Covid-19 Community Journal Club
April 15th, 2020

School of Medicine, Cardiff University

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

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

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

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

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

Table of Contents Page

Clinical

Transmission

Diagnostics and Therapeutics

Innate and Adaptive Immune Responses

Diao *et al*., Unpublished. Available on BioXRiv 2020.
Link: https://www.medrxiv.org/content/10.1101/2020.02.18.20024364v1.full.pdf

Multi-epitope vaccine design using an immunoinformatics approach for 2019 novel coronavirus in China (SARS-CoV-2) ***29***
Ye Fenget *et al*. (Unpublished, bioRxiv), 2020
Link: https://www.biorxiv.org/content/10.1101/2020.03.03.962332v1.full

Immuno-Pathogenesis

Highly pathogenic coronavirus N protein aggravates lung injury by MASP-2 mediated complement over-activation ***30***
Gao *et al*./preprint on medRxiv/2020
Link: https://www.medrxiv.org/content/10.1101/2020.03.29.20041962v2.full.pdf

Immunopathological characteristics of coronavirus disease 2019 cases in Guangzhou, China ***31***
Yaling Shi *et al*. (Medrxiv), 2020
Link: https://www.medrxiv.org/content/10.1101/2020.03.12.20034736v1

The role of IL-6 in monitoring severe case of coronavirus disease 2019 ***32***
Tao Liu *et al*. (Medrxiv), 2020
Link:https://www.medrxiv.org/content/10.1101/2020.03.01.20029769v2#Sec3

Virology

LY6E impairs coronavirus fusion and confers immune control of viral disease ***33***
Pfaender *et al*., 2020
Link: https://www.biorxiv.org/content/10.1101/2020.03.05.979260v1

Structure, Function, and Antigenicity of the SARSCoV-2 Spike Glycoprotein ***34***
Walls *et al*. (Cell), 2020
Link: https://www.sciencedirect.com/science/article/pii/S0092867420302622

SARS-CoV-2 Cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor ***36***
Hoffmann *et al*. (Cell 181, 1-10), 2020
Link: https://www.sciencedirect.com/science/article/pii/S0092867420302294

Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. ***37***
Xiuyuan Ou *et al*. (Nature Communications), 2020
Link: https://www.nature.com/articles/s41467-020-15562-9

Vaccines

Sources of further information:

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

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

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

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

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

Clinical Characteristics of Coronavirus Disease 2019 in China
Guan *et al*. (NEJM), 2020
Link: https://www.nejm.org/doi/full/10.1056/NEJMoa2002032

Summary (100 words max):
This paper summarized the characteristics of a selected cohort of 1099 patients with COVID-19 infection, from different regions in China, including the demographic, the most common symptoms, length of incubation periods, and finding in test. (I guess in clinical setting, the data may be more important than conclusion, so I listed in main findings).

Main findings:

- **Most patients had an incubation time less than a week**: The median incubation period was 4 days (interquartile range, 2 to 7).
- **People of all ages can get infected**: The median age of the patients was 47 years (interquartile range, 35 to 58). 0.9% of the patients were younger than 15 years of age. But patients with severe cases are older (median=7 years) than non-server patients).
- Very few patients (2%) had direct contact with wildlife. Three quarters were Wuhan citizens or contact history. Outbreak of a family cluster and transmission from an asymptomatic patient were also observed.
- This paper did not list all possible approaches of transmission, but mentioned SARS-CoV-2 can be detected in the gastrointestinal tract, saliva, and urine.
- **Common symptom**: Fever (initial 43.8% and 88.7% for hospitalized patients), cough (67.8%), fatigue (38.1%), sputum production (33.7%), shortness of breath (18.7%), muscle or joint pain (14.9%), sore throat (13.9%), headache (13.6%), chills (11.5%), nausea or vomiting (5.0%), nasal congestion (4.8%), diarrhea (3.8%), haemoptysis (0.9%), conjunctival congestion (0.8%).
- **Coexisting illness rate**: total 23.7%, HT (15%), diabetes (7.4%), CHD (2.5%), HBV infection (2.1%), cerebrovascular disease (1.4%), COPD (1.1%), Cancer (0.9%), Chronic rental disease (0.7%), immunodeficiency (0.2%). the presence of any coexisting illness was more common among patients with severe disease than among those with nonsevere disease (38.7% vs. 21.0%).
- **Radiologic findings:** CT scan: The most common patterns on chest CT were ground-glass opacity (56.4%) and bilateral patchy shadowing (51.8%), whilst 17.9% patients with nonsevere disease and 2.9% with severe diseases had no abnormal radiologic finding.
- **Lab findings**: at admission, Lymphocytopenia (83.2%), thrombocytopenia (36.2%), leukopenia (33.7%). Increased CRP, ALT, AST, CK, D-dimmer were seen, although less common.
- **A primary composite end-point event** (indicating severity of the disease)
  occurred in 6.1% patients (all patients): 5.0% ICU, 2.3% mechanical ventilation, and 1.4% died.
  Occurred in 24.9% patients if there was an initial severe condition.
- **Treatment patients received**: intravenous antibiotic therapy (58.0%), oseltamivir (35.8%); oxygen therapy (41.3%), mechanical ventilation (6.1% in all patients; 32.4% of patients with severe disease received noninvasive ventilation, while 14.5% received invasive ventilation, 14.5%); systemic glucocorticoids (18.6%). 16.2% of patients were admitted to the ICU, and 2.5% died. Extracorporeal membrane oxygenation was performed in 5 patients (0.5%) with severe disease.
- **The median duration of hospitalization** 12.0 days (mean, 12.8).
- **Inpatient's Complication:** Pneumonia (91.1%), ARDS (3.4%) and shock (1.1%).

- Early isolation, early diagnosis, and early management might have collectively contributed to the reduction in mortality in Guangdong.

