides may result in ambiguity or error. The controls used in the sequencing pipeline (if any) are not mentioned and there is no cross-platform or external dataset validation to test the haplotype analyses described.
- Ascertainment bias in sampling is a clear issue, particularly with the general population screens. The authors state a concern that some people may have

participated in the general population testing because they were already concerned about symptoms and should have been excluded from the screening. Importantly, the bulk of the general population screening (10,797 individuals) was conducted in the capital, Reykjavik, thus potentially not being representative of the population as a whole. Screening was only extended to the wider Icelandic community between 1st-4th April and resulted in an additional 2283 samples. The overall sampling distribution is unclear.

- Changing restrictions on travel and quarantine requirements will have resulted in virus strains and infected groups being under-represented in the sampling data – e.g. people travelling from high risk areas were subjected to quarantine rules from an early stage and thus excluded from the study. The authors have, however, tried to be as transparent as possible about the data collected and the potential bias associated and this remains a real-time study of the spread and diversification of infection in a small population.
- Stratification of cohorts and sample details are minimal and so understanding of particular subgroup susceptibilities is precluded. Children are arbitrarily designated as <10 years, but there is no additional subdivision of the remaining 'adult' population by age as has been done with other studies. It is therefore not possible to assess associations of infection or symptom severity with age, ethnicity, employment status, comorbidities etc. There is also no means of determining whether incidence is linked to exposure or susceptibility.
- Testing was all conducted using nasopharyngeal and oropharyngeal samples. Since other studies have revealed sustained viral load in stool samples, it would have been interesting to do a comparative general population screen with these types of samples to see whether sensitivity of detection could be improved upon.

Clinical

Level of IL-6 predicts respiratory failure in hospitalized symptomatic COVID-19 patients

Tobias Herold *et al*., (MedRxiv) 2020.
Link: https://doi.org/10.1101/2020.04.01.20047381

Study compares baseline clinical and laboratory characteristics (PCT, IL-6, creatinine, comorbidities etc) of 40 patients hospitalised with PCR proven symptomatic COVID-19 infection, in order to identify variables that allow the prediction of patients with high risk respiratory failure and need of mechanical ventilation. IL-6 was identified as a potential marker that might be able to predict upcoming respiratory failure.

Main findings:

- 13/40 (32.5%) patients deteriorated during hospitalisation and required mechanical ventilation.
- All patients who required intubation were male.
- Pulse, markers of inflammation, LDH and creatinine at admission were associated with respiratory failure.
- Elevated IL-6 was strongly associated with the need for mechanical ventilation.
- The risk of respiratory failure for patients with IL-6 levels of ≥ 80 pg/mL was 92%; 22 times higher compared to patients with lower IL-6 levels.
- After reaching an IL-6 value of 80 pg/mL, the median time for mechanical ventilation was 1.5 days (range 0–4 days).

Highlights

- Suggestive of IL-6 potentially being a central pathogenic element of severe COVID-19 that should be used as a parameter for therapeutic intervention.

Clinical Impact

- Minimal – needs further studies.
- It would be useful to have reliable indicators of severity of disease to aid treatment pathways.

Important Methodologies

- Patients found positive for COVID-19 through PCR analysis.
- Characterisation of IL-6 – not referenced.

Limitations

- Manuscript has not been peer reviewed yet.
- Sample size of 40 patients from the same hospital.
- Only 13/40 required mechanical intervention and 12/13 showed increased IL-6. A much larger sample size would be needed to validate these findings.
- 72% of patients in the study were male.

- Time points of evaluation were not clear (widely varied between patients depending on severity).
- How they calculated the maximal levels of IL-6 for each patient during disease was unclear (predicted by comparing IL-6 values at first assessment to follow-up data on IL-6 – calculations not shown).
- Study states that patients requiring mechanical ventilation did not differ in age or respiratory rate. Values are only given as the median of the range tested, no specific detail.
- Patients tested were aged between 19-81. All patients requiring mechanical ventilation were the same age (as stated). There may be different predictive critical values of IL-6 depending on age. Therefore, patients should be split into groups.

Clinical and epidemiological characteristics of children with COVID-19

Stower *et al*. (Nature Medicine), 2020
Link: https://www.nature.com/articles/s41591-020-0846-z

Stower wrote a research highlight on the paper "Epidemiology of COVID-19 Among Children in China", by Dong *et al.* published in Pediatrics (DOI: 10.1542/peds.2020-0702). In the original paper, Dong *et al*. analysed 2,143 cases of children with COVID-19 that were reported to the Chinese Centre for Disease Control and Prevention. They described that children with COVID-19 experience mild disease when compared with adults, however, younger children are vulnerable to the disease and may experience some severe outcomes.

Main findings:

- 34.1% laboratory-confirmed cases, 65.9% suspected cases. Median age was 7 years (1 day to 18 years), 56.6% were boys, although there was no significant sex difference.
- 4.4% of the cases were asymptomatic, 51% were mild and 38.7% were moderate. 10.6% of children <1 year were classified as severe or critical, whilst only 3% of children >16 years were classified as such. One 14-year-old boy died.
- 46% were from Hubei province, 18.5% were from provinces that border Hubei province.
- Total numbers of paediatric patients increased extraordinarily between mid-January and mid-February, with the peak in 1st February.
- The median number of days from illness onset to diagnosis was 2 days (0–42 days).

Highlights

- Clinical manifestations of children with COVID-19 were less severe than in adults.
- Infants were vulnerable to the virus.
- Evidence of human-to-human transmission of disease (children did not visit the food market).

Clinical Impact:

- Minimal

Important Methodologies:

- Diagnostic criteria stratification for children

Limitations:

- Lack of correlation with clinical characteristics.
- Incubation period was not examined.

Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding

Xu *et al*. (Nature Medicine), 2020
Link: https://www.nature.com/articles/s41591-020-0817-4

Xu and colleagues reported epidemiological and clinical investigations on 10 paediatric SARS-Cov-2 infection cases. No children required respiratory support or intensive care, and symptoms were nonspecific. Chest X-rays showed no signs of pneumonia, which is a defining feature in adults. Most children persistently tested positive on rectal swabs after nasopharyngeal testing was negative.

Main findings:

- Symptom presentation included fever up to 39°C, coughing, sore throat, nasal congestion, rhinorrhoea and diarrhoea. One child was asymptomatic.
- No children presented other symptoms commonly seen in adult patients – lethargy, dyspnoea, muscle ache, headache, nausea, vomiting and disorientation.
- Chest X-rays did not present unilateral or bilateral pneumonia, they were either normal or showed only coarse lung markings.
- It was not observed pleural effusion, enlarged lymph nodes or other changes commonly observed in critically ill adults.
- Viral testing for other virus were negative for all patients.
- IL-17F, IL-22 and IL-6 were elevated in at least half of the patients.
- 8 patients had persistently positive tests of rectal swabs after their nasopharyngeal testing had become negative.
- Viral RNA measurements suggested that viral shedding from the digestive system might be greater and last longer than that from the respiratory tract

Highlights

- Compared to adult patients, paediatric patients had clinically milder symptoms and showed fewer alterations in radiological and laboratory testing parameters.
- Gastrointestinal tract may shed virus and faecal–oral transmission may be possible.
- Rectal swab-testing may be more useful than nasopharyngeal swab-testing to judge effectiveness of treatment

Clinical Impact:

- Moderate

Important Methodologies:

- Use of cycle threshold (Ct) values of the serial rectal and nasopharyngeal swab tests to approximately indicate viral load

Limitations:

- Small number of patients (n=10).
- Lack of evidence of replication-competent virus in faecal swabs.

SARS-CoV-2 detection in patients with influenza-like illness

Kong *et al*., (Nature Microbiology) 2020
Link: https://www.nature.com/articles/s41564-020-0713-1

In an effort to better understand the status of COVID-19 patients with mild illness (no breathing difficulties or hypoxia, representing 80% of confirmed COVID-19 cases in China), Kong *et al*., retrospectively analysed throat swabs from 640 patients with influenza-like illness (ILI). Samples were collected from the Children's Hospital of Wuhan and Wuhan No. 1 Hospital under the sustained sentinel surveillance of ILI implemented since 2005. This study re-analysed throat swabs collected during the epidemic between the 6th October 2019 and the 21st January 2020. Nine patients tested positive for SARS-CoV-2.

