COVID-19 Community Journal Club No. 4

May 7th, 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.

New this week: "Neuroscience and Mental Health" with contributions from Nichola Brydges, James Galloway and Gréta Utassy.

****Plus an **interview with Dame Deirdre Hine**, former Chief Medical Officer for Wales and author of the report into the 2009 Swine Flu Pandemic****

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

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An interview with Dame Deirdre Hine

Dame Deirdre Hine, graduated as a doctor in 1961 and worked as a hospital doctor, a GP and then as a public health consultant before becoming the first woman Chief Medical Officer and the first woman president of the Royal Society of Medicine. In 2010, she was invited to lead on an independent review into the 2009 swine flu pandemic. Here, she talks to Dr Emma Jones about the various roles she has played during her career and her thoughts on the current COVID-19 pandemic.

Emma: Did you always want to practice Medicine? Of the many important roles you've had during your career, which one are you most proud of?
Dame Deirdre: When I was about 14 my mother asked me what I wanted to do when I grew up. I told her I thought I would like to be a nurse. She replied that that was very good but asked why I didn't want to be a doctor. I said I didn't think girls could be doctors. She told me that girls could be anything they wanted to be as long as they worked hard. Also, at that age, like many adolescent girls, I was convinced that I was so unattractive that no one would want to marry me and I should look for a career that would be completely absorbing. I decided on medicine as fitting the bill!
It is difficult to single out the role I am most proud of but perhaps it was as Director of Breast Test Wales, the All Wales breast cancer Screening Service which, together with a small team of excellent colleagues I established from scratch to be a high quality, women - friendly service for the early detection of breast cancer.

Emma: What's the best piece of advice any one ever gave you (and who was it from)?
Dame Deirdre: Clearly the best advice I was ever given was that from my mother as described above. However, it is closely followed by that given me by my husband some years later which was "Never duck a challenge"

Emma: You trained in public health. What drew you to working in public health?
Dame Deirdre: I came into Public Health almost by default as my ambition on qualifying was to become a hospital nephrologist. I thought the kidney was a fascinating organ! However it was clear that at that time a hospital medicine career was difficult to combine with marriage and a family and as my future husband had by then been waiting 6 years for me to be able to marry him I decided that a career in community health as a possible 9-5 option was sensible. After completing pre-registration hospital jobs in medicine and surgery and a brief period as a GP Locum I was accepted on the Postgraduate course leading to the Diploma in Public Health. This was a part-time course over 2 years and I combined it with a part-time appointment as a Clinical Medical Officer employed by Glamorgan County Council undertaking Antenatal and Child Health clinics, School Medical Examinations and Occupational Health examinations of Local Authority employees and the Police Force, Later

following the 1974 reorganisation of the NHS I was appointed to a consultant level post as a Specialist in Community Medicine at the new South Glamorgan Health Authority. This turned out to be a far from 9-5 job with a demanding on-call rota!

Emma: What was the most challenging public health crisis you had to deal with and why?
Dame Deirdre: The most challenging public health crisis I ever had to deal with was undoubtedly the Bovine Spongiform Encephalopathy/ Creutzfeld Jacob Disease problem. An epidemic of BSE emerged as a new disease of cattle in the early 1980s. This was at first not thought to be transmissable to humans and thus throughout most of the decade no precautions against its transmission were thought necessary. In early 1990 I was appointed as Chief Medical Officer at the Welsh Office. One of the first issues that was presented to me was the communication from the Department of Health (DH)in London that they were making a public statement that "British beef is completely safe to eat". I was aware that BSE had been found to have crossed the species barrier into cats. I therefore assembled the relevant members of my senior staff together with colleagues from the Cardiff Public Health Laboratory Service to discuss the matter. I was fortunate to have very experienced Environmental Health, Occupational Medicine, Bacteriology and Epidemiology colleagues. After much discussion we concluded that we could not endorse the DH statement and I made DH aware of our reservations. Needless to say, this brought down on us the wrath of that Department together with that of the Ministry of Agriculture, Fisheries and Food (MAFF). Also, for a short time that of the Welsh Office Agriculture Group though having heard our reasoning they fell in behind us. I did not issue any assurance on the safety of beef in Wales. It was not until March 1996 that it was confirmed that a rise in cases of CJD was likely to have been due to infection from BSE in cattle. The Report of the BSE Public Inquiry chaired by Lord Phillips published in October 2000 contains the following passage. "We were struck by the quality of independent thinking that the Welsh Office medical team led by the CMO for Wales Dr Deirdre Hine applied to the issues raised by BSE. A similar combination of skills at national level in Whitehall Departments could well have been fruitful."!!

Emma: Did you have a close relationship with the medical officers from England and the other devolved nations when you were Chief Medical Officer for Wales?
Dame Deirdre: I did have a close and fruitful relationship with the CMOs of the other 3 nations, we met regularly to exchange ideas and proposals. Inevitably some of these relationships were closer than others – for example I was very friendly with Dr Etta Campbell the CMO of Northern Ireland who was the only other woman to have been appointed as a CMO at that time. Also, a close colleague was Sir Kenneth Calman, first as CMO of Scotland and later of England. He and I co- authored the Report on Cancer that changed the structure of Cancer care in England and Wales. This became known as the Calman Hine Report.

Emma: Why were you asked to write the report on the 2009 Swine Flu pandemic?
Dame Deirdre: I am not sure why I was selected to write the Review of the H1N1 Pandemic. I had retired in 1997 and although I did various pieces of work thereafter, I was known to have the time and the relevant experience. I was appointed by the Health Secretaries of the

4 nations, in practice by Alan Johnson SoS Heath England and reported to Andy Burnham who succeeded Johnson on his transfer to the Home Office.

Emma: What were the main recommendations you made for future pandemics and have any of those recommendations been followed during this Coronovirus pandemic? What is your assessment of how the government has handled this coronavirus pandemic?
Dame Deirdre: My report contained 28 recommendations but perhaps the most important point was contained in the letter to the Ministers which appears as the introduction. In it I warned that although this had been a mild pandemic there was every likelihood that a more severe pandemic was possible and that would strain resources to a much greater extent. I cautioned against complacency on the part of government. Many of my recommendations appear to have been followed in during this pandemic. Notably the arrangements for a united 4 nations approach to both action and information. Also, my recommendation for the main actors, both official and ministerial, to have back up substitutes in case of fatigue or infection. My recommendation of a greater role for Behavioural Science has also evidently been actioned. I did advise a greater role for local responsibility and expertise to be used rather than everything being controlled rigidly centrally. A concept of local subsidiarity where this was appropriate. This has not been so evident leading to Sir Paul Nurse's call for for the use of the "little ships" concept in the mobilisation of laboratory capacity. Many of my recommendations concerned the purchase and administration of a vaccine. We had an effective vaccine available earlier in this pandemic since it was essentially a 'flu virus. But the organisation of its delivery was delayed by negotiation with GPs about its administration. I recommended that efforts on the production of a vaccine should be matched by planning for its administration. I do hope that this is currently in hand. On communication with the public I noted and endorsed the use of regular press conferences as a way of keeping the public abreast of an inevitably changing situation. This has clearly been followed.

Emma: What do you think is the best way of effectively bridging the gap between scientists, medics and government politicians, especially when time is of the essence i.e. how do we get all the small boats to plot and steer a collective course in a timely manner?
Dame Deirdre: I think the Cabinet Office mechanism including the Cobra Committee (more accurately called the Civil Contingencies Committee (CCC), the Scientific Advisory Group on Emergencies (SAGE) and its subcommittees, is highly effective in producing and providing advice to the Cabinet itself, which is where the decisions on action have to be made. The Ministers and civil servants can then co-ordinate the "small boats" action on the ground.

Emma: Did you attend SAGE meetings? What are your thoughts on the current make up of SAGE? Are there key advisors (such as yourself) notably missing from the team? Do you think it's appropriate for the Prime minister's chief political adviser Dominic Cummings and government data scientist Ben Warner to attend SAGE meetings on the Coronovirus pandemic? Were there any government advisors on SAGE during the Swine flu pandemic?
Dame Deirdre: I did not attend SAGE as the pandemic was over before I took up my appointment. I do not know the current membership of SAGE, but I published the membership of the 2009 Sage in my report. If there is any missing element it might be that of an experienced local public health physician to keep the overwhelmingly academic

membership “grounded”. The membership does include the Chief Scientific Adviser and the Chief Medical Officer, both of whom are “government advisers”, but both of whom are experts of similar scientific calibre to the other members of SAGE. It is unclear whether Cummings and Warner were in attendance in a purely observer/listening capacity or whether they asked questions and took part in discussions. If the former there could be no breach of the independence of the Group but if the latter it would severely compromise its independence.

Emma: The Chief Medical Officer and Chief Scientific Officer are political appointments. As someone who has been the Chief Medical Officer, can you tell us whether it is difficult to maintain scientific objectivity whilst resisting political agendas whilst holding the role?
Dame Deirdre: The Chief Medical Officer and Chief Scientific Adviser are not strictly political appointments. They are appointed, as are all civil servants, by a Civil Service Commission Panel on the basis of their professional standing. Their appointments are “signed off” by Ministers on the recommendation of a panel of their peers. It is essential, and not difficult, to maintain scientific objectivity when advising Ministers even when this advice goes against their political agenda. The value of professional advisers is wholly dependent on their willingness to “speak truth to power”. It is for Ministers to then decide whether to take the advice if it goes against their political agenda. If the advice is not acted on and the problem is serious the possibility of resignation could arise. This did happen to me on one occasion – the Minister concerned backed down and I stayed in my post!

Emma: Do you think the COVID-19 crisis has been over politicised and has this adversely affected the way the government has responded to the pandemic? Put another way, do you think the government should worry less about their public performance and more about their direct impact on the pandemic?
Dame Deirdre: In that the public performance of the government will inevitably have a direct impact on a pandemic I think this question presents a false dichotomy! Public trust is an essential element in determining the way in which it behaves (as witnessed in the current crisis) and that trust is very dependent on the perception of the ability, honesty and transparency demonstrated by Government Ministers.

Emma: How have you been spending your time during lockdown?
Dame Deirdre: I have found it difficult in that, like everyone else I have been cut off from family and friends and from my various societies and other activities, particularly my daily swim at my health club. As I live alone following the death of my husband 18 months ago I have had to embrace a new routine of daily walks, gentle gardening and housework (in the absence of my cleaning lady}. I have also developed some “lockdown projects” including an attempt at writing my Memoirs (purely for family consumption and definitely not for publication !) and cataloguing 80 years worth of family photographs, Recently I have reluctantly accepted invitations to contribute to newspaper articles, radio and TV programmes about COVID-19 based on my review of the H1N1 pandemic.

Emma: We know that you like to travel but do you think that the measures the government has introduced in response to the acute threat of COVID-19 (e.g. imposing lockdown) will

empower them to tackle the more chronic threat of climate changes e.g. by introducing legislation to restrict air travel?
Dame Deirdre: I doubt whether the introduction of lockdown will influence the Government's attitude to tackling climate change. However, it is clear that unless an effective vaccine emerges the need for social distancing will greatly reduce everyone's ability to travel by air. For myself, like the Queen, I feel my days of long-haul air travel are over, but I live in hope that car travel will be revolutionised in the near future by widespread adoption of electric vehicles and possibly hydrogen powered cars. I recently changed my own diesel powered Golf for an electric version out of a feeling of guilt!