Highlights

- It provides information regarding clinical characteristics of COVID-19 patients, and compare the differences between COVID, SARS, MERS and seasonal flu. This information can help in diagnosis, especially when early admission of patients. (The radiological part is interesting)
- It provides a general picture regarding how much percentage of patients may develop to severe cases, and many medical resources and equipment were needed (e.g. 0.5% of severe patients needed ECMO support here). That may help the managers prepare and distribute resources according to local scenarios.
- Some information about the symptoms of this disease, the length of incubation time and the where the virus can be detected. Such information may be helpful to improve prevention.

Clinical Impact:

- Moderate.

Important Methodologies:

- Subject: 1099 Covid-19 patients, including both hospitalized patients and outpatients, in Wuhan (43.9%) or outside Wuhan, infection was confirmed between December 11, 2019, and January 29, 2020; (not random selection)
- Cohort.
- The primary composite end point: admission to an intensive care unit (ICU), the use of mechanical ventilation, or death
- Secondary end points: the rate of death and the time from symptom onset until the composite end point and until each component of the composite end point.
- RT-PCR assay on nasal and pharyngeal swab specimens to determine infection.
- R: for analysis

Limitations:

- Patients were not randomly selected.
- The authors did not categorize the patients to Wuhan and non-Wuhan group. Patients in epicentre may get poorer treatment than others as the medical resource was overwhelmed occupied, and therefore the outcome may be significantly different.
- Some information of patients were incomplete. For example, the incubation period data was based on record of 291 patients. (However, the longest record of incubation period in China is 27 days). Also, Recall bias may be present.
- At time of data cutoff, many people who did not reach end point were still in hospital, their condition might further develop. Hence, the final duration of hospitalization and death toll may be higher.
- The author think their study cohort 'may represent the more severe end of Covid-19', as many mild and asymptomatic cases were treated at home. It is true. However, we should notice this study also included many patients with mild symptoms.
- Treatment options were limited here and this paper did not assess the efficiency of different treatment. For example, I noticed high proportion of patients had been given antibiotics. I'm not sure what kind of antibiotics was provided, and the outcome. Thus, I think this paper has moderate impact to guide clinical treatment.

Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study
Zhou *et al*. The Lancet 2020
Link: https://www.sciencedirect.com/science/article/pii/S0140673620305663

Summary
Zhou et al report the clinical characteristics of 191 patients from 2 hospitals in Wuhan who had been admitted or died between 29th Dec 2019 and 31st Jan 2020. Retrospective cohort with exclusion of 613 patients still admitted, not confirmed CoV2 RNA or with missing data. Demographic, clinical, treatment and lab data were reported. The cohort is separated into survivors and non survivors. Mortality rate for admitted patients was 28.2%. There was a high rate of cardiovascular comorbidites, which were associated with increased mortality. Non-survivors had higher levels and progressive increases in inflammatory markers and shedded virus to death. In survivors median viral shedding was 20 days (max 37 days).

Main findings:
- 54/191 admitted patients died, cohort mean age 56 years 62% male
- Comorbidites were present in ½ of patients hypertension, diabetes and coronary artery disease are the most common
- On admission patients who died had multiple features suggesting a more pronounced inflammatory state (albumin, kidney injury, anaemia, LDH radiology).
- Markers associated with poor outcome at admission- leucocytosis and lymphopaenia inflammatory markers procalcitonin, IL6, ferritin, coagulopathy- prothrombin time, D dimer, cardiac- troponin & CK- continue to worsen during illness in non-survivors
- Illness onset to discharge 22 days (IQR 18-25), to death 18.5 days (IQR 15-22)
- Antiviral immunoglobulin and corticosteroid treatments and Oxygen, Non-invasive mechanical ventilation and mechanical ventilation more commonly given to non-survivors (but this was the sicker group at admission)
- Interplay between inflammation, atherosclerosis +/- angiotensin converting enzyme 2 in the poor outcome with COVID

Highlights
- Defining clinical features of COVID in admitted individuals- *This should be one of the main references for clinical characteristics of COVID at present*

Clinical Impact:
- Massive- informed case definitions of COVID, groups to shield, ICU admission criteria, isolation following discharge criteria and COVID guidelines, new lab tests rolled out for COVID as standard practice (procalcitonin quantiative D dimer)

Important Methodologies:
- Retrospective cohort from 2 hospitals combined as a single small cohort (I think the first time the Lancet has published such a cohort following MMR)
- Alternate day throat swabbing for viral shedding

Limitations:
- Early report not prospectively gathered Selection bias (admitted sicker patients)
- Not possible to draw meaningful conclusions from treatments given
- PCR positivity does not equate to viable virus

Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study
Tan, L. *et al*. (Signal Transduction and Targeted Therapy), 2020
Link: https://doi.org/10.1101/2020.03.01.20029074

Summary (100 words max): Based on a retrospective study of 162 CVID patients from a local hospital in Wuhan, Tan et al. report an inverse correlation between the dynamic changes of blood lymphocyte percentage (LYM%) and disease severity. The authors analysed the LYM% in 15 death cases, 15 severe cases as well as 40 moderate cases of COVID-19 patients. A Time-LYM% model (TLM) was established according to the descriptive studies and was validated in 92 hospitalized cases.

The authors determined that Lymphopenia can be used as an indicator of disease severity and prognosis of COVID-19 patients, and that TLM is worth of application in the clinical practice.

Main findings:

- Results from death and severe cases showed that LYM% in blood test were inversely associated with the progression and severity of COVID-19.
- LYM% in patients with moderate COVID-19 remained higher than 20% 10-12 days after symptom onset. In contrast, LYM% was lower than 20% in severe cases.
- LYM% in severe cases was higher than 5% 17-19 days after the onset of the disease, while it fell below 5% in death cases.

Highlights

- The paper is of limited significance as it is reporting what has already been described by other papers, that severe COVID cases are characterised by lymphopenia.

Clinical Impact:

- Minimal

Important Methodologies:

- The data from routine examinations performed on patients was used: complete blood count, coagulation profile, and serum biochemical test (including renal and liver function, creatine kinase, lactate dehydrogenase, and electrolytes)

Limitations:

- In order for the model described to be of use it would need to be validated on a larger dataset.
- There should be a mechanistic study done on how COVID leads to lymphopenia.