Main findings:

- Patient cohort comprised 315 males and 325 females with an age range of 9 months to 87 years (median age 8 years, mean age 22.7 years)

- The study coincided with the winter peak of influenza illness with increased numbers across all age groups; particularly the 5–14 years of age group increased over 24-fold. The percentage of ILI patients in all outpatients increased from 1.07% to 9.44%

- SARS-CoV-2 RNA was detected in nine patients from samples collected in January 2020 when the seasonal influenza (mainly A/H3 and B/Victoria) remained active; however, no coinfection was detected

- Of the patients testing positive for SARS-CoV-2, the gender ratio was 1.25 (five males versus four females), and they were all adults (age range: 35–71 years)

- COVID-19 was gradually expanding among the ILI cases during January. In the last week of observation, the frequency of SARS-CoV-2-positive cases had exceeded that of influenza virus among the group of patients older than 30 years

Highlights:

ILI data for the 2019–2020 winter was significantly higher in comparison to previous years suggesting the necessity to distinguish between influenza-infected and suspected COVID-19 patients

Clinical Impact:
Minimal

Important Methodologies:
Detection of SARS-CoV-2 by real-time RT-PCR from processed frozen samples collected from ILI patient throat swabs

Limitations:
Small sample size (n=640). Frequency of SARS-CoV-2 positive cases (>30 years of age) in last week of study represented only 5/19 patients in comparison to 0/19 influenza-positive patients

Diagnostics and Therapeutics

Indomethacin has a potent anti-viral activity against SARS CoV-2 in vitro and canine coronavirus in vivo

Xu *et al.* bioRxiV 2020
Link: https://doi.org/10.1101/2020.04.01.017624

Pre-clinical testing of Indomethacin (Cyclopentone COX inhibitor) to see if it can be re-purposed to prevent replication of SARS CoV-2. Tested *in vitro* VERO E6 cells (monkey epithelial kidney line) transfected with a SARS CoV-2 psuedovirus and observed a reduction in viral replication. Indomethacin treatment *in vivo* for dogs infected with canine coronavirus (CCV, some sequence homology with SARS CoV-2) provided a faster recovery than ribavirin and a comparable one to serum/Hgb/IgG/interferon treated dogs.

Main findings:

- A SARS CoV-2 pesudovirus with a luciferase reporter was used to infect VERO E6 cells that were given various doses of Indomethacin or Aspirin after 1 hour. 48 hours later luciferase activity was assessed.
- The supernatants of these pseudovirus "treated" cells were added to fresh VERO E6 cells and the luciferase activity was assessed again 48 hours post-infection.
- Indomethacin reduced viral particle production (IC50 at 1µM) and appeared to clear most of the pseudovirus after 48 hours. VERO E6 cell proliferation was unaffected.
- Aspirin had no impact on viral particle production, suggesting a mechanism of action independent of COX inhibition.
- 26 dogs within 2-3 days of CCV symptom onset were given an additional treatment to the "symptomatic treatment" (not defined). These additional treatments were ribavirin or anti-CCV serum/canine haemoglobin/canine blood IgG/interferon or indomethacin.
- Indomethacin worked better than ribavirin but did not differ from the serum/Hgb/IgG/interferon group. All surviving dogs tested negative for CCV.

Highlights:

- Indomethacin, but not Aspirin, can reduce viral particle production *in vitro* and match efficacy of current treatments (serum/IgG/interferon) in canine coronavirus.

Clinical Impact:

- Low- has not been tested in human cell lines and complete mechanism unknown.

Important Methodologies:

- Generation of a SARS CoV-2 pesudovirus/luciferase-based assay to screen anti-viral activity of drugs *in vitro*. Could be applied to multiple cell lines and converted to HTS.

Limitations:

- Not yet peer-reviewed.
- Indomethacin intervention was given almost immediately after infection *in vitro* would need to know if it can block viral replication if given later.

- Viral load of canine's not measured and symptomatic treatment in dogs not defined, is it also drug based?
- Can't see SD error bars on graphs despite mean values being used.

Tocilizumab treatment in severe COVID-19 patients attenuates the inflammatory storm incited by monocyte centric immune interactions revealed by single-cell analysis.

Chuang Guo *et al.* (bioRxiv), 2020
Link: https://www.biorxiv.org/content/10.1101/2020.04.08.029769v1

Previous studies have shown that dysregulation of the immune system may lead to inflammatory storm in COVID-19. Tocilizumab treatment targeting interleukin 6 receptor has shown promising clinical results in severe COVID-19 patients. Study uses 5 peripheral blood samples from 2 severe COVID-19 patients at 3 consecutive time-points from the severe to recovery stages during Tocilizumab treatment. The blood samples at severe stage were collected within 12 hours of Tocilizumab was given. The study looks at single cell transcriptomes of peripheral blood mononuclear cells isolation at three stages in COVID-19 patients treated with Tocilizumab.

Main findings:

- After receiving Tocilizumab treatment, body temperature of the patients returned to normal after 24 hours.
- Plasma B cells, effector CD8+ T, proliferative MKI67+CD8+ T cells and NK cells were significantly enriched in patients versus control.
- Cytokine storm related genes, such as TNF, IL10, CCL3 and IL6 were found significantly higher expressed in severe stage-specific monocytes.
- A monocyte subpopulation which existed only in patients at severe stage was observed.
- Author suggests that inflammatory storm caused by this monocyte subpopulation was suppressed after the Tocilizumab treatment as genes involved in "acute inflammatory response" and "leukocyte chemotaxis" were significantly decreased at recovery and healthy stage.
- Data proposes that Tocilizumab may function to effectively block the inflammatory storm in severe COVID-19 patients.

Highlights

- Illustrates molecular basis of cell-cell interactions in the peripheral blood of COVID-19 patients, which may lead to a better understanding of the mechanisms of inflammatory storm of the disease.
- Demonstrates that Tocilizumab treatment doesn't affect the antiviral effects of the body.

Clinical Impact:

- Minimal

Important Methodologies:

- Profiling of peripheral immune cells of COVID-19 patients using single-cell transcriptome sequencing. scRNA-seq data of PBMCs from severe COVID-19 patients is available at Genome Sequence Archive at BIG data Center.

Limitations:

- Data is from 2 patients (n=2); control data is from published single-cell profiles of healthy PBMC from the 10X website.

Classical drug digitoxin inhibits influenza cytokine storm, with implications for COVID-19 therapy

Harvey B. Pollard *et al*. (Medrxiv), 2020
Link: https://www.biorxiv.org/content/10.1101/2020.04.09.034983#Sec3

Harvey B. Pollard et al. report the significant reduction of lung cytokines (IFNγ, MCP1, MIP2, GRO/KC, TNFα, TGB-β) expression along with digitoxin administration in cotton rat infected by intranasally instilling influenza strain A/Wuhan/H3N2/359/95. The effect seems to differ among cytokines and be digitoxin dose dependent. It suggests further investigation into digitoxin therapy for COVID-19.

Main findings:

- To evaluate effects of digitoxin (maximum dose equivalent to human dose routinely used to treat heart failure) in cotton rat lung after intranasally instilling influenza strain A/Wuhan/H3N2/359/95 virus.
- Digitoxin administration is significantly associated with the reductions in the expression of 6 cytokines (IFNγ, MCP1, MIP2, GRO/KC, TNFα, TGB-β) involved in TNFα/NFκB signalling in viral infection-induced cytokine storm.
- Digitoxin effect on expression seems to differ among cytokines with the highest reduction in IFNγ (68.9%), followed by MCP1 (54.9%), GRO/KC (46.6%), TNFα (38.4%), MIP2 (32.2%), and TGB-β (15.3%).
- Digitoxin effect on cytokines expression seems to be dose dependent
- IL-1α reduction is not significant but trends toward significance with highest digitoxin dose

Highlights

- Influenza strain A/Wuhan/H3N2/359/95 induced cytokine storm blocking effect of digitoxin in cotton rat lung suggests a potential candidate of digitoxin for further investigation into COVID-19 therapy.

Clinical Impact:

- Minimal, further studies are needed to elucidate the role of digitoxin in this circumstance.

Important Methodologies:

- Digitoxin was prepared as a stock solution in 95% ethanol and diluted in PBS before administration.
- Drug protocol: digitoxin was administrated one day before viral instillation and daily used until harvest (day 7).
- Characterization of lung cytokines and chemokines (from frozen lung samples) by ELISA

Limitations:

- Evaluation was taken at only one time point. Cannot exclude the baseline cytokine characteristics differences among rats.
- Cannot confirm direct effect of digitoxin use in inhibiting cytokine storm as the antiviral effects of digitoxin could contribute to influenza dependent cytokines levels.
- No experiment on coronaviruses including COVID-19.

A Trial of Lopinavir–Ritonavir in Adults Hospitalized with Severe Covid-19.