Emma: What do you see as the main public health threats for future generations?
Dame Deirdre: This is a difficult one! Clearly the effects of climate change must figure in such a list, with possibilities of food shortages and new pandemics. Equally I would be concerned about rising levels of mental health problems. Obesity is already a serious problem in the western world and although I hope we can find cures for cancer and cardiovascular disease, the pressing concern is to find ways of combatting the most common cause of death currently ie dementia.

News and Views

Editorial Concern—What policy makers need to know about COVID-19 protective immunity.

Altmann *et al* April 27, 2020.
Link: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30985-5/fulltext

Altmann and colleagues discuss how despite the pressure mounting on governments and their scientific advisors to provide strategies on how to lift lockdown, scientific policy must be guided by extensive seroprevalence data and a comprehensive understanding of immune correlates of protection are needed. Key areas for focus include:

- A better understanding of how the presence of SARS-CoV-2 specific antibodies translates to protective immunity. Total measurable antibody is not the same as protective virus-neutralising antibodies.
- Developing an understanding of both the correlates of progression to severe disease and the immune parameters that result in high protective virus-neutralising antibody titres are key. Most of the antibody data to date relates to patients with severe disease where 90% develop IgG antibodies within the first two weeks of infection and this correlates with clearance of virus. Limited data from milder infection suggests that only around 10% develop specific IgG's. This data needs to be repeated in much larger seroprevalence studies, but it may suggest that mild infection will not offer adequate herd immunity and mass vaccination will be required.
- Determining how long protective immunity following infection will last. Studies from related coronaviruses suggest that antibody responses wane after a year, whereas T cell responses can be detected at 4-years post infection.

SARS-CoV-2 Serology: Much Hype, Little Data

Farnsworth, C W. & Anderson, N W. *Clinical Chemistry,* 2020
Link: https://academic.oup.com/clinchem/advance-article/doi/10.1093/clinchem/hvaa107/5826351

Calls for widespread serological testing have gained popularity, with three roles being suggested: (1) Diagnosis, (2) Identification of convalescent plasma donors, (3) Population screening for purposes of determining immunity and exposure.

Serological testing for diagnostic purposes at initial patient presentation (3-5 days post-symptom onset) is unlikely to be useful given anti-SARS-CoV-2 IgG appear 7-14 days after symptom onset; IgM-based assays exhibit high false positive rates
Plasma of recovered patients with high anti-SARS-CoV-2 titres may be the ideal population for convalescent serum donation, however, most serologic assays provide no indication of

antibody titre and more research is needed to establish whether SARS-CoV-2-specific antibodies are neutralizing.

Population screening has been suggested to better establish infection levels and to allow serologically 'protected' individuals to return to work. Due to the current low seroprevalence of COVID-19, specificity of assays used for population-based screening must be more than >99% with small confidence intervals (i.e. between 99.0-99.9) to ensure a high positive predictive value.

Laboratories must rigorously validate assays to assure they are suitable for their ultimate use and to prevent misdiagnosis and misinformation.

COVID-19: the case for health-care worker screening to prevent hospital transmission

Black *et al*. Lancet 2020
Link: https://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(20)30917-X.pdf

Black and colleagues present the case for routine screening of healthcare workers (HCW) during the SARS-CoV-2 pandemic in this letter to the Lancet. Principle aims are to i) reduce unnecessary HCW quarantine, ii) reduce atypical/asymptomatic carrier spread and iii) protect HCWs. The context is high rates of asymptomatic carriage (series vary between 51.7% on the Princess Diamond cruise ship to 87.9% in an obstetric series). Modelling suggests 44% of secondary cases were infected by index cases who were asymptomatic at the time of spread. Hospitals are a high risk for transmission; in a series from Wuhan 41% of infections were acquired in hospital and 50% of Royal Gwent A&E staff have tested positive. It is therefore argued that asymptomatic HCW screening will decrease infection rates to patients and staff to understand the scale of the problem. As the background prevalence of COVID is reducing there is currently an opportunity to focus increased testing capacity on asymptomatic HCW with weekly or fortnightly screening.

My only argument with the case presented this letter is that it proposes screening of staff working in 'high risk areas'. A series from Newcastle reports administrative NHS staff are as likely to contract COVID as patient facing staff. **All these individuals are essential to the running of a hospital particularly as routine work is re-instituted.**

Predictive Mathematical Models of the COVID-19 Pandemic- Underlying Priniciples and Value of Projections

Jewell *et al*. JAMA 2020
https://jamanetwork.com/journals/jama/fullarticle/2764824

Jewell and colleagues discuss the discrepancies between the views of producers (epidemiologists) and consumers (politicians, media, and public) of mathematical models in this JAMA viewpoint.

Epidemiological models aim to give a basis the benefits of a range of interventions available to these consumers. The greatest predicted benefit needs to be set against the scale of the possible outcomes. The baseline predictions of long term mortality are often seized upon whilst the main benefit and aim of the epidemiologist is often to inform the short to medium interventions. Early interventions are likely to have the greatest impact on COVID however the early models of an emerging and evolving disease will inevitably be imperfect due to the quality of data. More complex models may be no better if key biological factors are not understood. A major challenge is accurate evaluation of mortality which is imperfectly collated across the world. The larger the country the more problematic to develop models as there is greater heterogeneity on multiple levels including disease spread based on population density, population comorbidity and impact on varying interventions such as social distancing.

The following key factors are suggested for developing and reporting SARS-CoV-2 models
Models need to be dynamic and if accurate predictions are required this can only be given in the short term
Assumptions need to be clear and discussed
Transparency of confidence intervals need to be discussed
Models should incorporate new measures of accuracy as these become available
Public reporting needs to be appropriately circumspect

"*Models do not determine the timeline, the virus makes the timeline*"

Perspective – Bill Gates

Responding to Covid-19 — A Once-in-a-Century Pandemic?

Gates, B., N Engl J Med 382;18 nejm.org April 30, 2020
Link: https://www.nejm.org/doi/full/10.1056/NEJMp2003762

Perspective from Bill Gates detailing our global preparedness for the current SARS-Cov-2 pandemic and potential for improved responses to future epidemics. Echoes 2018 NEJM perspective '*Innovation for Pandemics'* and 2015 NEJM perspective: *'The Next Epidemic — Lessons from Ebola'.* The 1% case fatality of SARS-Cov-2 results from transmission by asymptomatic individuals and the susceptibility of otherwise healthy adults. Governments must cooperate to establish an effective disease surveillance database of expertise and stocks to expedite rapid disease detection and establish fast and efficient vaccine and therapeutics pipelines. Healthcare services in poorer countries must be strengthened and supported by global knowledge and funding.

Gates proposes strategies for tackling the immediate crisis, as well as preventing future recurrence and/or incidence of additional biological threats.

The Key Points
* SARS-Cov-2 has a 1% case fatality – greater than the 1857 influenza pandemic (0.6%), but less than the 1918 Spanish flu outbreak (2%).
* Severity linked to asymptomatic transmission and broad-spectrum susceptibility.
* Gates' 2018 post-Ebola (2015) disease modelling of the spread of a lethal airborne pathogen predicted 33 million deaths within 6 months at a cost of $4 trillion to the global economy.
* Efforts must be made via public-private partnerships to develop both effective early warning systems and means of rapidly responding to the appearance of new disease.
* International cooperation and data sharing is essential.
* The Coalition for Epidemic Preparedness Innovation was established in 2017 by public private partnership (Gates Foundation, Wellcome Trust, Mastercard) to prioritise vaccine discovery for key global diseases.
* Vaccine development requires government funding to mitigate risk.
* New methods for quick and safe manufacture and screening of extant drugs libraries would expedite disease interception. RNA/DNA vaccines may be designed and scaled up quicker than traditional vaccines. Broad-spectrum anti-virals are more universally useful than targeted treatments.
* To obviate future pandemics, healthcare in poorer nations must be strengthened to enable faster detection and reporting of new disease as well as limiting global transmission and significant mortality.

Highlights:

Emphasis on global responses required by governments and private enterprise to facilitate rapid disease detection and therapeutic intervention to prevent future pandemics.
Despite warnings from previous disease outbreaks and the establishment of some organisations and funding to prepare for new diseases, we have not yet developed means of rapidly scaling up and mobilising new vaccines and therapeutics on a global scale.
Economic and knowledge-based support of low and middle income nations is essential for future global preservation.

Limitations:
Perspective based on the personal experience and opinions of Bill Gates and the Bill and Melinda Gates Foundation.
Modelling and current assumptions of disease transmission based on accurate and honest incidence reporting that may only be possible in hindsight. Uncertainty about the origins and first human transmission obscure accurate disease modelling. Disparate testing strategies and methods, as well as disease and fatality (and transparency of) reporting in different countries confound assessment of effective interventions.
Heavy reliance on substantial government funding, international data sharing and not-for-profit enterprise may not be realistic in the long-term once the current focus of necessity dissipates. Recent history would support a more pessimistic forecast.

Neurosciences and Mental Health

Psychological interventions for people affected by the COVID-19 epidemic.

Duan & Zhu, Feb 18th 2020, The Lancet
Link: https://www.thelancet.com/journals/lanpsy/article/PIIS2215-0366(20)30073-0/fulltext

This commentary summarises emergency psychological crisis interventions across China in relation to the COVID-19 pandemic. The authors state that medical institutions and universities have opened online counselling services for patients, their family members and medical staff, but that mental health needs are not being adequately handled. They argue barriers to successful intervention include lack of planning, lack of coordination between medical departments wasting resources, lack of timely diagnosis, poor follow-up treatments and evaluations, shortage of professionals and restricted accessibility to infected individuals. Improvements could be made by creating coordinated intervention plans before disasters occur, increasing cooperation between community health and mental health and establishing psychological intervention teams.

Highlights
1. COVID-19 has the potential to produce significant impacts on mental health
2. Emergency psychological crisis interventions are not well organised and managed in China

3. Several steps can be taken to plan and improve such services, preferably before a crisis occurs

Clinical Impact?
Emergency psychological intervention practices need to be urgently improved to safeguard those affected by COVID-19.

Vicarious traumatization in the general public, members, and non-members of medical teams aiding in COVID-19 control.

Li et al. March 10th, 2020, Brain Behavior, and Immunity
Link: https://doi.org/10.1016/j.bbi.2020.03.007

This study investigates vicarious traumatization (induction of trauma from contact with patients/stories of trauma) resulting from the COVID-19 pandemic on the general public, front-line nurses (provide direct care for COVID-19 patients) and non-front-line-nurses in China using a mobile app-based questionnaire. Surprisingly, front line nurses had lower vicarious traumatization scores than the other groups. This was explained by isolating general public having more time to gather knowledge about the lives and struggles of COVID-19 patients through digital means, and front-line nurses having greater psychological preparation. The authors suggest increased attention should be directed at psychological problems of medical staff and the general public in relation to the COVID-19 pandemic.

Highlights
1. The vicarious traumatization scores for front-line nurses were significantly lower than those of non-front-line nurses
2. The vicarious traumatization scores for the general public were significantly higher than those of font-lines nurses
3. Strategies that aim to prevent and treat vicarious traumatization in medical staff and general public are necessary

Clinical Impact?
The psychological impact of dealing with COVID-19 should be further investigated, and interventions aimed at reducing trauma provided where necessary and possible.

Important Methodologies?
Digital-based apps are useful and safe in the current climate.

Limitations?
It is possible that greater traumatization scores may be observed in front-line nurses weeks or months down the line. For example, PTSD does not always appear immediately after the event.