Will children reveal their secret? The coronavirus dilemma
Luca Cristiani/ Editorial in Eur Respir J/ 2020; in press
Link: https://erj.ersjournals.com/content/early/2020/03/26/13993003.00749-2020

Summary: Reports of SARS-CoV-2 infections in children (<19 years) are below 1% in China (of 44,672 confirmed cases) and Italy (of 62,844 cases) and mortality is extremely rare. Some studies suggest increased vulnerability in infants (< 1 year), but data is confounded by inconsistent testing. Mild disease in children may result from higher constitutive ACE2 expression, as well as raised lymphocyte counts and active immune status engendered by childhood vaccination programs and frequent infections. Declining immune function and reduced expression of ACE2 and steroid hormones in chronic disease and ageing may underpin the more severe pathologies observed in infected adults.

Main findings: This is a review editorial and so there are no primary data.
The report compares confirmed SARS-CoV-2 infections in China and Italy to determine a disproportionately low incidence of severe disease and death amongst children (<19 years) in both countries, despite the more usual susceptibility of children to other respiratory infections.
The authors speculate that reduced disease severity is linked to higher ACE2 expression in children (predicted from studies of expression in ageing rats and a preprints paper extracting RNA expression data from the GTEx Portal) since lower ACE2 in adults is associated with hypertension, chronic heart failure and lung injury; pathologies implicated in SARS-CoV-2-induced mortality. The authors also propose the more active immune status of children (following repeated childhood infections and immunisations) provides a more effective defence against SARS-CoV-2. Whilst severely affected adults demonstrate raised neutrophil and suppressed lymphocyte counts following infection, children maintain high lymphocyte counts, allowing effective progression from the innate immune response (that can result in a detrimental cytokine storm with sustained activation in adults) to an adaptive response with resolution of inflammation. Infants may be less protected owing to a lower immune experience than older children.

Highlights: Summary of potential mechanisms by which children may be protected from the most serious clinical consequences of SARS-CoV-2 infection.

Clinical Impact: Minimal. No primary data or new statistics or novel observations/conclusions.

Important Methodologies: None. An editorial article.

Limitations: Review of clinical data from China and Italy without any comparison with datasets from other countries or previous CoV outbreaks.
Acknowledgement of the contribution of inconsistent testing and recognition of disease in children as a source of conflicting reports.
Admission that low incidence of childhood infection may preclude immunological exploration at scale.
Some extrapolation from animal models (rat ACE2 expression) and non-peer-reviewed preprints (Chen J. et al 2020) that may be unfounded. No experimental validation of theoretical hypotheses.

Other Related Studies of Covid-19 in children:
Yang P (J Infect. 2020 Mar 3) paper described only 416 children with confirmed SARS-CoV-2 infection in China (72,436 total cases). 76.1% had fever, 70.4% had evidence of infection by chest imaging. Most common presentations were fever, cough, vomiting, diarrhoea and digestive symptoms. Critical illness was only reported with underlying conditions. Neonatal infection was reported as possible.

Lu X (NEJM letter 2020 Mar 18) revealed <1% 72,314 cases in China were in children under 10 years. Asymptomatic infection was not uncommon, but 7% infected children had radiologic features of pneumonia, despite being asymptomatic.
This suggests infection rates in children may be severely under-reported?

Multiple reports (**e.g. Xing Y-H J. Microb. Imm. Infect. 2020 Mar 8**) indicate prolonged viral shedding into stool samples of children. Viral load could be detected > 4 weeks post-infection in stool samples, compared with ~ 2 weeks post-infection in the respiratory tract.

This has implications for prolonged infection risk via faecal contamination and raises questions concerning the care and quarantine of children and the decontamination of sewerage and water supplies. Faecal samples may be useful clinical samples? These combined data also suggest that GI infection and symptoms may be more prevalent amongst children (linked to differential ACE2 expression in GI tract?), allowing improved recognition and diagnosis amongst this group?

The knowledge that MERS-CoV enters human cells via DPP4 and the SARS-CoV enter via ACE2 may suggest differential consequences of infection based on the predominance of cells expressing these membrane proteins in children versus adults? DPP4 is a marker of senescent cells and ACE2 is expressed most highly on type II alveolar cells (AT2) that are increasingly senescent in fibrotic lung disease. SARS-CoV infection of senescent cells with active cytokine and chemokine expression (SASP) might exacerbate the pathology in adults and limit effective immune response. In children, the presence of senescent cells in lung tissue is rare without underlying chronic disease and hence viral infection would more likely provoke an effective immune response, resulting in clearance and resolution of inflammation? It would be interesting to compare the immunological consequences of infection of senescent versus proliferative alveolar cells?

Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia
Qun *et al*. (The New England Journal of Medicine), 2020
Link: https://www.ncbi.nlm.nih.gov/pubmed/31995857

Summary (100 words max):
This paper analysed data from the first 425 confirmed cases of Covid-19 in Wuhan from December 2019 to January 2020. Using epidemiological techniques, they found that the median age was 59 years and 56% were male. 55% of cases were linked to the Huanan Seafood Wholesale Market. The mean incubation period was 5.2 days. In conclusion, this paper primarily showed evidence for human-to-human transmission.

Main findings:
- The patients with earlier onset were slightly younger, more likely to be male, and much more likely to have been in contact with the Huanan Seafood Wholesale Market.
- The R_0 for Covid-19 was estimated at 2.2, meaning that on average each patient was spreading the infection to 2.2 other people.
- The duration from illness onset, before January 1st, to first medical visit for 45 patients was 5.8 days.

Highlights:
- The R_0 for Covid-19 is less than that of SARS, R_0 of 3.
- A case was reported in an individual of 15 years of age.

Clinical Impact:
- Minimal

Important Methodologies:
- R_0 calculation.

Limitations:
- Small number of patients.
- Due to the novelty of the virus the classification of the symptoms of the virus may not be completely accurate.

Potent Antiviral Activities of Type I Interferons to SARS-CoV-2 Infection
Mantlo *et al*. bioRxiv preprint
Link: https://doi.org/10.1101/2020.04.02.022764.