Cao *et al*. *T*he New England Journal of Medicine. 2020
Link: https://www.nejm.org/doi/full/10.1056/NEJMoa2001282

Bin Cao *et al* conducted a randomised, controlled trial of 199 hospitalised SARS-CoV-2 patients to evaluate the safety and efficacy of lopinavir-ritonavir in severe Covid-19 disease. Lopinavir is a HIV type 1 aspartate protease inhibitor that has shown inhibitory activity against SARS-CoV *in vitro* and MERS-CoV in animal models, and ritonavir is suggested to increase the plasma half-life of lopinavir. 99 patients were randomly assigned to the lopinavir-ritonavir group, and 100 to the standard care group.

Main findings? **-**There was no significant difference in time-to-clinical improvement (i.e. length of time between group assignment and improvement) between groups.

- The lopinavir-ritonavir group had lower mortality numbers by 28 days post group assignment than the standard care group, and lopinavir-ritonavir patients had a shorter stay in ICU compared to standard care patients (6 days vs 11 days).
- 35% of patients (69/199) that tested positive (by RT-PCR) for viral RNA before admission to study had a negative result (by RT-PCR) after consent was taken. Viral RNA load over time did not differ between groups.
- Both groups reported similar percentage of patients that experienced adverse events throughout the study; respiratory failure, acute kidney injury and secondary infection were more common in the standard care group whereas adverse gastrointestinal events were more common in the lopinavir-ritonavir group.

Highlights- authors suggest that the difference in mortality between groups was greater when treatment was administered within 12 days of onset of symptoms, indicating starting treatment even earlier could have had a greater effect.

Clinical Impact? Minimal

Important Methodologies? RT PCR to confirm SARS-CoV-2 (data not down), measurement of viral load via RT-PCR (throat swab).

Limitations? – Throat swabs for viral RNA load taken intermittently (1, 5, 10, 14, 21, 28 days) More measurements taken in first 5 days might have been more informative.
-Study showed a 22.1% mortality rate which is higher compared to other 'initial descriptive' studies (11-14%).
-Throat swabs have been shown to have lower viral load compared to nasopharyngeal swabs, therefore accuracy of data is thrown into question.
-Studies suggest there are variable effective concentrations (EC_{50}) of lopinavir in vitro depending on cell type used (for SARS-CoV), therefore was the correct dose given to patients?
-Not a blinded study.

Compassionate Use of Remdesivir for Patients with Severe Covid-19.

Grein *et al*. The New England Journal of Medicine. April 10, 2020.
Link: https://www.nejm.org/doi/full/10.1056/NEJMoa2007016

Patients suffering from severe Covid-19 recruited from America, Canada, Europe and Japan were given Remdesivir, a nucleotide analogue metabolised to an analogue of ATP that inhibits RNA polymerases. Covid-19 patients were divided into groups receiving non-invasive ventilation (i.e. ambient air, nasal high flow oxygen and NIPPV) or invasive ventilation (invasive mechanical ventilation and extracoropeal membrane oxygenation (ECMO)). Patients were given an initial dose of 200mg IV, then subsequent doses of 100mg daily for 9 days. Follow up was at least 28 days.

Main findings - 18-days post start of treatment, 36 patients (68%) had an improvement in 'oxygen-support class'- 57% (36/53) of those on invasive mechanical ventilation were extubated and 75% (3/4) of those on ECMO were taken off.
- Clinical improvement, as measured by a 'decrease by either 2 or points on the 6-point scale or live discharge', was seen in 68% of patients.
- 60% (32 patients) reported mild adverse effects during follow up including rash, elevated hepatic enzymes and renal impairment; 23% (12 patients) showed serious adverse effects, including septic shock, acute kidney injury and multiple-organ-dysfunction syndrome. Adverse events were more common in those receiving invasive ventilation.

Is it different to what has been reported already? Remdesivir has shown efficacy against SARS-CoV and MERS-CoV infections *in vitro* (2017). Prophylactic and therapeutic administration of Remdesivir has been carried out in SARS-CoV (2017) and MERS-CoV (2020) infected mice with great success, reducing viral titres in the lung and reduced acute lung injury.

Highlights The mortality rate of remdesivir was substantially lower than seen in other case studies (13%). Additionally, the safety profile has been deemed favourable following a study using Remdesivir in healthy volunteers and Ebola virus patients. Also shows efficacy even in a small cohort- 47% (25/53) of patients were discharged at some point after treatment.

Clinical Impact? Substantial.

Important Methodologies? RT-PCR to confirm SARS-CoV-2 infection (data not shown), measurements of serum ALT, AST throughout treatment.

Limitations? -Minimal laboratory testing was carried out other than serum hepatic enzyme levels and creatine levels. No data on viral load was collected.
-Cohort was small, with patients skewed towards male (75%, 40/53 patients). There was also no randomised control group and the study uses the need for invasive ventilation as a 'proxy for disease severity'.

COVID-19 and emerging viral infections: The case for interferon lambda

Prokunina-Olsson *et al*. (Journal of experimental medicine), 2020
Link: https://rupress.org/jem/article/217/5/e20200653/151664/COVID-19-and-emerging-viral-infections-The-case

Prokunina-Olsson et al. reviews the potential use of IFNλ in the clinic as an anti-viral biological therapy. IFNs activate protective mechanisms aimed at both virus control and elimination. Type I IFNs have been explored as antiviral agents but IFNλ, a type III IFN, may be a better alternative as its receptor expression is limited to epithelial cells and some immune cells. Limited receptor expression will minimise systemic inflammatory damage and other side effects. Therefore, the existing pegylated- IFNλ1 drug has potential for COVID-19 treatment and is being investigated in the clinic.

Main findings:

- IFNs activate protective mechanisms aimed at both virus control and elimination.
- Distinctive actions of type I and type III IFNs are achieved through the engagement of separate nonoverlapping heteromeric receptor complexes: IFNAR for type I IFNs expressed on all cells and IFNs IFNL complexes for type III IFNs which is restricted to epithelial cells and a subset of immune cells, including neutrophils.
- Due to these specific expression patterns, type I IFNs provide a systemic response, and IFN- λs guard epithelial surfaces
- Pegylated IFN-λ1 is the only IFN-λ currently available as a therapeutic agent.
- IFN-λ treatment showed potency against a variety of viruses, including SARS-CoV1 and MERS-CoV in vitro.
- No increased risk of lung infection was observed in previous clinical trials of pegylated IFN-λ which don't include assessments during active COVID-19 disease.
- This approach could provide immediate protection to those at high risk of being infected or during early stages of infection, while patients show no sign of an inflammatory reaction, especially in the lungs.

Highlights

- IFNλ receptor expression is more limited than for type I IFNs therefore IFNλ therapy made meet an unmet need for boosting the antiviral host response with less risk of inflammatory damage.

Clinical Impact:

- Potential of IFNλ therapies in the clinic such as pegylated-IFNλ1 in current trials.

Important Methodologies:

- N/A

Limitations:

More information on IFN-λ biology is needed:

- What are the effects of IFN-λ on inflammatory responses and mechanisms of tissue damage and repair and how these activities should be measured in the clinical trials with peg-IFN- λ1 in development for COVID-19?
- Bacterial superinfections can be associated with severe cases of COVID-19 although this varies between clinical studies. While type I IFNs often suppress antibacterial action of immune cells, IFN-λ could employ other routes to facilitate bacterial superinfection?
- For SARS-CoV-2, it is still debated whether alveolar macrophages or endothelial cells are productively infected and could serve as a virus reservoir not accessible to IFN-λ antiviral action for lack of IFNLR1 which may limit the efficacy of IFNλ treatments.
- We need to understand whether the virus induces the endogenous expression of IFN-λ and/or blocks IFN-λ responses.

The establishment of reference sequence for SARS-CoV-2 and variation analysis

Wang *et al*. / Journal of Medical Virology / 2020
Link: https://onlinelibrary.wiley.com/doi/full/10.1002/jmv.25762

The group compared full length SARS-CoV-2 virus sequences reported from different regions worldwide to establish a reference sequence and identify regions of mutations for use in the design of PCR probes and primers to improve diagnostic testing.