Neurologic Features in Severe SARS-CoV-2 Infection

Helms, J *et al.* (2020) NEJM

Link: https://www.nejm.org/doi/full/10.1056/NEJMc2008597

A prospective study of 58 patients who were admitted to 2 ICUs with acute respiratory distress syndrome (ARDS) due to Covid-19. The median age of the patients was 63 and all tested positive for SARS-CoV-2 via RT-PCR assay of nasopharyngeal samples. 84 % of patients presented with some kind of neurological feature. These data further highlight the range of symptoms that can result from SARS-CoV-2 infection.

Main Findings

- Confusion as per CAM-ICU was recorded in 65% (26/40) patients with agitation in 69% (40/58), corticospinal tract signs in 67% (39/58).
- All 58 patients returned a positive RT-PCR test of their nasopharyngeal swabs for SARS-CoV-2.
- MRI of 13 patients demonstrating encephalopathic features showed 8 had enhanced leptomeningeal spaces.
- 11 patients who underwent perfusion imaging had bilateral frontotemporal hypoperfusion.
- 7 patients had CSF samples examined and all returned negative test for SARS-CoV-2.
- Of 45 patients who had been discharged 15(33%) displayed dysexecutive syndrome.

Highlights

- ARDS due to infection with SARS-CoV-2 is associated with encephalopathy, confusion, agitation and corticospinal tracts.

Clinical Impact

- Moderate

Important Methodologies

- Prospective observational study testing neurological symptoms in consecutive patients.
- Use of validated tests to determine symptoms e.g. CAM-ICU.

Limitations

- Unable to identify which of the neurological features reported were specific to SARS-CoV-2 infection and which were due to critical-illness encephalopathy, cytokines or the effect of medication withdrawal.
- No follow up tests to see how neurological symptoms track with progression of the infection.
- Not all tests were carried out at the same point in the patient's hospitalisation.

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

Mao, L., Jin, H., Wang, M., Hu, Y., Chen, S., He, Q., Chang, J. *et al.* JAMA Neurology; Mao, L., Jin, H., Wang, M., Hu, Y., Chen, S., He, Q., Chang, J. *et al.*

Link: https://jamanetwork.com/journals/jamaneurology/fullarticle/2764549

Neurologic manifestations which make up a previously not investigated symptom group of COVID-19 was covered by collecting data from 214 confirmed cases in a hospital in the centre of the outbreak. They showed that 36.4% of all patients had nervous system symptoms with significantly larger proportion (45.5%) of severe cases presenting them. Out of all symptoms tested for, impaired consciousness, acute cerebrovascular disease and skeletal muscle injury. Interestingly, the most publicly known symptoms (fever, cough) were shown to be more common in non-severe cases, while less than half of the severe cases presented them.

Highlights

1. Neurologic manifestations occurred in 36.4% of all patients
2. Fever and cough were more common in non-severe than in severe cases.
3. Hypertension was the only comorbidity looked at that was significantly more common in severe cases.

Clinical Impact?

Patients, especially ones with severe COVID-19 infection, commonly show neurologic manifestations which, when present, could be used for a faster clinical diagnosis leading to better chances of treatment and prevention of transmission.

Important Methodologies?

Retrospective, observational case series that were recorded between 16.01.2020. and 19.02.2020. including 214 consecutive hospitalised patients.

All neurologic manifestations were reviewed and confirmed by two neurologists, with a third one asked in the case of any disagreements.

Limitations?

The diagnosis was made mainly relying on the subjective symptoms of the patients as patients going out for further examination would have increased the chances of cross-infection.

Guillain–Barré Syndrome Associated with SARS-CoV-2

Toscan, G *et al.* (2020) NEJM

Link: https://www.nejm.org/doi/full/10.1056/NEJMc2009191

This observational series presents 5 patients who developed the neurological syndrome Guillain–Barré syndrome (GBS) 5-10 days following the onset of coronavirus disease 2019 (Covid-19). At the onset of GBS 4 patients returned nasopharyngeal swabs positive for SARS-CoV-2, all 5 patients were negative for SARS-CoV-2 in CSF. The onset of GBS is associated with other infections such as Epstein-Barr virus and the latency between viral infection and GBS onset similar in these patients as with other infections. GBS may contribute to reduced

vital capacity in cases where results from chest imaging do not explain the degree of respiratory insufficiency

Main Findings

- 5 patients from approximately 1200 admitted with Covid-19 in a 3-week period developed GBS.
- Onset of GBS occurred 5-10 days following presentation of Covid-19 symptoms
- 4 of the patients had a positive nasopharyngeal swab for SARS-CoV-2, the one patient with a negative swab returned a positive serologic result.
- 3 patients produced neurophysiological recordings consistent with an axonal variant of GBS, with the other 2 patients presenting recordings consistent with a demyelinating variant.
- All patients were treated with IVIG with varying outcomes, 3 patients neurological symptoms worsened following commencement of IVIG treatment.
- 4 weeks post treatment 2 patients were still receiving mechanical ventilation, 2 were undergoing physical therapy for flaccid paraplegia and 1 was discharged and able to walk independently.

Highlights

- Guillain–Barré syndrome may develop following Covid-19 infection similar to other viral illnesses and latency to onset.

Clinical Impact?

- Moderate

Important methodologies

- Collection of nasopharyngeal swab and CSF for SARS-CoV-2 testing
- Classification of GBS subtype via neurophysiological recordings such as EMG

Limitations

- Small sample size prevents robust characterization of Covid-19 associated GBS

Diagnostics and Therapeutics

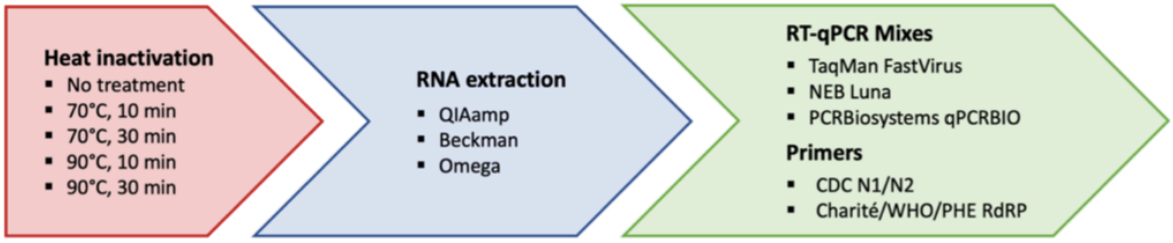
SARS-CoV-2 diagnostics workflows including viral heat inactivation

Lista *et al*, MedRxiv, 2020

Link: https://www.medrxiv.org/content/10.1101/2020.04.22.20074351v1

In this preprint, Lista *et al* compare a column based RNA extraction kit with two magnetic bead kits for SARS-CoV-2 viral RNA isolation. Next, they compare three different 1 step RT-qPCR kits. They also compare the use of primers that target 2 different viral genes, the N gene (primers recommended by Centre for Disease Control) and the RdRP gene (as per Public Health England Guidelines). Finally, the implemented a heat inactivation protocol to allow other laboratories without CL-3 facilities to perform SARS-CoV-2 diagnostic testing

safely. The methods can be easily implemented in other laboratories with SOPs made readily available at https://osf.io.uebvj/.



Main findings:

- RNAdvance Blood (Beckman) and Mag-Bind Viral DNA/RNA 96 Kit (Omega Bio-Tek) could be used as suitable alternatives to generate similar results to the QIAamp Viral RNA Mini Kit (QIAGEN)
- TaqMan Fast Virus 1 Step Master Mix (Thermofisher) and Luna Universal Probe One-Step RT-qPCR (NEB) kits were most sensitive; with qPCRBIO Probe 1-Step Go Lo-ROX (PCR Biosystems) being used as a good alternative.
- N1 and N2 primer probes are more sensitive than RdRP primer probes, particularly at low viral titers
- Heat inactivation can be used as an alternative to chemical inactivation of SARS-CoV-2 in swab samples and does not affect viral RNA detection

Highlights

- SOPs have been freely available to reduce the need of CL-3 laboratory facilities and allow CL2 facilities to help with diagnostic detection of SARS-CoV-2

Clinical Impact:

- Limited: provides diagnostic testing pipelines that can be implemented with ease in other laboratories, article is a preprint so should not be used as a guide for clinical practise

Important Methodologies:

- Heat inactivation of SARS-CoV-2
- RNA extraction using QIAamp, RNAdvance or Mag-Bind kits
- One step RT-qPCR using qPCRBIO, TaqMan Fast Virus or Luna kits

Limitations:

- Preprint has yet to be peer reviewed

An Infectious cDNA Clone of SARS-CoV-2

Xie *et al.* (Cell, Host & Microbe) 2020.
Link: https://www.sciencedirect.com/science/article/pii/S1931312820302316?via%3Dihub

Xie *et al*. reverse engineer an infectious cDNA clone of SARS-CoV-2 and a mNeonGreen reporter virus. Both were found to replicate as efficiently as an original clinical isolate (USA) and remain stable after five passages. These reagents can be utilised to study SARS-C0V-2 and used to develop future treatments.

Main findings:

- Generate a SARS-CoV-2 infectious cDNA clone by reverse engineering seven complimentary cDNA fragments to assembly the full genome.
- RNA transcribed from the clone was highly infectious (2.9×10^6 pfu/mL)
- When compared to an original clinical isolate the infectious clone had similar plaque morphology, viral RNA profile and replication kinetics.
- Molecular markers were retained and no mutations were acquired.
- mNeonGreen SARS-CoV-2 remained stable over five passages.
- mNeonGreen SARS-CoV-2 can be used to evaluate antiviral activity of IFN and is sensitive to high concentrations of IFN-alpha (1000/333 Units/mL).

Highlights

- Production of an infectious clone of SARS-CoV-2 and reporter to be used to screen anti-viral inhibitors and investigate effects of various mutations of transmission and vaccine development.

Clinical Impact:

- None

Important Methodologies:

- Reverse engineering of infectious SARS-CoV-2 clone and reporter virus.
- Western blot – protein expression of specific viral proteins

Limitations:

- Only tested in Vero E6 cells (monkey kidney).
- Used a low passage number (5) to assess stability.
- Not tested on interferon competent cells.

Development of CRISPR as an antiviral strategy to combat SARSCoV-2 and influenza

Abbott *et al*., (Cell), 2020
Link: https://www.cell.com/pb-assets/products/coronavirus/CELL_CELL-D-20-00736.pdf

Abbott *et al*., report an antiviral strategy using **P**rophylactic **A**ntiviral **C**RISPR in hu**MAN** cells (PAC-MAN), for repurposing RNA-guided RNA endonuclease activity of Cas13d in human lung epithelial cells as a form of genetic intervention to target SARS-CoV-2, influenza A virus (IAV) and potentially all coronaviruses. A bioinformatic pipeline defined highly conserved regions across many SARS-CoV-2 and IAV genomes enabling the design and screening of CRISPR RNA

(crRNA) pools. The Cas13d system effectively targets and cleaves RNA sequences of synthesised SARS-CoV-2 fragments and live IAV demonstrating PAC-MAN as a potentially powerful new approach for inhibiting viral function and replication.