Summary (100 words max):
Mantlo et al uses a simple *in vitro* assay to demonstrate that SARS-CoV-2 replication can be inhibited by treatment with recombinant human interferon α or β at clinically tolerable doses.

Main findings:
- In SARS-CoV-2 infected Vero cells reach a peak viral titer 24hrs after infection (see limitation below).
- Pre-treatment of Vero cells with either recombinant IFNα or IFNβ resulted in a significant reduction of viral titers.
- IFNα treatment resulted in an EC50 of 1.35IU/ml, IFNα/β treatment resulted in an EC50 of 0.76IU/ml

Highlights
- Suggests that unlike other pathogenic viral strains including SARS-CoV, SARS-CoV-2 maybe unique in its susceptibility of IFNα/ β treatment of patients

Clinical Impact:
- Minimal

Important Methodologies:
- Viral growth in Vero cells for TCID50 and CPE (data not shown in paper for the latter)

Limitations:
- Study is limited in its methodology and draws some large conclusions from very limited data.
- Discussion of results does not accurately reflect the data presented, with clear discrepancies in the data shown in figures 2 and 3.
- Data related to the CPE of the virus is discussed but no data shown.
- Viral growth curves only performed in Vero cells, with only 4 time points between 0 – 48hours, impossible to be sure that the peak of infection is at 24hrs with the data presented.
- IFNα/β treatment only given to Vero cells which are not able to produce IFNα/β . Should have been repeated in a cell line that produces IFNα/ β to determine if addition of IFNα/β has an effect on viral titers and would therefore be of clinical benefit.

Coronavirus Disease 2019: Coronaviruses and Blood Safety
Chang *et al*., (Transfusion Medicine Reviews), 2020
Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7135848/pdf/main.pdf

Summary (100 words max):
Chang *et a*l., review the current evidence and understanding of the potential transmission of human endemic coronavirus infections via blood transfusion: Severe Acute Respiratory Syndrome (SARS-CoV), Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and the current pandemic Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) causing COVID-19. Studies report viral RNA detection from plasma or serum of patients infected with SARS-CoV, MERS-CoV or SARS-CoV-2 during different periods after the onset of symptoms. Emerging evidence suggests a number of COVID-19 patients are asymptomatic, highlighting a theoretical risk for transmission through blood transfusion.

Main findings:
- Viral RNA detected in plasma and lymphocytes of SARS-CoV patients indicating the potential for transmission through blood products. No nation, organisation (WHO or American Association of Blood Banks) recommended screening for SARS-CoV in donors. SARS-CoV antibodies discovered in healthy blood donors and non-pneumonic paediatric patients during SARS outbreak
- Viral RNA detected in the serum of MERS patients although no virus was isolated. Not known whether live MERS virus was in the serum, so patients` blood may have been non-infectious
- Low viral RNA detected in plasma or serum of COVID-19 patients from days 2-3 after onset of symptoms with no significant difference between intensive care patients and patients with mild symptoms
- Younger adults who can donate blood tend to have milder COVID-19 symptoms than older adults
- Rate of infectivity of COVID-19 patients during incubation period remains uncertain

Highlights:
Emphasises the need for data on viral load in plasma, serum and lymphocytes of COVID-19 patients with mild disease for future screening of blood donors

Clinical Impact:
High

Important Methodologies:
- Patients with no fever and asymptomatic carriers identified in China, increasing the possibility that COVID-19 patients or virus carriers could donate blood
- Not known whether recommendations for transfusion deferral implemented during SARS-CoV and MERS outbreaks are sufficient for SARS-CoV-2

Limitations:
Not clear whether the data obtained from SARS-CoV, MERS-CoV or SARS-CoV-2 infections were solely from the Chinese population. This may have implications for other population groups.

Detection of SARS-CoV-2 in Different Types of Clinical Specimens
Wang *et a*l., (Journal of the American Medical Association) 2020
Link: https://www.ncbi.nlm.nih.gov/pubmed/32159775

Summary (100 words max):
Wang *et al.,* characterised SARS-CoV-2 RNA from multiple clinical isolates collected from 205 patients with COVID-19. As clinical diagnosis is normally confirmed by rRT-PCR of nasopharyngeal swabs, whether viral RNA can be detected in other tissues that present a potential route of transmission is unknown. This study collected pharyngeal swabs from patients at day 1 to 3 after hospital admission. Blood, sputum, faeces, urine, and nasal samples were collected throughout the illness. SARS-CoV-2 was most often detected in the lower respiratory tract but also in the faeces of a small number of patients, indicating potential transmission via the faecal route.

Main findings:
- Bronchoalveolar lavage fluid specimens showed the highest positive rates (93%), followed by sputum (72%), nasal swabs (63%), fibrobronchoscope brush biopsy (46%), pharyngeal swabs (32%), faeces (29%) and blood (1%). None of the urine specimens tested positive

- Of the 26 patients that had multiple specimens collected, viral RNA was detected in single specimens from 6 patients (respiratory specimens, faeces, or blood), while 7 patients excreted virus in respiratory tract specimens and in faeces (n=5) or blood (n=2).