Main findings:

- Published SARS-CoV-2 sequences were classified into stage 1 (pre 06.02.2020 = 63) and 2 (post 06.02.2020 = 32) and a reference sequence was constructed based on the most common nucleotide in each position of stage 1 sequences
- Comparing stage 1 sequences to other coronavirus sequences showed that all CoV-2 sequences clustered tightly together with the reference sequence in the middle, suggesting good representativeness

- Overall homology among stage 1 sequences was 99.99% and 15 clinical isolates were identical to the reference sequence
- Sequences from stage 2 showed > 99.9 homology to the reference genome
- The lowest homology was detected in 2 strains isolated in China (99.91% ad 99.79%)
- Few mutation hotspots were identified that should be avoided in the design of primers and probes

Highlights

- Mutations at nucleotide and amino acid level are generally rare
- The majority of isolates shared >99.9% homology to the reference sequence
- Hot spots or mutation do exist e.g. nt28144 of ORF 8 (mutation rate of 30.53%), nt8782 of 1a (mutation rate of 29.47%)
- The significance of these variations is unclear
- Differences of some published primer/probe sequences compared to reference sequence were detected and could explain false negatives in nucleic acid detection for diagnosis

Clinical Impact:

- High for development of diagnostics

Important Methodologies:

- Classification of sequences into stage 1 (pre 06.02.2020) and 2 (post 06.02.2020) and homology analysis and sequence alignment of stage 1, selecting the most common nucleotide in each position for the reference genome and comparison of the reference genome to stage 2 sequences
- Use of ClusterlW program for sequence alignment and phylogenetic tree construction based on stage 1 sequences
- Use of Primer 7.0 and Mega to compare variations at nucleotide and amino acid levels
- Alignment of published PCR primers/probes with reference sequence

Limitations:

- All viral sequences are derived from databases (NCBI/GISAID), the accuracy by which they are reported is not known
- A small number of sequences (95) has been analysed, although this as based on what has been published

Virological assessment of hospitalized patients with COVID-2019

Wölfel *et al*. Nature (2020)
Link: https://www.nature.com/articles/s41586-020-2196-x

Wölfel et al. provide detailed virological analysis of nine patients with mild COVID-19, reporting active SARS-CoV-2 replication in upper respiratory tract. Prolonged shedding of

viral RNA from sputum was observed even after symptoms had ended. Neutralising antibodies were produced in all patients by day 14 but did not lead to rapid elimination of virus. Instead, seroconversion correlated with gradual decline of viral load in sputum.

Main findings:

- Findings specifically based on SARS-CoV-2 infection as all patients were tested against a panel of pathogens that cause respiratory viral infection and no co-infection was detected in any patient.
- Pharyngeal SARS-CoV-2 shedding was very high at onset of symptoms (peaking on day 4 at 7.11×10^8 RNA copies/throat swab), indicating efficient early transmission even during mild symptoms.
- Successful live virus isolation was dependent on viral load as samples containing less than 10^6 copies of viral RNA never yielded a viral isolate.
- Infectious virus could be isolated from throat and lung samples of patients but not from stool samples (in spite of containing high levels of viral RNA).
- Blood and urine samples of patients did not yield SARS-CoV-2.
- Distinct viral genotypes in throat and sputum samples of same patient suggests that upper and lower respiratory tract are independent sites of SARS-CoV-2 replication. Live virus isolated from the throat is not due to passive shedding from the lung.
- In 50% of the patients, seroconversion occurred by day 7 (in all by day 14) but was followed by gradual (rather than rapid) decline in viral load.
- In spite of full resolution of COVID-19 symptoms (over three weeks post onset), viral RNA could be detected in stool and sputum samples from two-third of patients.

Highlights:

- Replication & validation of findings - all samples collected were tested for SARS-CoV-2 by two independent laboratories.
- Simple throat swabs provide sufficient sensitivity at mild/prodromal stage of SARS-CoV-2 infection.
- COVID-19 patients with less than 10^6 copies of viral RNA in their sputum sample on day 10 post onset of symptoms could potentially be discharged into home-based isolation from hospitals with limited bed capacity due to low residual risk of infectivity (based on cell cultures).
- Indicates that vaccine approaches mainly focussed on targeting onset of antibody production will need to elicit particularly strong & lasting neutralising-antibody response as SARS-CoV-2 was not rapidly eliminated at time of seroconversion.

Clinical Impact: High

Important methodologies:

- Recombinant immunofluorescence assays helped determine specific reactivity against recombinant coronavirus spike proteins.
- Identification of active SARS-CoV-2 in upper respiratory tract (and in absence of histopathology) using RT-PCR to detect replicative intermediate forms of viral RNA.

Limitations:

- Small sample number (n = 9).

- Functional studies required to understand extension of SARS-CoV-2 tropism to the throat (hypothesized that replication in upper respiratory tract is due to presence of 'polybasic furin-type cleavage site' in spike protein).
- Only mild cases studied – no severe cases observed (increased viral load beyond first week of symptoms could have prognostic value/significance).

Neutrophil extracellular traps (NETs) as markers of disease severity in COVID-19

Yu Zuo *et al.* (medRxiv preprint), 2020
Link: https://www.medrxiv.org/content/10.1101/2020.04.09.20059626v1.full.pdf+html

Zuo et al. report that sera from patients with COVID-19 (n=50 patients, n=84 samples) have elevated levels of cell-free DNA, myeloperoxidase(MPO)-DNA, and citrullinated histone H3 (Cit-H3); the latter two are highly specific markers of NETs. Highlighting the potential clinical relevance of these findings, cell-free DNA strongly correlated with acute phase reactants including C-reactive protein, D-dimer, and lactate dehydrogenase, as well as absolute neutrophil count. The data revealed high levels of NETs in many patients with COVID-19, where they may contribute to cytokine release and respiratory failure.

Main findings:

- Sera from patients with COVID-19 have elevated levels of cell-free DNA, myeloperoxidase(MPO)-DNA, and citrullinated histone H3 (Cit-H3).
- cell-free DNA strongly correlated with acute phase reactants including C-reactive protein, D-dimer, and lactate dehydrogenase, as well as absolute neutrophil count.
- MPO-DNA associated with both cell-free DNA and absolute neutrophil count, while Cit-H3 correlated with platelet levels.
- both cell-free DNA and MPO-DNA were higher in hospitalized patients receiving mechanical ventilation as compared with hospitalized patients breathing room air.
- Sera from individuals with COVID-19 triggered NET release from control neutrophils in vitro.

Highlights

- Highlights the need for future studies to investigate the predictive power of circulating NETs in longitudinal cohorts, and determine the extent to which NETs may be novel therapeutic targets in severe COVID-19.

Clinical Impact:

- Minimal

Important Methodologies:

- Quantification of cell-free DNA in sera using the Quant-iT PicoGreen dsDNA Assay Kit
- Quantification of citrullinated histone H3 in sera using the Citrullinated Histone H3 (Clone 11D3) ELISA Kit
- Quantification of MPO-DNA complexes

- Human neutrophil purification
- NETosis assays - SYTOX Green; NET-associated MPO; microscopy

Limitations:

- Whilst this study is one of the first studying the levels of srum markers for NETs in hospitalised COVID-19 patients, however there is no indication of what the marker levels will be like in milder cases of COVID-19.
- The triggers of NETosis in COVID-19 are potentially myriad and will require further investigation.

Estimating false-negative detection rate of SARS-CoV-2 by RT-PCR

Wikramaratna *et al*. MedRxiV
Link: https://doi.org/10.1101/2020.04.05.20053355

Using publicly available data from 2 studies, this paper analyses the probability of false-negative RT-PCR results in 30 SARS-Cov-2 patients who received multiple tests and were identified as positive at least once. Data from both studies were disaggregated per patient and the site from which swabs were taken has also been taken into account. The authors found that the probability of a false-negative test result increases with time after symptom onset. This has implications for the identification of infected patients as well as the discharge of convalescing patients who could still be infectious.

Highlights/Main findings:

1. The Probability of a positive result for SARS-CoV-2 infection decreases with time since the onset of symptoms. For nasal swabs:

   94.39% on day 0
   67.15% on day 10
   2.38% on day 31

   Time of testing and time passed from the onset of symptoms until a test can be taken is essential to correctly identify SARS-CoV-2 infection. If infected individuals are tested late, the false-negative rate is anticipated to be 4 times largen than when patients are tested early.

2. Swabs taken from the throat immediately upon symptom onset were predicted to be 6.39%less likely to yield a positive result than a nasal swab.

3. Testing again 24 hours after a negative results decreases the probability of a false-negative result.

4. Assuming that there are no or very few false positive test results, the number of positive tests underestimates the count of infected individuals in a group of tested individuals, which in turn has implications for estimates of case and infection fatality

risks in the wider population. Serological tests are more likely to determine the level of exposure to SARS-CoV-2 in a population.

Clinical Impact?
Moderate
The results are hardly surprising and false-negative testing is already being taken into consideration in hospitals. WHO guidelines only allow discharge of a patient after 2 negative tests in a row. However, the findings of this paper stress the importance of early and repeated testing, which is becoming increasingly difficult with raising numbers of patients.