Main findings:

- Bioinformatics pipeline revealed 6 crRNAs able to target 91% of 3,051 sequenced coronaviruses and 6 crRNAs targeting 92% of 91,600 IAV strains
- Proof-of-concept study demonstrating that targeting the positive-sense genome and viral mRNAs with Cas13d, PAC-MAN degrades viral genome templates for replication and viral gene expression
- crRNA pools targeting multiple, highly conserved regions in the same virus or different strains of coronavirus makes the system better able to buffer against viral evolution and escape and provide broad, pan-coronavirus protection
- PAC-MAN successfully cleaved SARS-CoV-2 fragments and reduced the amount of viral RNA from IAV in human lung epithelial cells *in vitro* to provide pan-coronavirus and IAV protection

Highlights:

- Demonstrates a potential pan-coronavirus strategy to target not only viruses that circulate in humans, but also those that are found in animal reservoirs

Clinical impact:
Moderate

Important methodologies:

- Provides a resource map to show how the top 6 crRNAs (PAC-MAN-T6) target the coronavirus phylogenetic tree. Altogether, the PAC-MAN-T6 can target all known human coronaviruses with broad coverage against other animal coronaviruses
- To evaluate Cas13d, created two cell reporters expressing synthesised fragments of SARS-CoV-2 fused to GFP. Created a stable A549 cell line expressing Cas13d through lentiviral infection and detection of Cas13d by co-expression with mCherry

Limitations:

- Requirement to validate the efficiency and specificity of crRNAs for inhibiting infection of respiratory tract cells with live SARS-CoV-2 viruses
- Test PAC-MAN system *in vivo* and after viral infection

A SARS-CoV-2 protein interaction map reveals targets for drug repurposing

Gordon, D.E. *et* al. (2020). Nature – Accelerate Article Preview.
Link: https://www.nature.com/articles/s41586-020-2286-9

There is potential to repurpose existing drugs to overcome SARS-CoV-2 infection. 332 human proteins have been shown to physically interact with various proteins produced by the SARS-

CoV-2 genome. Multiple biological processes, including translation and vesicle trafficking, were found to be involved. Analysis using both literature and chemical databases revealed 69 compounds, ranging from FDA-approved drugs to preclinical molecules, which could target 63 of these interacting proteins. Testing the antiviral activity of many of these drugs revealed 2 broad classes that were particularly effective: protein biogenesis inhibitors and ligands of the Sigma1 and Sigma2 receptors.

Main Findings

- 332 human proteins were found to interact, with high-confidence, to a SARS-CoV-2 encoded protein. Comparison to the proteome of 29 healthy human tissues (Wang *et* al. 2019) revealed that interacting proteins were enriched in lung tissue.
- 40% of interacting proteins were associated with vesicle trafficking pathways.
- Highlighted interaction proteins included **HDAC2**, **NUP98-RAE1**, **G3BP1/2**, **Sec61**, **Cullin 2** and **eIF4F translation complex** – many of which are druggable targets or pathways.
- Innate immune signalling pathways are targeted by viral proteins e.g. IFN and NF-kB.
- 62/322 interacting proteins are "druggable".
- 69 available compounds, ranging from FDA-approved drugs, investigational new drugs and preclinical molecules, can modulate targets.
- 2 class of molecules: **Protein Biogenesis inhibitors** (Zotatifin, ternatin-4 and PS3061) and **ligands of the Sigma1 and Sigma2 receptors** (haloperidol, PB28 and hydroxychloroquine) emerged as particularly antiviral.
- Efficacy of protein biogenesis inhibitors' viral infectivity was in the 10 - 100 nM range
- Sigma ligands have clear separation between antiviral and cytotoxic effects.

Highlights

- Novel approach for drug discovery
- Reveals potential pathways implicated during a SARS-CoV-2 infection and also currently available drugs that could be repurposed. Some highlighted (e.g. hydroxychloroquine) are already being used in clinical trials.

Clinical Impact?

High.

Important Methodologies

- Affinity-purification mass spectrometry (AP-MS) was used to analyse the interactions
- 2 antiviral assays to test drugs – immunofluorescence-based and qRT-PCR on viral RNA.

Limitations?

- AP-MS lyses cells for analysis so transient interactions may have been missed.

Complement as a target in COVID-19?

Risitano *et al*. (medRxiv), 2020
Link: https://www.nature.com/articles/s41577-020-0320-7.pdf

Risitano *et al*. reports on the potential use of C3 targeting therapies to reduce inflammation which causes acute respiratory distress syndrome in COVID-19 patients. Complement is an integral part of innate immune responses to viral infection. C3 component signalling is positioned upstream which argues for broader anti-inflammatory potential of C3 blockade. Downstream elements such as C3a, C5a and IL-6 present will be blocked by C3 inhibition which may ameliorate lung injury. Indication that C3 inhibition may be preferred to C5 inhibition.

Main findings:

- A recent study of SARS-CoV, closely related to SARS-CoV-2, found activation of C3 exacerbates disease in SARS-CoV-associated ARDS.
- C3-deficient mice infected with SARS-CoV exhibited less respiratory dysfunction, despite equivalent viral loads in the lungs, and this was associated with decreased lung infiltration of neutrophils and inflammatory monocytes and lower levels of cytokines and chemokines in the lungs and sera.
- C3 inhibition possible combination with IL-6 inhibiting agents to reduce inflammation in COVID-19 patients?
- AMY-101 C3 inhibitor currently being tested in COVID-19 patients.
- A recent preprint showed lung biopsy samples from patients with severe COVID-19 exhibited widespread complement activation, characterized by C3a generation and C3-fragment deposition. Also showed increased serum C5a and treatment of patients with an anti-C5a antibody led to immediate clinical improvement .
- Good safety profile of C5 inhibitors.
- C5 inhibition can be partial, allowing residual terminal pathway activity to skew efficacy in cases of excessive complement activation, often seen in infections. Also C5 inhibition will not affect C3.

Highlights:

- Anti-C3 agents for reducing the activation of innate immunity in the lung and controlling the maladaptive inflammatory response

Clinical Impact:

- Minimal short-term impact-possible potential for C3 inhibiting agents in future

Important Methodologies:

- Evidence from *in vivo* mouse studies and clinical data

Limitations:

- Clinical data on the role of complement activation in the development of SARS-CoV-2-associated ARDS are scarce

- No C3 inhibiting agent currently approved for use
- Only a proportion of COVID-19 patients develop aggressive disease therefore there is a need for clinical indicators and biomarkers to predict where treatment using inflammatory inhibitors is necessary.

Critical role of type III interferon in controlling SARS-CoV-2 infection, replication and spread in primary human intestinal epithelial cells

Megan L. Stanifer *et al*., bioRxiv preprint (2020)
Link: https://www.biorxiv.org/content/10.1101/2020.04.24.059667v1

Stanifer et al. provide a comprehensive analysis of SARS-CoV-2 infection in human intestinal epithelial cells (IECs). They demonstrate that intestinal epithelium fully supports entry, replication and assembly of *de novo* virus particles, highlighting that 'enteric phase' of SARS-CoV-2 could potentially contribute to pathogenesis of COVID-19. They propose that active SARS-CoV-2 replication in the gastrointestinal tract could explain the elevated viremia observed in patients and account for high levels of viral RNA detected in stool samples (even after complete resolution of COVID-19 symptoms).

Main findings:

- Intestinal epithelium is a site of productive SARS-CoV-2 infection (supported by the fact that IECs highly expresses ACE2 - the virus entry receptor).
- Primary and transformed human IECs fully support SARS-CoV-2 infection *in vitro*.
- Organoids derived from colons of two independent donors also supported SARS-CoV-2 infection, replication and dissemination.
- Interferon (IFN) inhibits SARS-CoV-2 infection in human IECs *in vitro*.
- Type III IFN was significantly more efficient in inhibiting/controlling replication and spread of SARS-CoV-2 than type I IFN-mediated response.
- Depletion of type III IFN receptor significantly increased susceptibility to SARS-CoV-2 (approximately by a factor of 7).
- Organoids significantly upregulated type III IFN following SARS-CoV-2 infection (type I IFN was not produced).
- Pre-treating cells/organoids with exogenous IFNs prior to SARS-CoV-2 infection dramatically inhibited infection, diminished viral replication and a significantly reduced *de-novo* assembly of infectious viral particles.

Highlights:

- SARS-CoV-2 could potentially be transmitted by faecal-oral route.
- Type III IFN response is vital for controlling SARS-CoV-2 infection of gastrointestinal epithelium

Clinical Impact: Medium/High

Important methodologies:

- Use of human intestinal organoids as a model to investigate SARS-CoV-2 infection.
- Identified intestinal epithelial cell lines as the best/ideal culture model to propagate SARS-CoV-2.

Limitations:

- Additional functional assays are required to:
    1) characterise SARS-CoV-2 enteric lifecycle in order to understand if infection of the gastrointestinal tract is due to transmission of virus by faecal-oral route or simply an outcome of virus dissemination from the lungs.
    2) directly compare immune responses in lung and gut during SARS-CoV-2 infection (e.g. using lung & intestinal organoids).
    3) correlate severity of COVID-19 with extent of enteric SARS-CoV-2 replication (could have prognostic value).

A Novel Protein Drug, Novaferon, as the Potential Antiviral Drug for COVID-19

Zheng *et al*. MedRxiv 2020
Link: https://www.medrxiv.org/content/10.1101/2020.04.24.20077735v1

Novaferon, a drug against chronic Hepatitis B in China, was assessed for its antiviral properties in vitro and in a clinical study compared to an existing drug treatment for Sarscov2. Primary endpoint was SARS-CoV-2 clearance rate on day 6 of treatment, and secondary endpoint was the time to the SARS-CoV-2 clearance in COVID-19 patients that had been hospitalized with moderate or severe disease. Novaferon reduced viral clearance in patients by 3 days compared to control treatment. This early clearance of SARS-CoV-2 might help to shorten the disease course and reduce viral shedding.

Highlights
Novaferon exhibited significant effects against SARS-CoV-2 in a randomized clinical study from Wuhan, China. In the study, 30, 30, and 29 patients were assigned into a Novaferon only group, a Novaferon plus Lopinavir/Ritonavir group or Lopinavir/Ritonavir only (control)group respectively.

1. **Primary endpoint:** On day 6, SARS-CoV-2 clearance rates in the Novaferon group and Novaferon plus Lopinavir/Ritonavir group reached 50.0% and 60.0% respectively, and were significantly higher than in Lopinavir/Ritonavirgroup (24.1%).

2. **Secondary endpoint:** The median time to SARS-CoV-2 clearance were 6 days for the Novaferon group, 6 days for the Novaferon plus Lopinavir/Ritonavir group, and 9 days for the Lopinavir/Ritonavir control group. This indicates a 3-day reduction of time to SARS-CoV-2 Clearance by Novaferon treatment.

3. During course of the study, none of the moderately ill patients in the Novaferon or the Novaferon plus Lopinavir/Ritonavir group progressed to severe illness. In contrast, 4 moderately ill patients in the Lopinavir/Ritonavir group progressed to severe illness.

4. No severe adverse events were observed.

Clinical Impact?
High

Important Methodologies?
Novaferon was given by oxygen-driven atomized inhalation for at 20 µg for 15 min twice a day. SARS-CoV-2 RNA was detected via qRT-PCR of nasopharyngeal swabs.
Limitations?

One hospital study only and low n number. Due to lack of medical staff during the Covid19 outbreak, adverse effects might not have been reported accurately.

Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial

Wang *et al*. (The Lancet), 2020
Link: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)31022-9/fulltext

Wang *et al*. did a randomised, double-blind, placebo-controlled, multicentre trial at ten hospitals in Hubei, China (trial registered with ClinicalTrials.gov – NCT04257656). Eligible patients were randomly assigned to receive intravenous remdesivir (200 mg on day 1 followed by 100 mg on days 2–10 in single daily infusions) or placebo infusions. While the administrated dose was well tolerated, remdesivir use was not associated with statistically significant clinical benefits. However, although not statistically significant, patients receiving remdesivir had a faster time to clinical improvement than those receiving placebo. This reduction in time requires confirmation in larger studies.