- 1% of blood samples had positive PCR test results, suggesting that infection sometimes may be systemic

- Transmission of the virus by respiratory and extra-respiratory routes may help explain the rapid spread of disease

Highlights:
- Testing of multiple tissues may improve the sensitivity and reduce false-negative test results

Clinical Impact:
Moderate

Important Methodologies:
- Pharyngeal swabs collected at days 1-3 after hospital admission followed with blood, sputum, faeces, urine, and nasal samples collected throughout the illness
- Sampling of viral RNA in bronchoalveolar lavage fluid and fibrobronchoscope brush biopsy from patients with severe illness or undergoing mechanical ventilation

Limitations:
- Lack of detailed clinical information available for some patients, precluding correlation of data with symptoms or disease course
- Small sample size for some tissues

An orally bioavailable broad-spectrum antiviral inhibits SARS-Cov-2 in human airway epithelial cell cultures and multiple coronaviruses in mice
Sheahan *et al*., Science Translational Medicine, 2020
Link: https://stm.sciencemag.org/content/early/2020/04/03/scitranslmed.abb5883

Summary (100 words max):
Sheahan et al. report here the use of a ribonucleoside analog β-D-N4-hydroxycytidine (NHC) in human airway epithelial cell lines and in primary human airway cultures to inhibit multiple coronaviruses. They also report the use of a bioavailable prodrug of NHC, EIDD-2801 in mice. NHC/EIDD-2801 were both found to be potently antiviral against SARS-CoV-2, MERS-CoV, SARS-CoV, multiple Bat-CoVs, and even against a CoV strain with resistance to the nucleoside analog inhibitor remdesivir. This study also supports the existing data suggesting the mode of action of NHC/EIDD-2801 to be through lethal mutagenesis in viral genomic RNA.

Main findings:
- NHC potently inhibited MERS-CoV infection in Calu3-2B4 cells, determined by quantification of reporter gene NanoLuciferase
- NHC inhibited SARS-CoV-2 infection in Vero cells and Calu3-2B4 cells, determined by plaque assay and quantification of viral genomic RNA by qRT-PCR
- NHC is active against MERS-CoV, SARS-CoV and SARS-CoV-2 in primary human epithelial cell cultures (HAEs) without cytotoxicity, determined by plaque assay and CellTitre Glo assay
- NHC is also active against Bat-CoVs in HAEs, determined by plaque assay suggesting emerging zoonotic CoVs may be susceptible to NHC treatment
- Prophylactic orally administerd EIDD-2801 was actively antiviral and prevented SARS-CoV replication and disease progression in C57BL/6 mice
- Therapeutic EIDD-2801 potently antiviral against SARS-CoV in vivo but degree of benefit dependent on time of initiation post infection
- NHC and prodrug EIDD-2801 drive mutagenesis in viral genomic RNA (but not host cell RNA) and this correlates with reduced viral load, supportive of lethal mutagenesis mode of action

Highlights
- Provides pre-clinical data for testing in non-human primate models and human clinical trials

Clinical Impact:
- Limited

Important Methodologies:
- CoV infectivity in vitro assessed by nanoluciferase quantification and plaque assay
- Primer ID NGS to identify RT-PCR mutations
- CoV infectivity in vivo assessed by monitoring body weight, pulmonary function by whole body plethysmography and lung haemorrhage score as well as viral titration by plaque assay

Limitations:
- Lack of drug efficacy testing in CoV aged mouse models that recapitulate the age-related increase in pathogenesis observed in humans
- Kinetics of disease compressed in mice compared to humans – peak window for treatment may vary

- Does not recapitulate SARS-CoV-2 pathogenesis observed in humans due to incompatibility of virus spike glycoprotein with murine ACE2 receptor

In Vitro Diagnostic Assays for COVID-19: Recent Advances and Emerging Trends
Vashist. (Diagnostics), 2020
Link: https://www.mdpi.com/2075-4418/10/4/202

Summary (100 words max):
Vashist presents a critical evaluation of *in vitro* diagnostic (IVD) assays for COVID-19. He discusses the current use of real-time reverse transcriptase PCR tests and their failure to detect all cases. He goes on to discuss advances in serological assays able to detect IgG and IgM antibodies (lateral flow immunoassay based tests and automated chemiluminescent immunoassay tests). However, these antibodies are only present in the second week post SARS-CoV-2 infection and highlights the importance of biomarker discovery for early stages of clinical infection.

Main findings:
- Current RT-PCR based assays are still missing infected cases and unable to detect COVID-19 in early stages of infection which is critical to prevent widespread transmission
- Advances in RT-PCR assay duration shortened (Cepheid, Xpert® Xpress SARS-CoV-2 test in 45 minutes)
- Important new advances include point-of-care molecular assay (Abbott ID NOW™) which gives results in 5 minutes
- Lateral flow based immunoassays (IA) have been developed which detect IgG and IgM antibodies in response to SARS-CoV-2 infection.
- Advantages of IA include minimal sample volume and rapid testing
- Disadvantages of IA include very-few peer reviewed evaluations and IgG and IgM antibodies only detected at 2 weeks post infection.
- Other developments include chemiluminescence immunoassays (CLIA), manual ELISA kits and reverse transcription loop-mediated isothermal amplification (RT-LAMP)
- Highlights need for detection of other biomarkers for early stages of infection
- Highlights importance of evaluating new tests before use in clinical diagnosis on a wider scale.

Highlights
- Summarises available *in vitro* diagnostic assays for COVID-19

Clinical Impact:
- Minimal

Important Methodologies:
- No methods-review of available tests

Limitations:
- More information on each test and usage would have been beneficial

SARS-CoV-2 specific antibody responses in COVID-19 patients
Nisreen *et al*. (Emerging Infectious Diseases), 2020
Link: https://wwwnc.cdc.gov/eid/article/26/7/20-0841_article

Summary (100 words max):
Nisreen et al. reported the validation and development of serological assays for the detection of SARS-CoV-2 neutralizing, spike- and nucleocapsid-specific antibodies. Showed that spike protein S1 is more specific than S protein. RBD and N proteins also specific for SARS-CoV-2. However, all proteins (S1, N and RBD) cross-reactive with SARS-CoV patient sera. Also found that commercial IgA and IgG S1 based ELISA kits differ in sensitivity and specificity but importantly consistently cross reactive with HCoV-OC45 patient sera.