Important Methodologies?
Statistical analysis: binomially-distributed (logit-link) generalised additive mixed models (GAMM).

Tested hypotheses:

- The probability of a positive result will change through time after symptom onset
- Different swab locations may have different detection probabilities
- Each study may have a different baseline detection probability

Limitations?

- Only 30 patients analysed. There is more data that could be used for this analysis and would provide more robust results.
- Not enough evidence to rule out the impact of variation among labs performing RT-PCR such as different SOPs etc.
- Data analysed all comes from symptomatic patients. Relevance of testing in asymptomatic patients and viral load were not taken into account.

Antibodies

Evaluation of Nucleocapsid and Spike Protein-based ELISAs for detecting antibodies against SARS-CoV-2

Liu *et al*. (Journal of Clinical Microbiology), 2020
Link: https://jcm.asm.org/content/jcm/early/2020/03/27/JCM.00461-20.full.pdf

Liu et al aim to determine whether two ELISA kits based on recombinant SARS-CoV-2 nucleocapsid protein (rN) and spike protein (rS) for detecting IgM and IgG antibodies can be used in place of PCR-based nucleic acid detection for SARS-CoV-2 diagnosis. Patient cohort: 214 patients (laboratory-confirmed positive for COVID-19) and 100 healthy blood donors as controls. Sensitivity of rS-based ELISA for IgM detection was significantly higher than rN-based ELISA. An increase of positive rate was observed with an increase of number of days post-disease onset.

Main findings:

- The percentage of positive results with IgM, IgG and IgM and/or IgG detected by rN-based ELISA were 68.2%, 70.1% and 80.4% respectively
- The percentage of positive results with IgM, IgG and IgM and/or IgG detected by rS-based ELISA were 77.1%, 74.3% and 82.2% respectively
- Positive rates of IgM and IgG for rN-based ELISA was low at days 0-5 and 6-10 post onset (<60%). The IgM and/or IgG positive rate was 88.9% at 11-15 days post onset and more than 90% at later stages of the disease
- Positive rates of IgM and IgG for rS-based ELISA was low at days 0-5 and 6-10 post onset (<60%). The IgM and/or IgG positive rate was ~80% at 11-15 days post onset and more than 90% at later stages of the disease
- Decreased positive rate of IgM at >35 days post onset
- No positive result was found in both rN- and rS-based IgM and IgG ELISAs in the healthy blood donor samples
- The sensitivity of the rS-based ELISA for IgM detection was significantly higher than rN-based ELISA but no significant difference was observed in the IgG and total antibodies.

Highlights

- Evaluates immunoassays for the detection of antibodies against SARS-CoV-2 and found a positive rate of over 80% at 11-15 days post onset.

Clinical Impact:

- Minimal

Important Methodologies:

- rN- and rS-based ELISA

Limitations:

- The proposed methodology can only be used in patients after 10 days post onset, before this the successful detection is low
- Requires blood sample

Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019

Zhao J. *et al* (Clin Infect Dis), 2020
Link: https://www.ncbi.nlm.nih.gov/pubmed/?term=Antibody+responses+to+SARS-CoV-2+in+patients+of+novel+coronavirus+disease+2019

In this study they study the seroconversion rate for antibodies against SARS-CoV-2. Also the measure the sensitivities and dynamics of the whole antibody, IgM and IgG in different time after onset and compare it to the sensitivity of RNA test in 173 patients that were tested positive for COVID-19 and grouped considering different parameters such as critical or no-critical.

Main findings:

- The seroconversion of Ab was significantly quicker than that of IgM and IgG
- No difference in seroconversion between critical and non-critical patients
- The sensitivity of Ab overtakes RNA sensitivity test after day 8 onset. Reaching nearly the 90% for Ab and decreasing to 54% for the RNA test.
- Antibodies may not be sufficient to clear the virus

Highlights

- The show the importance of Ab testing in diagnosis and its relevance in clinical management of COVID-19 patients

Clinical Impact:

- High

Important Methodologies:

- Antibody measurement. They measure total ab and the IgM they used an immunoassay based on a double-antigens sandwich (ELISA). Whereas for the IgG the used an indirect ELISA kit based on a recombinant nucleoprotein antigen.
- Statistics. The Ab level vs severity association was estimated by generalizing estimating equations (GEE) model with logit link function

Limitations:

- For the Antibody measurement they used a different detection mode depending whether they were measuring IgG, IgM or Ab. Which might affect to the sensitivity rates.

Distinct early IgA profile may determine severity of COVID-19 symptoms: an immunological case series

Dahlke C. MedRxiv preprint
Link: https://www.medrxiv.org/content/10.1101/2020.04.14.20059733v1.full.pdf

Dahlke et al present analysis of the SARS-CoV-2 specific antibody response in specific B-cell populations. They show increased number of memory B cells at 15 days post-disease onset which persisted only in a severe case and expansion of plasmablasts at early timepoints in the mild case but only became apparent when symptoms subsided in severe case. Analysis of the presence of IgA, IgG and IgM SARS-CoV-2 specific antibodies in serum showed a strong and specific IgA response in the mild case and more delayed but eventually strong and broad IgA response in the severe case.

Main findings:

- Two patients, husband hospitalised (no ventilation) with moderate/severe case and wife displayed mild symptoms 6 days later (but high viral shedding).
- Flow cytometry performed on PBMCs. Memory B-cell population (CD19+CD24+CD38-/low) increased 15 days post onset in both cases and persisted to 32 days in severe case.
- Plasmablast expansion (CD19+CD27+CD38+) at day 3 in mild case and only when symptoms had resolved in severe case.
- SARS-CoV-2 full proteome peptide arrays. Severe case, day 6 few IgG and IgA (only targeting ORF1ab polyprotein) reactive peptides. Response increased towards day 22 after virus clearance targeting spike, membrane, ORF8 and nucleocapsid proteins.
- Mild case displayed stronger IgA and more broad response soon after symptom onset (targeting spike, envelope, nucleocapsid and ORF1ab proteins) that decreased during course of disease.

Highlights

- First longitudinal sample analysis of B-cell epitope development at various stages of disease and across different disease severity.

Clinical Impact:

- Moderate- more cases needed

Important Methodologies:

- B-cell immune-phenotyping of PBMCs on flow
- SARS-CoV-2 whole proteome peptide microarray
-

Limitations:

- Very small sample size (2 patients)
- Arrays cannot identify antibodies that bind conformational or discontinuous epitopes
- Different time points analysed in patients
- Graphs show very different axis and confuses comparisons

Potent human neutralizing antibodies elicited by SARS-CoV-2 infection

Ju *et al* bioRxiv preprint
Link: https://www.biorxiv.org/content/10.1101/2020.03.21.990770v2

Ju et al demonstrates that plasma derived from SARS-CoV-2 infected patients has neutralization activity directed against the receptor binding domain (RBD) of the spike protein. Using single cell sorting, SARS-CoV-2 RBD specific B cells were isolated and BCR sequencing was performed, enabling the generation of 206 SARS-CoV-2 RBD monoclonal antibodies (mAbs). The mAbs are from diverse families of heavy and light chains with no apparent enrichment for specific families in the repertoire. Antibody neutralising potency is determined by competition of the cellular receptor ACE2 with the RBD of SARS-CoV-2. Of the mAb clones selected for further analysis, two had the ability to block the ACE2 – RBD binding with over 98% efficiency. This demonstrates the ability of the host to mount a virus-specific humoral response that is capable of neutralising viral entry.

Main findings:

- Plasma derived from 8 SARS-CoV-2 infected patients binds to the SARS-Cov-2 spike protein to varying degrees, with some cross-reactivity with the spike protein of the related SARS-CoV and MERS-CoV.
- Plasma binding to the RBD is only observed for SARS-CoV-2 in some of the patients tested, there is no cross reactivity with SARS-CoV RBD or MERS-CoV RBD.
- Plasma from patients had varying degrees of neutralising ability for SARS-CoV-2 but minimal neutralization of SARS-CoV or MERS-CoV.
- RBDs from different SARS strains are likely to be immunologically distinct.
- 206 SARS-CoV-2 RBD specific mAbs were generated from the 8 patients
- The distribution and frequency of VH usage was diverse between the patients and no standout single or group of VH families was identified. Patients appear to have immunologically distinct responses to SARS-CoV-2
- Using selected mAbs they determined that an Ab's neutralising potency is determined by its ability to compete with ACE2 receptor and the RBD for binding.
- None of the mAbs tested cross-reacted with the RBD from either SARS-CoV or MERS-CoV.
- Suggest that the Ab response to the RBD is virus specific and any cross-reactivity occurs outside of the RBD.