Main findings:

- 255 patients screened, of whom 237 were eligible: 158 were assigned to receive remdesivir and 79 to receive placebo.
- Patients received either intravenous remdesivir (200 mg on day 1 followed by 100 mg on days 2–10 in single daily infusions) or the same volume of placebo infusions for a total of 10 days.
- Lopinavir-ritonavir was co-administrated in 18% of patients at baseline; 66% of patients received corticosteroids.
- Median time from symptom onset to starting study treatment was 10 days.
- The remdesivir group was not associated with significantly clinical benefits.
- Although not statistically significant, patients receiving remdesivir had a numerically faster time to clinical improvement than those receiving placebo (5 days).
- Mortality at 28 days was similar in both groups.
- Viral load decreased over time similarly in both groups.
- Adverse events were reported in 66% of patients in the remdesivir group vs 64% in the placebo group; severe adverse events were reported in 18% of patients in the remdesivir group vs 26% in the placebo group.

Highlights

- Despite the dose regimen being adequately tolerated, intravenous remdesivir did not significantly improve the time to clinical improvement, mortality, or time to clearance of virus in patients with COVID-19.

Clinical Impact:

- Moderate

Important Methodologies:

- Viral load testing: at baseline, specimens were tested for detection of E-gene, RNA-dependent RNA polymerase gene and N-gene; on subsequent visits, samples were quantitatively and qualitatively assessed for E-gene.

Limitations:

- Study did not reach its target enrolment, therefore there is an insufficient power to detect assumed differences in clinical outcomes.
- Due to hospital bed availability, most patients were enrolled later in the course of disease.
- Reduction in time to clinical improvement in patients treated earlier needs confirmation in larger studies.

Virus Tropism

Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis

Hamming *et al*. (Journal of Pathology), 2020
Link: https://onlinelibrary.wiley.com/doi/full/10.1002/path.1570

Recently, a metallopeptidase named angiotensin-converting enzyme 2 (ACE2) has been identified as the functional receptor for SARS-CoV.
Hamming et al. report the immunolocalization of angiotensin-converting enzyme 2 (ACE2), the functional receptor for SARS-CoV, in human tissues. The authors studied the localization of ACE2 protein in various human organs (oral and nasal mucosa, nasopharynx, lung, stomach, small intestine, colon, skin, lymph nodes, thymus, bone marrow, spleen, liver, kidney, and brain).

Main findings:

- the surface expression of ACE2 protein on lung alveolar epithelial cells and enterocytes of the small intestine.
- ACE2 was present in arterial and venous endothelial cells and arterial smooth muscle cells in all organs studied.
- ACE2 is abundantly present in humans in the epithelia of the lung and small intestine, which might provide possible routes of entry for the SARS-CoV.

Highlights

- The epithelial expression of ACE2, together with the presence of ACE2 in vascular endothelium, provides a first step in understanding the pathogenesis of the main SARS disease manifestations. Whether the abundant expression in the vascular system may also serve as a route of spread and replication should be investigated further in functional studies applying blockade of the ACE2 protein.

Clinical Impact:

- Minimal

Important Methodologies:

- Tissues were chosen to represent organ systems where the SARS virus has been detected in humans and in experimentally infected macaques.
- Immunohistochemistry and ACE2 localization

Limitations:

- Whilst this study is one of the first characterizing the expression of ACE2 in different organs, the physiological role of ACE2 in most tissues has not been elucidated.

Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2

Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z *et al.* Cell
Link: https://www.sciencedirect.com/science/article/pii/S009286742030338X

Wang *et al* show the crystal structure of SARS-Cov-2 spike protein in complex with human ACE2 (resolution 2.5Å) and determine that binding is similar to SARS-CoV in complex with hACE2. They also investigate whether monoclonal and polyclonal antibodies raised against SARS or MERS-CoV are able to effectively bind SARS-CoV-2 spike protein.
For background, coronavirus spike glycoprotein (including SARS-CoV and MERS-CoV) is cleaved into S1 and S2 subunits. S1 can further divided into C-terminal domain (CTD) and N terminal domain (CTD); the SARS-CoV CTD is called the receptor binding domain (RBD) in this paper.

Highlights

1. Structure of SARS-CoV-2 CTD shows two subdomains: one a conserved core domain with 5 antiparallel B strands and a conserved disulphide bond, the other an external subdomain predominantly consisting of a disulphide-stabilised flexible loop. SARS-CoV-2 C-terminal domain uses the latter to recognise hACE2 N-terminal domain.
2.The virus-receptor interface (SARS-CoV-2 CTD and hACE2) is largely mediated by polar contacts created by hydrophilic residues; this complex has more residues and contacts at its interface with hACE2 than SARS-RBD:hACE2 (21 residues vs 17, and 288 vdW contacts vs 213). SARS-CoV-2 CTD also has a four-fold stronger binding affinity for hACE2 than SARS-

RBD. Kd numbers are as follows: SARS-CoV-2 S1- 94.6±6.5nm, SARS-CoV-2 CTD 133.3±5.6nm, SARS RBD 408.7±11.1nm.
3. Study shows significant structural homology and sequence identity (~73.9%) between SARS-CoV-2 CTD and SARS-RBD. So much so that soluble SARS-RBD blocks interaction between SARS-CoV-2 ligand and hACE2.
4. However, murine monoclonal antibodies against SARS-CoV S1 showed no interaction with SARS-CoV-2 spike protein. Additionally, polyclonal antibodies against SARS-RBD and MERS-RBD showed no binding to SARS-CoV-2 CTD. This demonstrates a clear difference in epitope features.

Clinical Impact? Minimal. SARS-CoV 2 structure in complex with hACE2 as already been shown. Does inform clinical studies that cross-binding or neutralisation is not possible by mono/polyclonal antibodies raised against SARS-CoV or MERS-CoV, but again this has already been reported.

Important Methodologies? Flow cytometry, confocal fluorescent microscopy, surface plasmon resonance assays.

Limitations? Authors mention that since paper has been revised, other groups have shown that polyclonal antibodies against SARS-CoV S (in both mice and patients) were able to effectively neutralise SARS-CoV-2 S-mediated entry into cells, in contradiction to this paper.

SARS-CoV-2 Productively Infects Human Gut Enterocytes.

Lamers *et al.*, bioRxiv preprint, April 25th, 2020.
Link: https://doi.org/10.1101/2020.04.25.060350

Summary

Viral RNA can be found in rectal swabs, even after nasopharyngeal testing has turned negative, implying gastro-intestinal infection. Human small intestinal organoids (hSIOs) are established from primary gut epithelial stem cells, can be expanded indefinitely in 3D culture and contain all proliferative and differentiated cell types of the *in vivo* epithelium. Study exposes ileal hSIOs grown under four different culture conditions to SARS-CoV and SARS-CoV-2. hSIOs grown in Wnt-high expansion medium (EXP) overwhelmingly consist of stem cells and enterocyte progenitors. Organoids grown in differentiation medium (DIF) contain enterocytes, goblet cells and low numbers of enteroendocrine cells (EECs). Addition of BMP2/4 to DIF (DIF- BMP) leads to further maturation. In the final condition, induced expression of NeuroG3 with doxycycline to raise EECs numbers. Samples were harvested at multiple timepoints post infection and processed for the analyses. The SARS-CoV-2 receptor angiotensin converting enzyme 2 (ACE2) is highly expressed on differentiated enterocytes. This study shows that in hSIOs, enterocytes were readily infected by SARS-CoV and SARS-CoV-2, demonstrated by confocal- and electron-microscopy. Consequently, significant titres of infectious viral particles were measured. mRNA expression analysis revealed strong induction

of a generic viral response program. They conclude that intestinal epithelium supports SARS-CoV-2 replication.

Main findings

- SARS-CoV and SARS-CoV-2 readily infect organoid-derived human airway epithelium cultured in 2D over a 96hr time course. The viruses targeted ciliated cells, but not goblet cells.
- Both SARS-CoV and SARS-CoV-2 productively infected hSIOs as assessed by qRT-PCR targeting the E gene and by live virus titrations on VeroE6 cells.
- Infectious virus particles and viral RNA increased to significant titres for both viruses in all conditions.
- As EXP medium supported virus replication, enterocyte progenitors appeared to be a primary viral target.
- ACE2 mRNA expression differed greatly between the four conditions.
- Infected cells invariably displayed proliferative enterocyte progenitor-phenotypes (EXP) or ApoA1+ enterocyte-phenotypes (DIF).
- Low expression of Type I and III interferons induced in SARS-CoV-2 infected organoids but not in SARS-CoV infected organoids.

Clinical Impact

Minimal – initial characterisation of an *in vitro* human organoid cell model.

Important Methodologies

- Determination of virus titres using qRT-PCR.
- RNA Sequencing.
- Transmission electron microscopy.
- Multiplex cytokine ELISA.

Limitations

- The article is a preprint and has not been peer reviewed.
- No reference to how the MOI was determined.
- Further characterisation required to draw conclusions.

Human iPSC-Derived Cardiomyocytes are Susceptible to SARS-CoV-2 Infection

Sharma *et al*./preprint on bioRxiv/2020

Link: https://www.biorxiv.org/content/10.1101/2020.04.21.051912v1

Sharma *et al*. aimed to investigate the impact of SARS-CoV-2 infection on human cardiomyocytes. They demonstrated the ability of SARS-CoV-2 to enter and replicate within human cardiomyocytes *in vitro,* causing functional alterations and cytopathic effects. The group are aiming to establish a model system to study the mechanisms of cardiomyocyte infection and potentially create a cardiac-specific antiviral drug screening platform.

Main findings:

- Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) can be infected by SARS-CoV-2 *in vitro.*
- Infected cells stained positive for a double stranded RNA (dsRNA) intermediate unique to positive sense RNA virus infection as well as the "spike" capsid protein responsible for viral entry.
- A proportion of infected cells stained positive for both, dsRNA as well as the apoptosis marker cleaved caspase-3 suggesting virally induced cell death
- Infected hiPSC-CMs stopped beating after 72 hours of SARS-CoV-2 infection, whereas control cells continued to contract.

Highlights

- SARS-CoV-2 is able to infect human stem cell derived cardiomyocytes *in vitro* and the hiPSC-CMs model could be useful in a cardiac-specific antiviral drug screening process

Clinical Impact:

- Limited, might provide a useful method for cardiac-specific antiviral drug screening

Important Methodologies:

- Differentiation of hiPSC-CMs from a human induced pluripotent stem cell line via a GSK3β inhibitor to initiate mesoderm specification, followed by a Wnt inhibitor to initiate cardiac specification and selection by glucose deprivation
- Inoculation of hiPSC-CMs with SARS-CoV-2 at an MOI of 0.1 (approximately 1 viral particle introduced per 10 cells)
- Immunofluorescence
- Beat rate analysis comparing infected to control hiPSC-CMs

Limitations:

- Manuscript has not been peer reviewed yet
- While hiPSC-CMs do seem to share features of primary human cardiomyocytes, they are electrophysiologically, structurally, and genetically immature in comparison to real life adult counterparts
- the *in vitro* cardiomyocyte culture lacks any immune components present *in vivo*
- how well does a MOI of 0.01 represent actual clinical disease?