Main findings:
- S1 spike protein was more specific than S protein in detecting SARS-CoV-2 specific antibodies
- S1 does cross react with SARS-Cov patient sera
- Anti-nucleocapsid and anti-RBD IgG ELISAs cross reacts with SARS-CoV but not with other beta-coronaviruses (eg MERS-CoV)
- N and RBD inhouse ELISA more sensitive than S1 ELISA
- Cross reactivity with SARS-CoV unlikely to be major issue due it not circulating in population since 2003.
- Tested commercial IgA and IgG S1 based ELISAs shown to cross react with HCOV-OC45 patient sera
- IgA-based ELISA showed higher sensitivity but lower specificity than the IgG-based ELISA
- Antibody levels higher in patients with severe infection than mild ones

Highlights
- Outlined consistent false-positive detection with commercial IgA and IgG S1 based kits (Cross reactive with HCOV-OC45)
- Validated sensitivity and specificity of N, S1 and RBD proteins for SARS-CoV-2 detection (All are cross reactive with SARS-CoV)
- Found association with higher antibody levels in patients with severe COVID-19 infection than mild ones

Clinical Impact:
- Minimal

Important Methodologies:
- ELISA utilizing S, S1, N and RBD proteins
- Plaque-reduction neutralization test as reference for ELISAs

Limitations:
- Larger cohort of patients required to confirm whether varying degrees of severity association with antibody levels
- Sensitivities of the developed assays need to be further validated with a larger cohort as currently only used 3 PCR confirmed COVID-19 patient sera were used

The landscape of lung bronchoalveolar immune cells in COVID-19 revealed by single-cell RNA sequencing
Liao *et al*. (medRxiv preprint), 2020
Link: https://www.medrxiv.org/content/10.1101/2020.02.23.20026690v1
Editorial: https://www.nature.com/articles/s41577-020-0303-8

Summary (100 words max):
Acute respiratory distress syndrome (ARDS) and robust cytokine storm are the hallmark of severe COVID-19 cases. Using single-cell RNA sequencing of bronchoalveolar lavage fluid, this preprint study from Liao et al. found that the depletion of tissue-resident alveolar macrophages and the accumulation of monocyte-derived inflammatory macrophages associate with disease severity. Inflammatory macrophages adopted interferon-signalling and monocyte-recruiting chemokine programmes that may drive ARDS. Increased clonal expansion of CD8+ T cells was found in mild cases; this may reflect viral clearance due to the induction of virus-specific cytotoxic T cells, as is seen in influenza virus infection. Overall, these data support therapeutic strategies that target the myeloid cell compartment, such as IL-6 inhibitors, to treat COVID-19-associated inflammation.

Main findings:
- Increased inflammatory macrophages are replacing tissue-resident macrophages in the lung of severe COVID-19 patients – which is essentially a readout of enhanced inflammation
- Highly expanded and functional competent tissue resident clonal CD8+ T cells in mild COVID-19 patients, but not in severe – may indicate a delayed response to infection
- Severe cases likely had higher viral titre than mild cases (SARS-CoV-2 transcripts picked up severe but not mild) – more evidence for a delayed response

Highlights
- RNA-seq dataset of lung immune cells available for further analysis and collation

Clinical Impact:
- Minimal

Important Methodologies:
- Single cell RNA-seq
- T-cell receptor sequencing

Limitations:
- Not yet peer reviewed – specifically with regards to the way data integration was carried out (I'm not an RNA-seq expert – but removing batch effects with n as low as they had seems wrong)
- Only 3 severe and 3 mild patient samples compared with existing data from 8 healthy controls, also UMAP plots not included per patient sample
- Analysis is biased toward in-depth analysis of certain cell subsets, if the immune microenvironment was revealed, why doesn't the paper mention neutrophils or eosinophils even once? Data analysis was rushed until a difference was found and then published – it is possible some macrophage subsets are neutrophils
- No data availability

- No cell counts from BAL, only % of each cell from total thus the conclusion is invalid (i.e. if 1 billion monocytes were recruited this would mask the signal from tissue-resident macrophages without removing them)
- No functional determination of lymphocyte functionality – just based on RNA expression

Characteristics of Peripheral Lymphocyte Subset Alteration in COVID-19 Pneumonia
Fan Wang *et al*, The Journal of Infectious Diseases, March 30th 2020
Link: https://academic.oup.com/jid/article/doi/10.1093/infdis/jiaa150/5813618

Summary (100 words max): Fan Wang et al report changes in lymphocyte subsets in 60 patients with confirmed SARS-CoV-2 infection. Differences in absolute counts of $CD4^+$ & $CD8^+$ T cells, B cells, $CD4^+/CD8^+$ T cell ratio and NK cells are reported. Among COVID-19 patients, severe cases had fewer total lymphocytes, $CD4^+$ T cells, $CD8^+$ T cells, and B cells. Those that responded to treatment showed increased lymphocyte count and increase $CD8^+$ T cell and B cell subsets. Correlations with inflammatory markers show a more obvious change in $CD8^+$ T cells than other lymphocyte subsets, indicating that $CD8^+$ T cells might act as a good indicator of disease severity.

Main findings:

- Significantly lower total lymphocytes $CD4^+$ T cells, $CD8^+$ T cells, B cells, and NK cells compared to healthy controls
- 19 (32%) of patients categorised as having severe illness and showed significantly lower total lymphocytes, $CD4^+$ T cells, $CD8^+$ T cells and B cells compared to those with mild illness; no significant difference in $CD4^+/CD8^+$ ratio ($p = .392$) and NK cells ($p = .177$)
- ESR, CRP, and IL-6 were abnormal in 71%, 72%, and 70% of patients on admission, respectively
- $CD8^+$ T cells negatively correlated with ESR, CRP, and IL-6 whereas $CD4^+/CD8^+$ ratio was positively correlated with the same inflammatory markers
- 50% of patients received more than 1 anti-viral and after 1 week 67% reached clinical response
- In responders total lymphocytes, $CD8^+$ T cells, and B cells increased significantly whereas in non-responders there was no significant change in any lymphocyte subset
- Posttreatment decrease in $CD8^+$ T cells ($p=0.011$), B cells ($p=0.010$), and increase in $CD4^+/CD8^+$ ratio ($p=0.032$) indicated a poor efficacy when controlling for age, sex, disease severity on admission, oxygen inhalation, antiviral treatment, and use of corticosteroid and immune enhancers