Highlights

- Indicates that B cells are capable of making highly specific antibodies capable of preventing viral entry by competing with the ACE2 receptor for binding to the RBD of SARS-CoV-2

Clinical Impact:

- Moderate

Important Methodologies:

- Identification and isolation of RBD-SARS-CoV-2 specific B cells from blood
- Cloning and expression of RBD specific monoclonal antibodies from patient B cells
- Neutralising activity of mAbs against pseudoviruses (containing spike proteins/RBDs from SARS strains) and live SARS-CoV-2

Limitations:

- The study only uses mAbs derived from 8 patients, 5 of these are related. A bigger cohort of patients is needed
- Only a select few mAbs from 1 patient are expanded for further study, more Abs from different patients need to be expanded to determine if these B cells are truly important for vaccine development.
- Use of pseudoviruses for the majority of studies may not accurately reflect the neutralizing capacity of these mAb's in a patient with SARS-CoV-2 infection.
-

The SARS-CoV-2 receptor-binding domain elicits a potent neutralizing response without antibody-dependent enhancement

Quinlan BD *et al*. (Unpublished BioRxiv). 2020.
Link: https://www.biorxiv.org/content/10.1101/2020.04.10.036418v1

The spike (S) protein of SARS-CoV-2 uses cellular receptor angiotensin-converting enzyme 2 (ACE2) to facilitate entry into cells for infection via its receptor binding domain (RBD). Immunisation with a SARS-CoV-2 RBD-Fc vaccine candidate exhibits a robust neutralising antibody response in rats, without antibody-dependent enhancement (ADE); whereby antibodies produced will facilitate viral entry and replication. This suggests that the SARS-CoV-2 RBD could be a candidate for a safe and effective COVID-19 vaccine.

Main findings:

- Sera from 4 rats immunised with a SARS-CoV-2 RBD-Fc vaccine candidate was able to potently neutralise retroviruses pseudotyped with the SARS-CoV-2 S protein.
- Immunisation with SARS-Cov-2 RBD-Fc did not produce neutralising responses against the RBD for SARS-CoV-1. This excludes the possibility of cross-protection for SARS-like viruses.
- The SARS-CoV-2 RBD-Fc elicits its neutralising response by binding the spike protein of SARS-CoV-2 directly.
- Antibodies produced by this vaccine showed no evidence of ADE in HEK293T cells expressing Fc receptors *in vitro,* compared to rat anti-Zika virus antisera; known for its ADE responses.

Highlights

- A candidate COVID-19 vaccine that is tolerated in rats has been produced. This vaccine does not exhibit antibody-dependent enhancement (ADE).

Clinical Impact:

- Moderate. Impacts for vaccine design/development.

Important Methodologies:

- An RBD fusion protein (RBD-Fc) was produced and purified into a vaccine formulation via conjugation to a Keyhole limpet hemocyanin (KLH) carrier protein, mixed with AS01 adjuvant formulation.
- The vaccine was injected IM (intramuscularly) into 4 female Sprague-Dawley rats in seven 2.5-fold increasing doses, once a day. A total of 500 μg of SARS-CoV-2 RBD-Fc was administered. This was repeated 30 days later. Serum harvested for analysis.

Limitations:

- Only 4 rats tested.
- Immunisation strategy used in this study is unfavourable for vaccination of the public.
- Rats were not tested directly in their immune response to SARS-CoV-2 virus, post immunisation, only sera were tested *in vitro* for antibody neutralising responses.

Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications

Fan Wu *et al*. Medrxiv.2020
Link: https://www.medrxiv.org/content/10.1101/2020.03.30.20047365v2

Wu et al. analysed plasma collected from 175 COVID-19 recovered patients with mild symptoms to characterise neutralising antibodies (Nabs) against SARS-CoV-2. NAbs were detected in patients from day 10-15 after the onset of the disease. The titers of NAbs correlated with the spike-binding antibodies targeting S1, RBD, S2 regions and were variable among the patients. The NAb titers were positively correlated with plasma C-reactive protein levels (CRP) but negatively correlated with the lymphocyte counts at the time of admission, indicating an association between humoral and cellular immune response. The correlation of NAb titers with various factors suggested that the interplay between virus and host immune response in coronavirus infections should be explored for the development of an effective vaccine.

Main findings:

-SARS-CoV-2 NAbs could not neutralize other viruses like SARS-CoV.
-Humoral immune response against SARS-CoV-2 occurs 10-15 days following infection. RBD and S2 domains are the main targets of NAbs.
-A proportion of tested patients were able to recover without developing high titer of virus specific NAbs.
-There was an increase in NAbs in relation to age suggesting high levels of NAbs might be helpful in recovery of elderly and middle-age patients

In elderly and middle-aged patients' lymphocyte counts were lowered while CRP level was increased suggesting humoral response might play a role to balance out the cellular dysfunction.

Highlights
The levels of NAbs against SARS-CoV-2 is variable in patients based on their age and their recovery is a coordinated response of humoral and cellular response.

Clinical Impact: minimal

Important Methodologies:

Pseudo typed-lentiviral-vector-based neutralization assay.
ELISA for Spike-binding antibody in plasma

Limitations:
Viral RNA was not detectable in patient's blood
The effect of NAbs on viral clearance was not directly evaluated in this study as patients with severe and critical condition were excluded

Virology

Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor.

Lan *et al*. (Nature), 2020
Link: https://doi.org/10.1038/s41586.020.2180-5

Lan et al. report the detailed crystal structure of SARS-CoV-2 receptor-binding domain (RBD) bound to ACE2 receptor. They identify residues essential for ACE2 binding and highlight similarities with existing SARS-CoV structures. Similarities between SARS-CoV-2 and SARS-CoV suggest an evolutionary convergence between the two viruses despite their apparent phylogenetic differences. They go onto map RBD residues in SARS-CoV-2 as potential targets for neutralising antibodies.

Main findings:

- Determine the crystal structure of SARS-CoV-2 spike receptor domain (RBD) (Thr333 to Gly526) bound to cell receptor ACE2 N-terminal peptidase domain (Ser19 to Asp615) at 2.45 A.
- SARS-CoV-2 – twisted 5-strand antiparallel beta-sheet with short connecting helices and loops forming a core.
- Structural analysis identified residues in SARS-CoV-2 RBD critical for ACE2 binding.
- Identify a strong similarity to SAR-CoV binding to ACE2 – share similar side chain properties. Subtle differences in ACE2 interaction may contribute to binding affinity differences (4.7 nM Vs 31 nM).
- A unique 'RRAR' furin cleavage site at S1/S2 boundary in spike protein may facilitate the rapid transmission of SARS-CoV-2.
- Conserved epitopes in non-RGB regions such as spike S2 subunit maybe potential targets for cross-reactive antibodies.
- Compare epitopes of SARS-CoV antibodies targeting RBD to help identify cross-reactive antibodies in the future.
- RBD is a critical region for receptor binding, antibody targeting conserved epitopes of RBD used for development of potent cross-reactive therapeutic targets.

Highlights

- Findings identify intrinsic sequence and structure differences between SARS-CoV and SARS-CoV-2 RBDs.
- Improve understanding of interaction between SARS-CoV-2 and ACE2 allowing the precise targetting of neutralising antibodies and assist in structural based vaccine design.

Clinical Impact:

- Limited

Important Methodologies:

- Expression and purification of SARS-CoV-2 RBD bound to ACE2.
- Crystallisation of RBD and ACE2 to high resolution.
- Surface Plasmon Resonance

Limitations:

- Whilst this study is one of the first characterizing the crystal structure of SARS-CoV-2 binding to ACE2 at high resolution no neutralising antibodies were identified in this study.

Structural basis of receptor recognition by SARS-CoV-2

Shang et al. (Nature), 2020
Link: https://www.nature.com/articles/s41586-020-2179-y

Shang et al. crystallized an engineered SARS-CoV-2 receptor binding domain (RBD) in complex with human ACE-2 receptor (hACE2) and revealed that the hACE2 binding ridge in SARS-CoV-2 takes a more compact conformation. Several residue changes in SARS-CoV-2 RBD found to stabilise two virus binding hotspots leading to a higher affinity for hACE2. The functional importance of these residues was confirmed by mutating them to SARS-COV residues and performing protein pull-down assays which revealed reduced hACE2 binding affinity of SARS-CoV-2 spike. SARS-CoV-2 may have evolved from bat coronavirus RaTG13 since found to share similar four residue motif in ACE-2 binding ridge.