Cell-intrinsic differences between human airway epithelial cells from children and adults

Elizabeth F. Maughan *et al*. (bioRxiv), 2020
Link: https://www.biorxiv.org/content/10.1101/2020.04.20.027144v1

Study investigates intrinsic molecular differences between paediatric and adult airway basal cells via bulk RNA sequencing studies in laser-capture microdissected whole epithelium, FACS-sorted basal cells and cultured basal cells, as well as in vitro cell proliferation experiment. Paediatric airway epithelial cells displayed higher colony forming ability, better in vitro growth and outcompeted adult cells in competitive proliferation assays. In RNA sequencing experiments, differences were seen in airway epithelial gene expression between samples from children and adults. However, genes known to be associated with SARS-CoV-2 infection were not differentially expressed between children and adults.

Main findings:

- Genes known to be associated with SARS-CoV-2 infection were not differentially expressed between children and adults.
- Results chart cell-intrinsic differences in transcriptional profile and regenerative capacity between proximal airway epithelial cells of children and adults.
- No significant differences (histochemistry or bulk RNA sequencing) in the proportion of cells between adult and paediatric steady state normal human airway epithelium in basal, mucosecretory or ciliated cell compartments.
- Differences were seen in the expression of genes associated with interferon responses and cell proliferation. Further, in growth experiments, paediatric cells outgrew adult cells which is suggested as a reflection of these differences.
- It has been observed that children suffer less severe symptoms than adult patients of COVID-19 which has been speculated that this might be due to their lower expression levels of the viral entry receptor, ACE2. The results presented imply that there are no major differences in the epithelial expression of viral infection-associated genes between children and adults in the proximal airways, and suggest that expression of *ACE2* and other genes implicated in SARS-CoV-2 cell entry are also comparable.
- Highlights a further area of study in the mechanisms by which airway basal cells lose their *in vitro* proliferative capacity with age, and whether this reflects *in vivo* loss of progenitor capacity.

Highlights:

- Investigated speculated relationship between ACE2 and COVID-19, however, evidence suggested that there is no relationship.

Important Methodologies:

- RNA sequencing datasets will be a useful resource for further investigations (GEO accession number GSE148818).

Virus Origin and Transmission

The Proximal Origin of SARS-CoV-2

Andersen *et* al (Nature Medicine), 2020
Link: https://www.nature.com/articles/s41591-020-0820-9.pdf

There is still considerable uncertainty as to the origins of the SARS-CoV-2 virus, other than the geographical location of Wuhan, China. Therefore, this study aims to identify the origin of the virus using comparative analysis from genomic data. Ultimately, their analysis clearly shows the SARS-CoV-2 virus not be the construct of laboratory or purposefully manipulated virus. This is shown by the main findings listed below. Moreover, they propose 2 theories for the origin of SAR-CoV-2. Firstly, natural selection in an animal host before zoonotic transfer. Secondly, natural selection in humans following zoonotic transfer.

Main findings:

- SARS-CoV-2 spike protein has a receptor binding domain (RBD) that has a high affinity for the ACE2, but with a very different compared to other SARS-CoV-like viruses. As a result, the high affinity binding must be a result of natural selection.
- Genetic data irrefutably shows that SARS-CoV-2 is not derived from any previously used virus backbone.
- Bats could serve as progenitor hosts for SARS-CoV-2 due to their 96% similarity to a SARS-CoV-like coronavirus from this species. As discussed, there are significant differences in the RBD which, prevent it from binding to human ACE2.
- It is possible that a SARS-CoV-2 progenitor may have jumped to the human population, acquiring the infective genetic components such as the specific RBD, during an undetected human-to-human infection process.

Spike mutation pipeline reveals the emergence of a more transmissible form of SARS-CoV-2

Korber B. BioRxiv 2020
Link: https://www.biorxiv.org/content/10.1101/2020.04.29.069054v1

In this study they try to identify dynamically changing patterns of mutation indicative of positive selection for Spike variants. By using GISAID they try to come up with changes in frequency of mutations that might signal positive selection and change in either viral phenotype or antigenicity which will support the immunology and vaccine communities. Also, set out to determine whether recombination is playing a role in SARS-CoV-2 pandemic evolution.

Main findings:

- They found two spike mutations of particular interest D614G and S943P. Also other additional sites of interest in spike which are L5F, L8V, V367F, G476S, V483A, H49Y, Y145H/del, Q239K, A831V, D839Y/N/E and P1263L

Highlights

- D614G mutation is associated with increased transmission.
- D614G may impact spike's infectivity by improving receptor binding, fusion activation or ADE antibody elicitation
- G614 mutation was slightly enriched among the ICU subjects (not statistically significant.
- Patient with G614 mutation had higher viral loads
- S943P mutation was a result of recombination of distinct viruses within a coinfected host
- The mutational sites A831 and D839 occur in the region of fusion peptide
- L5F and L8V mutation tends to persist when it arises although it's not frequently
- V367F, G476S, and V483A are found within the RBD domain
- P1263L near the end of the cytoplasmic tail of the spike protein maintains its frequently globally. It is mostly found in the UK and Australia, emerging as a single related linage

Clinical Impact: Medium

Important Methodologies:

- Data obtained from the sequence database GISAID.
- The computational method used to determine if recombination events could be identified in other geographically regional datasets -they used RAPR

Limitations:

- As it compares data obtained from a database the principal limitation of this study is the uncertainty regarding the time from infection at which sample was taken.
- GISAID data used in this study was frozen at April 13th
- Accessibility to data. i.e the status of the D614G mutation in China remained unclear as very few Chinese sequences in GISAID were sampled after March 1st.

An analysis of SARS-CoV-2 viral load by patient age

Terry C Jones *et al*. Zoonosen website.2020

Link: https://zoonosen.charite.de/fileadmin/user_upload/microsites/m_cc05/virologie-ccm/dateien_upload/Weitere_Dateien/analysis-of-SARS-CoV-2-viral-load-by-patient-age.pdf

Jones *et al*. analysed the data on viral load, as estimated by real-time RT-PCR threshold cycle values from 3,712 COVID-19 patients to examine the relationship between patient age and SARS-CoV-2 viral load. Patients were divided into two categories- based on age brackets and on broad social strata. Analysis of variance of viral loads in patients of different age categories found no significant difference between any pair of age categories including children. The data indicate that viral loads in the very young do not differ significantly from those of adults. **Children may be as infectious as adults**.

Main findings:

- No significant relationship between patient age and SARS-CoV2 viral load.
- No significant relationship between social strata and viral load.

Highlights

- The attack rate in children seems to correspond to that in adults, it is obvious that children are under-represented in clinical studies and less frequently diagnosed due to mild or absent symptoms.

Clinical Impact: minimal

Important Methodologies:

- RT-PCR quantification for viral load

Limitations:

- The sample size in the paediatric age groups is small (1-11 years of age).
- The observation that hospitalization occurs after some days of symptoms, a time when viral loads in throat swabs are beginning to decline
- Role of ethnicity

Innate and Adaptive Immunity

Heightened innate immune responses in the respiratory tract of COVID-19 patients

Zhou *et al.* Cell Host and Microbe 2020
Link: https://doi.org/10.1016/j.chom.2020.04.017

Performed metatranscriptomics on BAL (bronchoalveolar lavage) samples from 8 COVID-19 cases, 146 community-acquired pneumonia (CAP) patients and 20 healthy controls. Study of SARS-CoV-2-host response *in situ* highlighted a robust Interferon response and an upregulation of chemokines involved in recruiting Neutrophil and Monocytes. SARS-CoV-2 cases had the most gene expression changes in compared to healthy controls, and some genes did overlap with viral-CAP cases. Confirmed an increased Neutrophil to Lymphocyte ratio in SARS-CoV-2 cases. Propose the robust chemokine and interferon response in COVID-19 cases is pathogenic even though hypercytokinemia/“Cytokine Storms” is only seen in severe cases.

Main findings:

- Transcriptomic analysis of BAL samples sequenced human cells and transcriptionally active microbes, allowing host gene changes to be compared to patient viral load.
- COVID-19 cases had higher proinflammatory cytokines than healthy controls.
- Pathways upregulated by SARS-CoV-2 included; Interferon signalling (slightly ↑ in virus-like CAP), IL-17, TNF and NFκB signalling.
- Protein-Protein Interactions showed ribosome and chemokine signalling pathways were the most connected subnetworks.
- Heatmaps of cytokine genes showed neutrophil and monocyte recruitment related chemokines were higher in COVID-19 cases e.g. *CXCL8*, *CCL2* and *CCL7*.
- Heatmap shows interferon signalling genes, in COVID-9 cases, and includes several key transcription factors (*IRF7* and *STAT1*) and other genes related to antiviral activity *IFIT* and *IFITM*.
- Both the Cytokine and Interferon genes appeared to be more upregulated in samples were taken closer to symptom onset.
- Estimated that COVID-19 patients had a higher Neutrophil-to-Lymphocyte ratio than viral CAP and healthy controls.
- The highest ratio was seen in the case with the highest virus.

Highlights:

- Transcriptomic analysis of SARS-CoV-2-host response *in situ* highlighted a robust Interferon and chemokine response, recruiting Neutrophil and Monocytes.

Clinical Impact:

- Low, focuses on improving immunopathological understanding.

Important Methodologies:

- Metatranscriptomic dataset not released yet but should be available at https://bigd.big.ac.cn/gsa project number PRJCA002273.
- Using CIBERSORT to get estimates of proportions of cell types from transcriptomic data.

Limitations:

- Small number of SARS-CoV-2 patients sequenced and dataset not released yet.
- Transcriptomic changes need to be validated at the protein level.

STAT2 signalling as double-edged sword restricting viral dissemination but driving severe pneumonia in SARS-CoV-2 infected hamsters

Robert Boudewijns et al. (bioRxiv), 2020

Link: https://www.biorxiv.org/content/10.1101/2020.04.23.056838v1

Robert Boudewijns *et al*. report productive SARS-CoV-2 infection in the lungs of mice is limited and restricted by early type I interferon responses. In contrast, Syrian hamsters are highly permissive to the virus. STAT2 signalling was demonstrated to play a double – edged role in COVID-19 (restricting systemic viral dissemination but driving severe lung injury) in hamster model. Hamster could be a preferred over mouse as infection model for COVID-19 study (pathogenesis, antiviral or immunomodulators treatment).

Main findings:

- Compare the effects of SARS-CoV-2 infection in wild type (WT) mice of different lineages (BALB/c and C57BL/6), Syrian hamsters, and panel of matched transgenic mouse and hamster strains with a knockout (KO) of key components of adaptive and innate immunity.
- Low level of viral replication and inflammatory response in mice suggests it is a poor model for COVID-19 pathogenesis study or treatment and vaccine efficacy assessment. In contrast, after intranasal inoculation, Syrian hamster model had higher viral load and infectious titers in lung. Lung pathology in this model was shown to be resembled that in humans. Hamster, therefore, could be a good model for COVID-19 study.
- Interferon system restricted SARS-CoV-2 replication showed by transcriptomic analysis in Ifna1$^{-/-}$ mice (mice with a genetic ablation of their type I interferon receptor): upregulated classically enriched antiviral effector molecules (cGAS, Mx1, IFIH1/MDA-5, IRF3, OAS1, OAS3 and PKR/EIF2AK2) and downregulated upstream regulators (STAT1, STAT3 and STING/TMEM173). HCS treatment modulated the observed gene expression patterns (decrease Akt1, DDX58 mRNA levels)
- The increase in viral replication, tempered inflammatory response and lung injury in IL28R-a$^{-/-}$ (lack of type III interferon signalling) hamsters compared to WT reinforces the protective role of type III interferon in respiratory viral infection.
- In Syrian hamsters, STAT2 was demonstrated to play a critical role in mediating antiviral responses and restricting systemic dissemination of SARS-CoV-2, but driving severe pneumonia:
  . STAT2$^{-/-}$ subjects had higher titers of infectious virus in lung, higher viral load in blood and organs (spleen, liver, upper and lower gastrointestinal tract) compared to WT groups.
  . Lung pathology more attenuated in STAT2$^{-/-}$ hamsters compared to IL28R-a$^{-/-}$ subjects. Absence of pneumonia was shown by sensitive micro-CT in STAT2$^{-/-}$ hamsters.
  . Lung matrix metalloprotease (MMP)-9 level elevated in all infected hamsters and inversely correlated with histopathological findings in STAT2$^{-/-}$ ones. IL-6 and IL-10 (negatively regulated by STAT2) levels increased in lung of infected STAT2$^{-/-}$ and IL28R-a$^{-/-}$ hamsters.
  . No significant elevation of serum cytokines (IL-6, IL-10 and IFN γ) in infected hamsters.