Highlights

- Shows possible utility of $CD8^+$ T cells as a biomarker for disease severity

Clinical impact

- Minimal

Important methodologie

- FACS Canto Flow Cytometer used to acquired $CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, $CD16^+$ $CD56^+$ NK cells and reported as cells/µL
- Severe illness defined according to respiratory function; breathing rate, oxygen saturation at rest, and Pao2/Fio2
- EDTA collected and tested prior to initial treatment and after 1 week. Clinical response after 1 week of treatment was defined as 'symptom alleviation and improvement in radiological abnormalities on chest CT/radiograph'

Limitations

- Treatments varied significantly between patients; 47% received oxygen inhalation, 45% corticosteroid, 68% antiviral treatment (6 different agents used), and 38% immune enhancer therapy.
- Exact methodology used for Multivariate Analysis for identifying independent predictors of lymphocyte subsets for treatment efficacy not specified
- Outlier in IL-6 results in very large residuals in upper range and scaling obscures the correlation in lower ranges
- Although $CD8^+$ T cell counts and $CD4^+/CD8^+$ ratios looked promising when correlated with inflammatory markers and for comparisons made post-treatment, the 95% CI for ROC curves indicates that no particular cell subset nor an integrated indicator are reliable predictors of treatment outcome in this cohort.

Immune phenotyping based on neutrophil-to-lymphocyte ratio and IgG predicts disease severity and outcome for patients with COVID-19
Zhang *et al*. (medRxiv), 2020
Link: https://www.medrxiv.org/content/10.1101/2020.03.12.20035048v1

Summary (100 words max):
Zhang *et al.* immunophenotyped 222 patients within 35 days of symptom onset (defined by self/family-reporting and medical records). This retrospective study aimed to predicted disease severity in patients utilising the levels of anti-human SARS-CoV-2 IgG, IgM and the neutrophil-to-lymphocyte ratio (NLR). Patients with a high NLR and IgG^{high} had a lower recovery rate, including several deaths, and higher levels of pro-inflammatory cytokines. The $NLR^{high}IgG^{high}$ phenotype did not encompass all "severe" disease (seemed to be defined by required intervention & lower recovery rate) and the $NLR^{low}IgG^{low}$ group had the lowest disease severity.

Main findings:
- Anti-human SARS-CoV-2 IgG (IgG) antibodies were detected on 4th day of illness and peaked in the 4th week.
- Anti-human SARS-CoV-2 IgM (IgM) antibodies peaked earlier at 2 weeks but there were too few patients to include IgM in the immunophenotyping.
- Stratified patients into 4 response phenotypes using cut-off of 116.9 AU/mL for IgG and neutrophil-to-lymphocyte ratio (NLR) of 3.04.

$NLR^{high}IgG^{high}$	$NLR^{high}IgG^{low}$
$NLR^{low}IgG^{high}$	$NLR^{low}IgG^{low}$

- 72.3% of $NLR^{high}IgG^{high}$ cases were "severe" compared to 48.5% of $NLR^{high}IgG^{low}$.
- These NLR^{high} cases had higher pro-inflammatory cytokines (IL-2, IL-6, IL-10) and decreased $CD4^+$ T-cell count.
- However, 1/3rd $NLR^{low}IgG^{high}$ patients were classed as severe, suggesting a high NLR may not accurately identify severity in all cases.
-

Highlights
- Retrospective study immunophenotyping patients to identify high-risk patients. Found a high neutrophil-to-lymphocyte ratio and high anti-SARS-CoV-2 IgG to be most indicative of severe disease.

Clinical Impact:
- Mid, potential to be used to stratify patients but has yet to be tested in clinics.

Important Methodologies:
- Chemiluminescence Analysis measuring anti-human SARS-CoV-2 IgG and IgM measured by (Shenzhen YHLO Biotech Co, Ltd)
-

Limitations:
- Immunophenotype needs to be tested in independent cohort to confirm accuracy.
- Did not clearly define how patients were classified as "severe" or "non-severe".
- Viral titres were not monitored during infection or recovery.
- Some immunophenotype groups had very few "severe" cases.
- Haven't measured the IgG response in severe patients not given high-dose corticosteroids as a treatment.

Characterization of anti-viral immunity in recovered individuals infected by SARS-CoV-2
Ling Ni *et al*. Medrxiv.2020
Link: https://www.medrxiv.org/content/10.1101/2020.03.17.20036640v1

Summary (100 words max):
This work analyses SARS-CoV-2-specific humoral and cellular immunity in 6 patients who were newly discharged and 6 patients who were 2 weeks post discharge (follow-up patients). Both humoral and cellular immunity were detected in newly discharged patients, suggesting the involvement of innate and adaptive immunities participate in immune-mediated protection to viral infection. The follow-up patients exhibited high titers of IgG antibodies, but with low levels of virus-specific T cells.

Main findings:

COVID-19 patients mounted IgG and IgM responses to SARS-CoV-2 proteins, especially nucleoprotein (NP) and Spike receptor binding domain(S-RBD) and infected patients could maintain their IgG levels for at least two weeks.

Neutralizing antibody assay for antibodies against Angiotensin-converting enzyme 2 (ACE2), shows that newly discharged patients had protective humoral immunity to SARS-CoV-2 and follow-up patients had lower levels of neutralizing antibody titers.

There was no significant difference in terms of the percentages of T cells among those newly discharged, follow-up and the healthy donors

Elispot assay for IFN-gamma-secreting NP-specific T cells in patients 4 out of 6 newly discharged were much higher than other patients, suggesting that they had developed SARS-CoV-2-specific T cell responses.

The numbers of IFN-gamma secreting S-RBD specific T cells were much lower than those of NP-specific T cells, but they could be detected in more patients than those for other viral proteins. S-RBD thus not only elicited humoral immunity that may result in blockade of receptor binding during viral entry in host cells, but also induced T cell immune responses, suggesting S-RBD is a promising target for SARS-CoV-2 vaccines.

A significant correlation between the neutralizing antibody titers and the numbers of NP-specific T cells indicates that the development of neutralizing antibodies may be correlated with the activation of anti-viral T cells, thus suggesting a coordination of humoral and cellular immunity in SARS-CoV-2 clearance.