Main findings:

- SARS-2-CoV receptor binding motif (RBM) contains structural changes in hACE binding ridge caused by four-residue motif (residues 482-485: Gly-Val-Glu-Gly)
- Four-residue motif causes ridge to become more compact and form better contact with the N-terminal helix of hACE2
- Phe486 from SARS-CoV-2 RBM inserts into a hydrophobic pocket whereas SARS-CoV forms weaker contact due to corresponding residue being leucine which has smaller side chain
- SARS-2-CoV higher binding affinity than SARS-CoV as determined by surface plasmon resonance
- Two virus hotspots are more stabilized at RBM/hACE2 interface through interactions with SARS-Cov-2 RBM
- Gln493 and Leu455 stabilize hotspot-31 whereas Asn501 stabilizes hotspot-353
- Functional importance of these residues confirmed by protein pull down assay following Q493N mutation and N501T mutation (reverted residues to those found in Sars-CoV)
- Introduced mutations reduced the hACE-2 binding affinity of SARS-2-CoV spike
- Bat Ratg13 coronavirus has same four residue motif suggesting SARS-2-CoV may have evolved from RaTG13
- Showed that retroviruses pseudotyped with RaTG13 spike require hACE2 expression for entry on human cells

- RaTG13 spike also pulled down by hACE
- Showed retroviruses psedutoyped with SARS-CoV-2 spike require hACE2 expression on human cells
- RaTG13 spike is not cleaved at pseudovirus surface whereas SARS-CoV-2 spike is

Highlights

- Revealed functionally important epitopes in SARS-CoV-2 RBM that may aid in development of neutralising antibody drugs and RBD subunit vaccine design
- SARS-CoV-2 may have evolved from bat coronavirus RatG13
- The RBM of CoV-pangolin/Guangdong contains Leu455, the 482-485 loop, Phe486, Gln493 and Asn501 and may also bind hACE2

Clinical Impact:

- Limited

Important Methodologies:

- Coronavirus-spike-mediated pseudovirus entry assay
- Surface plasmon resonance to measure binding affinities

Limitations:

- Did not crystallize SARS-CoV-2/hACE2
- Crystallized chimeric RBD/hACE2 consisting of core SARS-CoV RBD including a short loop from SARS-CoV RBM to enhance crystallization. Kept only the RBM from SARS-CoV-2
- Chimeric RBD higher binding affinity than SARS-CoV-2 RBD
- No *in-vivo* models used
- CoV-pangolin/GD binding to hACE2 not experimentally tested

SARS-Cov-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells

Soeren Lukassen *et al*, The EMBO Journal, 2020
Link: https://stm.sciencemag.org/content/early/2020/04/03/scitranslmed.abb5883

SARS-CoV-2 has been previously reported to enter cells through binding to ACE2 receptor followed by cleavage of S protein by the transmembrane protease serine 2 (TMPRSS2), but dual blockage does not prohibit SARS-CoV-2 infection in vitro. It is unknown whether additional proteases are involved, but it has been reported that SARS-CoV-2 has a FURIN cleavage site in its S protein, similarly to MERS-CoV. Lukassen et al. report here the expression of the expression levels of ACE2, TMPRSS2 and FURIN across the different cell types of the lung tissue and bronchial branches. They show that transient secretory cells of the bronchial branches highly express ACE2 and TMPRSS2, and they hypothesise that these cells are more vulnerable to SARS-CoV-2 infection.

Main findings:

- In primary lung tissue samples expression of ACE2 and TMPRSS2 expression was highest in a subpopulation called AT2 cells
- In air liquid interface (ALI) cultures of human bronchial epithelial cells (HBECs), ACE2 and TMPRSS2 co-expression was found to be highest in a transient secretory cell type
- Transient secretory cell markers were also enriched for RHO-GTPases and their related pathways
- High expression of FURIN was found in lung tissue across all cell types with a preference for co-expression with ACE2 and/or TMPRSS2
- Between 40-50 years of age, expression of ACE2 in males is significantly higher (P=0.048) than in females

Highlights

- Provides a reference dataset for future studies of primary samples taken from SARS-CoV-2 patients and *in vitro* studies to assess the replication cycle of SARS-CoV-2

Clinical Impact:

- Limited – supports previous findings in the literature, limited novel data

Important Methodologies:

- Single nuclei isolation and RNA sequencing from healthy lung tissue derived from lung adenocarcinoma patients
- Air liquid interface culture of HBECs for single cell RNA sequencing

Limitations:

- Tissue donors have no history of SARS-CoV-2 infection
- 2 different RNA sequencing methods are used across different parts of the lung and the data is then combined
- Limited number of samples (12 total, 3 men and 9 women aged 44-79)
- Lack of validation at the protein level for the single cell transcriptomics dataset

SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion

Wang *et al.,* 2020. Nature Cellular and Molecular Immunology
Link : https://www.nature.com/articles/s41423-020-0424-9

Lymphocytopenia, particularly T cell reduction, is a common symptom in illness caused by types of coronavirus, including MERS-CoV, SARS-CoV and SARS-CoV-2. MERS-CoV has previously been shown to infect T cells via the DPP4 receptor, while SARS-CoV, for which the host receptor is ACE2, does not infect T cells. This study tested whether T cells are infected by SARS-CoV-2, which also uses the ACE2 receptor to enter host cells. This study used T cell lines; therefore, it remains necessary to test if primary T cells can be infected by SARS-CoV-2 and to determine how T cell infection has a detrimental effect on T cell numbers.

Main findings

- Both SARS-CoV and SARS-CoV-2 pseudotyped viruses could infect cell lines expressing high (293T) and moderate (Huh7) levels of ACE2 but not ACE2 negative cells (HeLa).
- Pseudotyped SARS-CoV-2 infected MT-2 and A3.01 T cells, which have low expression of ACE2; comparably T cell infection by pseudotyped SARS-CoV was negligible.
- Receptor mediated fusion with the Spike protein appears to be involved in T cell infection,
- Live virus infection of MT-2 T cells was detected at 1 hr and 24 hr, increasing from 5.51%-23.6%.
- Virus genome copy number peaked at 6 hr but infection did not result in viral replication.

Highlights

- Infection of low ACE2 expressing-T cells by receptor mediated fusion suggests that SARS-CoV-2 may infect T cells by a different receptor. A preprint has recently suggested SARS-CoV-2 enters T cells by CD147 (Wang et al., 2020).

Clinical Impact?

Minimal

Important Methodologies?

This paper does not include a methods section, some methods mentioned in the text include:

- Luminescence based measurement of infection using pseudotyped virus
- The mechanism of cell entry was investigated (by luminescence) using a peptide inhibitor of the Spike protein fusion domain (Xia et al., 2019). A fluorescence microscopy cell-cell fusion assay (Xia et al., 2020) was used to further investigate the entry mechanism, without including the peptide.
- Flow cytometry detection of SARS-CoV-2 infected T cells using an antibody to the SARS-CoV-2 nucleoprotein.
- Detection of cellular infection and viral replication by RT-qPCR of the viral genome from cell lysate and supernatant, respectively.

Limitations

- This use of model T cell lines rather than T cells isolated from primary PBMC samples is a major limitation of this paper. The T cell lines used are: MT-2: a HTLV transformed T cell line and A3.01, a lymphoblastic leukaemia cell line. It is not clear if SARS-CoV-2 could infect already transformed cells differently to primary T cells. Moreover, the MT-2 T cell line has been described to have a 'T-reg like' phenotype (Hamano et al., 2015).
- This paper does not have a methods section; experimental design, controls and statistical analysis is unclear.
- ACE2 mRNA, but not protein expression levels were measured.
- The virus entry mechanism was not comprehensively examined; inhibition of receptor-fusion entry was blocked by a peptide inhibitor of the spike protein fusion complex, whereas fusion was observed in the fluorescence based assay. Since the ACE2 receptor was not highly expressed by T cells, the non-receptor mediated endocytic pathway merits further investigation, in addition to further studies of alternate receptors.

- Flow cytometry gating not convincing, limited timepoints in infectivity experiment; figure legend (1.f) indicates infection was tested at 1 hr, 24 hr and 48 hr but the 48 hr timepoint dot plot is not shown.
- There were no controls shown in the timepoint experiment to compare detection of viral genome in cell lysate and supernatant of cells in which SARS-CoV-2 can successfully replicate.
- No functional analysis was carried out to determine what the virus did to infected T cells which might link it to the lymphopenia symptoms seen in COVID19; a previous study of MERS-CoV (Chu et al., 2016) showed that viral infection caused apoptosis.