Highlights

- Suggest hamster could be a preferred above mouse as infection model for COVID-19 study.
- STAT2 play a dual role in limiting viral dissemination but driving severe pneumonia in COVID-19.

Clinical Impact:

- Minimal

Important Methodologies:

- SARS-CoV-2 strain recovered from a nasopharyngeal swab taken from a symptomatic patient returning from Wuhan, China, isolated and passaged on HuH7 and Vero-E6 cells were used
- Lung tissues were fixed in 4% formaldehyde and embedded in paraffin then stained with haematoxylin and eosin to visualise and score lung damage.
- Characterization of plasma and lung cytokines by ELISA; MMP-9 quantification by gelatine zymography.
- Micro-computed tomography and image analysis with DataViewer and CTan software.
- Others: RT-qPCR, differential gene expression and bioinformatics analysis

Limitations:

- Being not homogeneous in age of hamster groups makes the results could be affected by baseline differences.

COVID-19 patients exhibit less pronounced immune suppression compared with bacterial septic shock patients

Kox *et al.* (medRxiv), 2020
Link: https://www.medrxiv.org/content/10.1101/2020.04.03.20049080v3

Summary (100 words max):
A previous study reported that 16% COVID-19 patients who died had developed secondary infections. Additionally, studies indicate that COVID-19 may be associated with a hyperinflammatory "cytokine storm". Based on this information, this study assessed HLA-DR expression levels of 24 COVID-19 patients admitted to ICU. Reduction of HLA-DR expression is a common marker for innate immune suppression and a positive identifier of sepsis. Nevertheless, despite reduced levels of HLA-DR in patients compared to healthy subjects, the extent of immune suppression was milder compared to sepsis.

Main findings:

- COVID-19 was confirmed by RT-PCR for SARS-CoV-2 and by CT-scan
- **COVID-19 patients displayed lower HLA-DR expression (11860 mAb/cell) compared to healthy subjects (15000-45000 mAb/cell); reduced levels were not as extensive as sepsis patients (5211 mAb/cell).**
- Circulating C-reactive protein levels were found to be decreased in COVID-19 patients.
- No differences were observed in procalcitonin, leukocytes or ferritin levels
- **None of the patients developed a secondary infection**

Highlights

- Offers evidence that the risk of secondary infection due to suppressed innate immune response is unlikely in COVID-19 patients.

Clinical Impact:

- Moderate

Important Methodologies:

- Expression levels of HLA-DR were determined by Anti-HLA-DR-Monocyte Quantibrite assay (BD Biosciences) – *on a Navios flow cytometer and software (Beckman Coulter).*
- mAb/cell were quantified using a standard curve constructed with Quantibrite phycoerythrin beads (BD Biosciences)

Limitations:

- Patient cohort was restricted to a limited age range and does not show any observations of younger age groups
- A large majority of the patients suffered from comorbidities at the time of the study. The fact that these comorbidities may be affecting the innate immune system cannot be ignored and this may be skewing the results obtained.
- 17% of the patients were immunocompromised – chronic use of immunosuppressive medication could also affect the results obtained.
- HLA-DR levels at an earlier time-point than day 11 would be interesting
- The n number in sepsis was 10x higher than that of COVID-19. More data would be more a better comparison.

Persistent SARS-CoV-2 presence is companied with defects in adaptive immune system in non-severe COVID-19 patients

Bing Liu *MedRxiv preprint server* (2020)
Link: https://www.medrxiv.org/content/10.1101/2020.03.26.20044768v1

Bing Liu *et al.* analysed inflammatory markers and lymphocyte subsets in patients who were still shedding SAR2-CoV-2 after 20 days, despite nearly complete resolution of symptoms (designated PP), and followed them as they either recovered (PPN) or remained persistent shedders of virus (PPP). Compared with newly-admitted, symptomatic patients (PA), PP had much improved peripheral blood parameters, comparable to healthy controls. PPP had markedly reduced B and T cells compared to PPN and healthy subjects. Paired results from 10 PP who converted to PPN demonstrated that the number of T and B cells significantly increased when the SARS-CoV-2 tests turned negative.

Main findings:

- Patients were selected on the basis of non-severe disease. PP patients were still shedding SAR2-CoV-2 after 20 days, despite nearly complete resolution of symptoms. After at least seven days, PP patients were retested for SAR2-CoV-2 and divided into

PPN, who were negative for SAR2-CoV-2 by PCR, and PPP who remained positive. PA refers to a separate cohort of patients, sampled upon admission to hospital with active symptoms of non-severe COVID-19.

- PP patients had much improved laboratory findings than PA patients, even though they had persistent SARS-CoV-2 shedding.
- This included counts of WBCs, neutrophils, lymphocytes (CD3+ T cells, CD4+ T cells, and NK cells), neutrophil-to-lymphocyte ratio, and levels of albumin, AST, CRP, SAA, and IL-6.
- The absolute numbers of CD3+ T cells, CD4+ T cells, and NK cells were significantly higher in the PP group than that in the PA group, and were comparable to that in healthy controls.
- The PPP subgroup had markedly reduced B cells and T cells compared to the PPN group and healthy subjects.
- PPP patients had significantly lower numbers of CD3+ T cells, CD4+ T cells, CD8+ T cells and B cells, but higher proportion of NK cells than PPN patients
- Paired results from 10 PP to PPN patients demonstrated that the number of CD3+ T cells, CD4+ T cells, CD8+ T cells and B cells significantly increased when the SARS-CoV-2 tests turned negative.

Highlights:

- Persistent SARS-CoV-2 presence in non-severe COVID-19 patients is associated with reduced numbers of adaptive immune cells.
- Lymphocyte counts in individual patients reflect the point at which they clear virus.

Clinical Impact:

- Monitoring lymphocyte subpopulations could confirm fully recovered COVID-19 patients and identify patients that are still infectious but have a false negative PCR test

Important Methodologies:

- Very simple lymphocyte counts can be used to monitor disease resolution

Limitations:

- Informed consent was waived by the hospital ethics committee
- Baseline data from time of admission to hospital was not available, so instead other patients were used as a baseline control (PA group)
- Narrative of paper is difficult to follow

Structure-based modelling of SARS-CoV-2 peptide/HLA-A02 antigens

Nerli *et al*. bioRxiv (preprint) March 2020

Link: https://doi.org/10.1101/2020.03.23.004176

Nerli *et al*. used their own computational platform, RosettaMHC, to model 9-mer and 10-mer SARS-CoV-2 peptide epitopes on HLA-A*02:01. They modelled 439 9-mer and 279 10-mer predicted epitopes on HLA-A*02:01 and made these publicly available on an online database (https://rosettamhc.chemistry.ucsc.edu). RosettaMHC and NetMHCpan were used to predict strong and weak binders. This methodology could be used to understand the link between peptide immunogenicity and the peptide/HLA complex structure in SARS-CoV-2 infection.

Highlights

1. Modelled binding of predicted 9-mer and 10-mer peptide epitopes from SARS-CoV-2 to HLA-A*02:01
2. Used RosettaMHC and NetMHCpan to predict strong and weak binders
3. Made their models publicly available

Clinical Impact? Low

Important Methodologies?

1. Identified all possible 9-mer and 10-mer peptides from SARS-CoV-2 protein sequences using NCBI.
2. Used NetMHCpan-4.0 to derive binding scores to HLA-A*02:01 – authors kept only peptides classified as strong or weak binders
3. Used PDB to identify HLA-A*02:01 structures to be used as templates for modelling – only kept structures below 3.5 A resolution and those with 100% identify to the HLA-A*02:01 heavy chain sequence
4. RosettaMHC is a comparative modelling approach which leverages existing high-resolution X-ray structures from already available pMHC complexes to create realistic 3D models for high affinity SARS-CoV-2 epitopes on HLA-A*02:01.

Limitations?

This is not peer reviewed.
A manuscript is in preparation describing the RosettaMHC methodology, therefore a detailed description of the modelling is lacking in this paper.
Further investigation needed to determine the relevance of the 9- and 10-mer epitopes identified.

Increased Expression of CD8 Marker on T-cells in COVID-19 Patients

Ganji *et al*. Blood Cells Mol Dis 2020
Link: https://www.sciencedirect.com/science/article/pii/S1079979620301364?via%3Dihub

Many studies have established that T cells are crucially involved in or affected by the response to SARS-CoV-2. Ganji and colleagues investigated immune cell counts and proportions in COVID19 patients admitted to the ICU, focusing on $CD4^+$ and $CD8^+$ T cell characteristics. While the frequency and ratio of $CD4^+$ and $CD8^+$ T cells were not different to healthy controls, CD8 was upregulated on $CD8^+$ T cells in COVID19 patients compared to controls. However, a larger study which includes phenotyping and functional analysis of $CD8^+$ T cells is warranted to determine the impact of these data.

Main findings

- Decreased total counts of white blood cells, lymphocytes, and platelets in COVID19 patients compared to healthy controls. While lymphocytopenia is very common in

COVID19, there isn't a consistent trend in white blood cell counts particularly since some studies report total WBC and others analyse specific subsets.
- No significant difference in frequency of $CD8^+$ and $CD4^+$ T cells in COVID19 patients compared to healthy individuals.
- CD4:CD8 ratio in COVID19 patients not significantly different from healthy donors. This is in line with some recent studies (Hou et al., 2020; Wang et al., 2020) however a number of preprints have reported an increased CD4:CD8 ratio (Anft et al., 2020; Weiskopf et al., 2020).

Highlights
CD8 expression was significantly increased, in terms of MFI, on $CD8^+$ T cells of COVID19 patients compared to healthy donors. The authors suggest that since the CD4:CD8 T cell ratio is not altered, CD8 upregulation could represent a mechanism of the cytotoxic T cell response to SARS-CoV-2, instead of proliferation.

Clinical Impact
Limited
Important Methodologies
- Automated haematology counter to assess white blood cell, lymphocyte, and platelet count.
- Flow cytometry (CD4 and CD8 T cells).

Limitations
- No other phenotyping/ functional analysis of the T cells was performed so there is no other indication of the activation/ exhaustion state or phenotype of the CD8 high T cells.
- No indications of when in the clinical course that the analysis was carried out.
- No isotype control is shown for either of COVID19 patients or healthy controls and the CD8 levels weren't assessed by any other methods.
- Small scale; 25 ICU patients and 25 healthy individuals of a similar age and sex. However no descriptive statistics of the patient and healthy control data are included.
- Patients who died of COVID19 were excluded from the study: it is not clear whether any measurements occurred before this outcome. Since reduced T cell numbers in COVID19 cases of increasing severity, including death, have previously been reported (Diao et al., 2020), it would have been useful to clarify/ include this readout.