Virus-host interactome and proteomic survey of PBMCs from COVID-19 patients reveal potential virulence factors influencing SARS-Cov-2 pathogenesis.

Liang *et al*., pre-print on BioRxiv.
Link: https://www.biorxiv.org/content/10.1101/2020.03.31.019216v1

Virus-virus protein interactions related to virus particle assembly and virus-host interactions related to protein synthesis, degradation, inflammation, and innate immunity are identified. Notably, non-structural protein (nsp) 9, 10, 12, 13 and 15 is linked to NRKF (transcriptional repressor of IL6 and IL8 synthesis). Expression of nsp9 and nsp10 induce IL6 and IL8 expression (related to neutrophil recruitment).

Proteome analysis of PBMCs from control and mild COVID-19 samples show differential expression of proteins involved in the innate immune response and priming of the adaptive immune system. Comparison of mild and severe COVID-19 samples reveal lower expression of adaptive immune response proteins.

Main findings:

- Yeast 2 Hybrid (Y2H) System: 19 protein-protein interactions (PPIs) between SARS-CoV2 proteins.
- Co-immunoprecipitation (Co-IP): 52 PPIs between SARS-Cov2 proteins.
    - Interaction of 8 PPIs between structural proteins and non-structural proteins (nsp), which the authors indicate is likely due to viral particle assembly.
    - Identification of nsp10-nsp14 and nsp10-nsp16 interactions whose analogues in SARS-CoV1 mediated methyltransferase activity validating their technique.
- Co-IP, liquid chromatography, and tandem mass-spectrometry (LC-TMS): 631 high confidence interactions between SARS-CoV2 proteins and 251 host-cell proteins.
    - These Include interactions previously identified by other studies using SARS-CoV proteins.
    - SARS-CoV2 proteins interacts with:
        - 40s proteomal proteins
        - T complex proteins
        - Proteasome related proteins

        - Proteins pertinent to inflammatory and innate immune responses.
            - nsp9/10/12/13/15-NRKF (repressor of IL6/8 synthesis)
            - nsp9/10-C1QBP (involved in complement C1 activation)
            - ORF6-G3BP2 (anti-viral stress granule protein)
            - ORF6-RAE1 (IFN inducible mRNA nuclear export protein)
- 4,274 proteins quantified in all 26 PBMC samples.
- Mild COVID-19 samples relative to healthy: 220 proteins differentially expressed. 115 increased, 110 decreased.
    - Identified pathways involved in the innate immune response (I.e. IFN type 1 signalling pathways, increased neutrophil chemotaxis and activation) and priming of the adaptive immune system (i.e. antigen processing and presentation).
        - Focus on CXCR2 and MMP25 upregulation (linked to LPS induced neutrophil response and indirect facilitation of transendothelial neutrophil migration, respectively).
- Mild COVID-19 samples relative to Severe COVID-19 samples: 553 proteins differentially expressed. 27 increased, 526 decreased.
    - Identified pathways involved in the adaptive immune response and complement activation were downregulated.
- In lung epithelial cells (A549 cell line) nsp9, 10, 12, 13 and 15 which were linked to NRKF (repressor of IL6/8 synthesis) was individually expressed.
    - nsp 9 and 10 induce expression of IL6 and IL8 whilst GFP (control), nsp 12, 13 and 15 do not.

Highlights

- The identification of interactions between nsp 9, 10, 12, 13, 15 and NKRF through the author's protein interactome (despite the lack of readout data, see limitations) is impressive when combined with their data showing that nsp9 and nsp10 can induce IL6 and IL8 expression in a lung epithelial cell line. This implies viral manipulation of neutrophil recruitment in COVID-19 pathogenesis.

Clinical Impact:

- Minimal

Important Methodologies:

- Yeast-2-Hybrid Assay
    - Protein-Protein Interaction Identification
- Co-immunoprecipitation
    - Protein-Protein Interaction Identification
    - Further Analysis by liquid chromatography and tandem mass-spectrometry.
- PBMCs (6 healthy donors, 22 mild COVID-19 patients and 13 severe COVID-19 patients) were lysed in denaturing buffer before proteome analysis.
- Flow Cytometry ("FACS analysis")
- qRT-PCR of nsp expressing lung epithelial cells

Limitations:

- The authors refer to extended data and tables throughout which are not in the pdf uploaded to BioRxiv. The authors should make this available as soon as possible.
- The authors do not distinguish which virus-virus protein interactions were identified by which method (Y2H or Co-IP) although they note some proteins were identified by both. I suspect this is in the extended data.
- No explanation is given for the difference in number of proteins identified by each method i.e. 19 by Y2H and 52 by Co-IP. Perhaps this is due to method sensitivity.
- There is no data shown showing how the protein-protein interactions networks were generated i.e. direct readouts from the yeast-2-hybrid assay and Co-IP.
- 41 PBMC samples were taken for lysis and proteomic analysis (6 control, 22 mild and 13 severe COVID-19 patient samples). However, the authors state *"4,274 proteins could be quantified in all 26 patient samples"* and do not address this discrepancy.
- The levels of IL6 and IL8 in mild, severe, and recovered patients by flow cytometry is shown in fig 3, d and e. No gating strategy, FMOs or method are provided for this and the y-axis is in pg/ml strangely. I suspect this is supposed to be ELISA data.
  The authors link IL6 and IL8 to neutrophil recruitment. Further, they link the % of leukocyte count made up by neutrophils to disease progression. As one of the most interesting aspects of this paper is the induction of IL6 and IL8 expression by two viral nsp, addressing errors in fig 3, d and e is particularly important.

Vaccines

Preliminary Identification of Potential Vaccine Targets for the COVID-19 Coronavirus (SARS-CoV-2) Based on SARS-CoV Immunological Studies

Ahmed, S.F. et al. *Viruses,* 2020
Link: https://www.mdpi.com/1999-4915/12/3/254/htm

Ahmed, S.F. et al. analyse publicly available experimentally-determined SARS-CoV-derived T cell and B cell epitopes for epitopes which are conserved in publicly available SARS-CoV-2 sequences, choosing to focus on Spike (S) and Nucleocapsid (N) proteins due to the long-lasting immune responses reported towards these proteins in SARS-CoV. They establish that 23% and 16% of known SARS-CoV T cell and B cell epitopes, respectively, are conserved in SARS-CoV-2 protein sequences. From these, they identify a set of T cell epitopes that have a high MHC allele global population coverage that can be used to guide preliminary phase SARS-CoV-2 vaccine development.

Main findings:

- High genetic similarity (over 90%) between the N-proteins of SARS-CoV-2 and SARS-CoV and reduced (76%) but still significant genetic similarity between the S-protein of the two viruses
- 27 T cell epitopes of SARS-CoV (derived using publicly available T cell assay data) were conserved in the protein sequences of SARS-CoV-2; 16 in the N-protein and 11 in the S-protein
- Based on available MHC binding assays for 19 of these epitopes, population coverage was estimated to be 59.76% in the global population and 32.36% in China
- 229 T cell epitopes of SARS-CoV (this time derived using publicly available MHC binding assay data) were conserved in the protein sequences of SARS-CoV-2; of these, 36 were in the N-protein and 66 in the S-protein
- From these 102 S-protein and N-protein T cell epitopes, a set of epitopes associated with 20 distinct MHC alleles was generated with population coverage of 96.29%
- 49 linear SARS-CoV-derived B cell epitopes (derived using publicly available B cell assay data) were conserved in available SARS-CoV-2 protein sequences, of which 23 were in the S-protein and 22 were in the N-protein
- 20 of the 23 epitopes in the S-protein were located in the S2 region involved in membrane fusion, suggesting that antibodies targeting these epitopes might be neutralizing

Highlights

- Generates a set of T cell epitopes that have high MHC allele population coverage that can be used in preliminary vaccine design studies

Clinical Impact:

- Minimal

Important Methodologies:

- Acquisition of SARS-CoV-derived, experimentally-determined human B cell and T cell epitopes from the NIAID Virus Pathogen Database and Analysis Resource (ViPR)
- T cell epitopes with high MHC allele population coverage identified using tools provided by the Immune Epitope database

Limitations:

- Some SARS-CoV T cells epitopes acquired from the ViPR were experimentally-determined based on positive MHC binding assays, indicating that these are antigens, not necessarily epitopes, given that they were not confirmed using T cell assays
- No *in-vitro* or *in-vivo* experiments were performed to consolidate *in-silico* predicted T cell and B cell SARS-CoV-2 epitopes; this would help refine the reported epitope set prior to vaccine testing.