Antibody responses to SARS-CoV-2 in patients with COVID-19

Quan-Xin et al. (Nature), 2020
Link: https://www.nature.com/articles/s41591-020-0897-1.pdf

Quan-Xin examined antibody responses to SARS-CoV-2 in 285 patients with COVID-19 and showed that 100% of patients tested positive for anti-viral IgG within 19 days of symptoms onset. Seroconversion for IgG and IgM occurred either sequentially or simultaneously. Both

IgG and IgM titers plateaued within 6 days after seroconversion. Serological testing may identify COVID-19 infected individuals that are asymptomatic and those who test negative for RT-PCR.

Main findings:

- Serum samples showed no cross-binding to the S1 subunit of the SARS-CoV spike antigen but did cross-react with nucleocapsid antigens of SARS-CoV
- Positive virus-specific IgG reached 100% approximately 17–19 days after symptom onset
- Positive virus-specific IgM reached peak of 94.1% approximately 20–22 days after symptom onset
- 13 days post symptom onset was the median day of seroconversion for both IgG and IgM
- 3 modes of seroconversion were identified including synchronous IgG and IgM seroconversion, IgM earlier than IgG and IgG earlier than IgM seroconversion
- IgG and IgM plateaued 6 days after the first positive measurement
- Plateau IgG levels varied widely (more than 20-fold) across patients
- No association between plateau IgG levels and the clinical characteristics of the patients
- 70.7% (29/41) of patients with COVID-19 met the WHO criteria for MERS-COV infection diagnosis of seroconversion and/or fourfold increase or greater in the IgG titers
- Early collection of serum samples is important for some patients to be diagnosed as 12.2% (5/41 patients) had already plateaued in IgG titer within 7 days of symptom onset
- Serology testing applied to 164 close contacts of COVID-19 patients
- 16 RT-PCR positive tested 100% for IgG and/or IgM
- 10 close contacts of COVID-19 patients who were asymptomatic also tested positive
- 7 close contacts who had negative RT-PCR results tested positive for IgG and/or IgM

Highlights

- Seven asymptomatic patients showed positive IgG and/or IgM even though RT-PCR negative
- This highlights importance of serological testing to achieve more accurate estimates of the extent of the COVID-19 pandemic

Clinical Impact:

- Medium

Important Methodologies:

- Magnetic chemiluminescence enzyme (MCLIA) for virus-specific antibody detection

Limitations:

- Difficult to determine the association between antibody response and clinical course due to limited number of patients in critical or severe condition analysed

- Did not test samples for virus neutralization and therefore the neutralizing activities of the detected IgG antibodies are unknown.

IgA-Ab response to spike glycoprotein of SARS-CoV-2 in patients with COVID-19: A longitudinal study

Andrea Padoan. (Clinica Chimica Acta), 2020
Link: https://www.sciencedirect.com/science/article/pii/S0009898120301819?via%3Dihub

The authors explore the kinetics of IgM and IgA antibodies to SARS-CoV-2 spike protein to aid investigations into antibody response and seroconversion in patients with rRT-PCR confirmed COVID-19 infection. They show that the IgA response increases early and peaks at 20-22 days. IgM levels peak at 10-12 days and significantly decline after 18 days. The IgA response is stronger and more persistent than the IgM response.

Main findings:

- Average levels of both antibodies increased from day 0 to day 6-8 (Day 0 = onset of symptoms (fever))
- IgA showed persistently higher levels compared to IgM, peaking at 20-22 days
- IgM levels peaked at 10-12 days and significantly declined after 18 days
- An IgA response to the S protein was detectable at week 1 in 75% of patients
- The values of IgG were also measured and were comparable to previous referenced study

Highlights

- Determining successful ways of measuring antibodies in the blood of patients will aid the production of vaccines, epidemiological studies and facilitate the identification of infected patients
- IgA response appears early and peaks at week 3

Clinical Impact:

- Minimal

Important Methodologies:

- Chemiluminescence immunoassay (CLIA) measuring SARS-CoV-2 specific IgM and IgG antibodies
- ELISA measuring SARS-CoV-2 specific IgG and IgA antibodies

Limitations:

- Only COVID-19 positive adults in study – no one under the age of 22 and no healthy controls
- Different numbers of samples are measured from IgA and IgM methods, indicating that they are not always measured from the same patient

- Different numbers of samples are measured from each time point, indicating the samples might not be taken from the same patients throughout the time course. Authors state the average follow up time for IgA was 7.5 days (SD 4.9) and for IgM was 4.6 days (SD 4.0), therefore indicating the patients measured at the beginning, day 0, where not the same patients measured at the end (day 40) - but this is not clear

Mechanisms of Disease

Gut microbiota may underlie the predisposition of healthy individuals to COVID-19

Gou et al. (Preprint), 2020
Link: https://www.medrxiv.org/content/10.1101/2020.04.22.20076091v1

The gut is indicated in COVID-19 as 60% of patients report GI symptoms; with these patients having more severe disease. The gut microbiota was investigated here, to understand its role in COVID-19 severity. A proteomic risk score (PRS) was created from 20 blood proteomic biomarkers, which were found to correlate with proinflammatory cytokines in older individuals. A core set of gut microbiota was found to accurately predict these markers using a machine learning tool. Faecal metabolomic analysis linked amino acid pathways with gut microbiota and inflammation. This suggests gut microbiota may be an underlying cause of normal individuals facing severe COVID-19 infection.

Main findings:

- Proteomic risk score (PRS) was found to correlate positively, with blood inflammatory markers TNFα and hsCRP in healthy individuals. In patients over 58 years old, all 4 inflammatory markers tested correlated positively with PRS score.
- 20 top predictive 'operational taxonomic units' (OTU) were identified in gut microbiota. 11 microbial OTUs were significantly associated with 10 inflammatory cytokines including IFNγ, TNFα, IL-6, IL-1β. 3 microbial species were negatively correlated with most of the inflammatory cytokines tested.
- 45 faecal metabolites relating to amino acids, fatty acids and bile acids showed significant associations with more than half of the microbial OTU's.
- 3 amino acid metabolic pathways were identified from faecal metabolites using metabolic pathway analysis, to elucidate possible biological mechanisms.
- Dairy consumption significantly contributed to the variance of the core OTU composition between individuals, as did a range of demographic/clinical factors.

Highlights

- Use of a 'proteomic risk score' (PRS) for COVID-19.

Clinical Impact:

- Moderate.

Important Methodologies:

- Proteomics.
- Metabolomics.
- LightGBM machine learning-based method.

Limitations:

- Only Chinese individuals tested, may not be applicable to whole population.
-

Reduction of lymphocyte at early stage elevates severity and death risk of COVID-19 patients: a hospital-based case-cohort study

Fei *et al*., medRxiv preprint. Version posted April 6, 2020.
Link: https://doi.org/10.1101/2020.04.02.20050955.

Summary (100 words max):

The authors use 192 PCR+ COVID19 patients to assess the correlation of decreased lymphocyte counts with lung CT scores, biological indices, disease severity and risk of fatality. Using well documented patient characteristics they assess the implication of age, gender, and co-morbidities upon decreased lymphocyte count correlations. The authors find a correlation of decreased lymphocyte count with increasing age (most clear at 70>) and cardiac and pulmonary co-morbidities. Decreased lymphocyte counts also correlate with poorer CT scores, multiple organ injury markers, likelihood of disease severity and fatality.

Main findings:

- 33.5 % of patients had a leukocyte count below the normal range and 7.9 % above the normal range upon admission and age had a significant effect on admission lymphocyte count.
- Decreased lymphocyte % correlates with age, cardiac and pulmonary disease.
- Patients with lymphocyte reduction had significantly lower lung CT scores (right, left and bilateral) but correlation data is quite spread.
- In patients with lymphocyte reduction, markers associated with multiple organ injury are present.
- More likely to be severely, or critically ill upon admission if lymphocyte count is reduced as well as an elevation in fatality rate by 26.54 %.

Highlights

- Importantly, the authors show counts for all major subsets of white blood cell upon admission. This will be importantly for future retrospective meta-analyses.
- Patients admitted with severe disease are more likely to have lower lymphocyte counts.

- Decreased lymphocyte counts correlate with severe disease in agreement with other medical pre-prints some of which establish that leukocyte counts can be used as predictors of patients likely to require ICU care.

Clinical Impact:

- Moderate to high. Patients presenting with low leukocyte and lymphocyte counts have a greater disposition to severe disease and a fatal outcome.

Important Methodologies:

- 192 PCR+ COVD19 patients (49.5 % M 50.5 % F, 22 – 87 years of age)
    - CT Image findings from two independent radiologists where scans were divided into individual lungs with upper, middle, and lower sections each scored according a points-based system of disease severity (1 to 4; increasing severity). Cumulative scoring provides each patient's scan a score out of 24.
    - Demographic data: gender, age, smoking history, exposure history.
    - Co-morbidities: cardiovascular disease, hypertension, chronic pulmonary disease, hepatic disease, diabetes, amongst others.
    - Symptoms: cough, fever, fatigue, diarrhoea
    - Biochemical indexes: routine blood including but not limited to O2 saturation, partial pressure of 02 and C02, fraction of FiO2, oxygenation index, total and direct bilirubin.

Limitations:

- Inconsistencies in the age groups referred to in the text and those presented in table 1. The only p values below 0.05 are smoking and age but the relevant text is unclearly written. Contradicting, in table 2 and 3 smoking is stated as having no correlation with lymphocyte count.
- The authors use both lymphocyte counts and lymphocyte % in tables 1 and 2 but do not define to which the lymphocyte reduction values in table 4 refer to.

Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: A report of five cases

Magro C. Translational Research Journal pre-proof
Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7158248/

A subset of severe COVID-19 patients present with delayed respiratory distress. They then manifest preserved lung function despite severely reduced O_2 levels in blood requiring prolonged ventilation. Magro et al., examined skin and lung tissue from 5 patients with severe COVID-19 (5 with respiratory failure and 3 with signs of microvascular thrombosis). They observed striking septal capillary injury with complement deposition in lungs and complement mediated microthrombiotic disease in skin. Complement proteins C4d and C5b-9 were shown to co-localise with COVID-19 spike glycoproteins in inter-alveolar septa and skin microvasculature in two cases.

Main findings:

- Severe- COVID-19 clinical features are distinct from classic ARDS (acute respiratory distress syndrome).
- Pulmonary abnormalities largely restricted to alveolar capillaries
- Some patients present with generalised thrombotic microvascular injury
- Significant complement deposition of terminal components C5b-9 (membrane attack complex), C4d and MASP2 in lung
- Deposition of C5b-9 and C4d in purpuric and grossly normal skin.
- C4d and C5b-9 co-localise with COVID-19 spike glycoproteins

Highlights

- Deposition of C5b-9, C4d and MASP2 in the microvasculature of lung and skin
- A subset of sustained, severe COVID-19 may be defined by a type of microvascular injury syndrome brought about by complement activation.

Clinical Impact:

- Moderate- small subset of cases but paves the way for further exploration of complement in COVID-19

Important Methodologies:

- Skin and lung tissue from 5 patients with respiratory failure (3 with purpuric skin rash).
- Wide range of age and degree of pre-existing immune suppression
- IHC for deposition of C5b-9 (membrane attack complex), C3d, C4d
- IHC for SARS-CoV-2 spike and envelope proteins
- Haematoxylin and eosin staining

Limitations:

- Five patients
- No control data shown
- Figures mislabelled
- Complement in serum measured in only one patient