Highlights: Effective clearance of virus may need collaborative humoral and cellular immune responses.

Clinical Impact: minimal

Important Methodologies: Anti-SARS-CoV-2 IgG/IgM ELISA
Neutralizing antibody assay to assess the existence of antibodies against ACE2.
Characterization of immune cell subsets by flow cytometry

Limitations: Increased NK cell frequency in follow-up patients needs further explanation/investigation

Functional exhaustion of antiviral lymphocytes in COVID-19 patients

Zheng *et al*. (Nature cellular and molecular immunology), 2020

Link: https://www.nature.com/articles/s41423-020-0402-2

Summary (100 words max):

Zheng et al. reports on total numbers and anti-viral functionality of NK and CD8+ T cells in mild and severe cases of COVID-19 and healthy controls. Reduced CD8+ T cell and NK cell numbers were found in COVID-19 patients compared to healthy controls. The functionality of CD8+ T cells and NK cells was assessed using the percentage of CD107a+, IFNγ+, IL-2+, granzyme B+ and TNFα+ cells. COVID-19 patients exhibited loss of anti-viral functionality compared to healthy controls. An increased percentage of cells expressing the inhibitory receptor NKG2A was associated with exhaustion. Convalescing patients post-treatment had increased numbers of T cells and NK cells and fewer NKG2A+ NK and NKG2A+ CTLs.

Main findings:

- Neutrophil count higher in severe compared to mild disease cases.
- Lymphocyte count significantly lower in severe cases compared to mild.
- CD8+ T cell and NK counts lower in severe compared to mild. Counts were also lower in all COVID-19 patients compared to healthy controls.
- The percentage of NKG2A+ CD8+ T cells and NK cells increased in COVID-19 patients compared to healthy controls.
- Functionality of cytotoxic lymphocytes was assessed between COVID-19 patients and healthy controls using the percentage of CD107a+, IFNγ+, IL-2+, granzyme B+ and TNFα+ cells. COVID-19 patients had reduced percentages of CD8+T cells and NK cells expressing these markers.
- Total number of T cells and NK cells increased in the convalescent period in 4/5 patients assessed.
- The percentage of NKG2A+ T cells and NK cells decreased in 5/5 patients in the convalescent period.

Highlights

- Paves the way for future characterization of CD8+ T cell and NK cell functionality in Covid-19 patients

Clinical Impact:

- Indication that targeting exhaustion markers may improve anti-viral immunity.

Important Methodologies:

- Characterization of CD8+ T cell and NK lymphocyte subsets by flow cytometry
- Details of patient whole blood analysis upon admission also provided

Limitations:

- Although a relatively large number of patients, a total of 68, were investigated in the study there was a disproportionate amount of mild cases (55) compared to severe (13). The age range of severe cases was limited to older patients compared to the mild cohort where there was a greater age range. The severe disease cohort also had a much higher male to female patient ratio compared to the mild cohort.

- No timeline is provided for when the patient samples were collected during the course of infection.
- No information provided about the healthy controls.
- Only 5 patients were assessed during the convalescent period and details are not provided as to their level of disease severity and what treatment they received.
- Samples stimulated with PMA/ionomycin only- other stimulations and unstimulated controls may be necessary to uncover functional differences.
- Patients were administrated with a variety of therapies including Kaletra, chloroquine phosphate, IFN and antibiotics.
- Paired/unpaired t tests used to compare groups but do not know if data is normally distributed.

Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients
Hong-Yi Zheng, *et al*. Cellular & Molecular Immunology (2020)
Link: https://www.nature.com/articles/s41423-020-0401-3

Summary (100 words max):
Zheng *et al.* report flow cytometry analysis of peripheral blood and analysis of inflammatory mediators in serum from people with mild (n=10) or severe COVID-19 (n=6) and healthy controls (n=6). Serum IL-6 was not increased in patients although sCD14 increased and granulocyte counts decreased. CD4+ T cells lacked IL-2, IFNγ or TNFα whilst CD8+ T cells had an exhausted phenotype. A combination of these markers along with patient demographics could distinguish between mild and severe COVID-19 and healthy controls. The authors hypothesise that COVID-19 damages CD4+ T cells' functionality and promotes excessive activation and subsequent exhaustion of CD8+ T cells.

Main findings:
- Granulocyte count decreased in severe infection compared to mild. No other changes in leukocyte counts
- Plasma IL-6, TNFα and sCD14 examined, of which only sCD14 was increased (in mild compared to healthy).
- In CD4+ T cells functional markers were decreased especially Granzyme B and IFNγ, more pronounced effect in severe group. TIGIT was increased in COVID-19 CD4+T cells
- In CD8+ T cells granzyme B and perforin were higher in COVID19 with a more pronounced effect in severe group
- CD4 T cells in patients were more likely to be non-functional (expressing no IL-2, IFNγ or TNFα). CD8+ T cells in patients were more likely to be exhausted (combination of CTLA4, PD1 and TIGIT expression).
- Correlation network analysis identified combinations of variables significantly related to COVID-19,
- Hierarchical cluster analysis showed that these factors could distinguish healthy, mild, and severe patients, independent of age and chronic ailments.

Highlights
- Identification of T cell phenotypic changes in COVID-19

Clinical Impact:
- May cast doubt on use of anti-IL-6 mAbs or stratification of patients by IL-6 levels.

Important Methodologies:
- Well defined flow cytometry antibody panels

Limitations:
- No evidence of informed consent from participants
- It is not clear what phenotypic changes were present in patients before infection and which were as a result of COVID-19. Timepoint of sampling in course of infection also unclear.
- Healthy controls were not age/sex matched to patients, nor did they have any pre-existing conditions as the patients did. Therefore phenotypic changes in patients may not be specific risk factors for COVID-19 severity.
- Sample sizes were small
- Cluster analysis not validated on a separate cohort

Reduction and Functional Exhaustion of T Cells in Patients with Coronavirus Disease 2019 (COVID-19)
Diao *et al*., Unpublished. Available on BioXRiv 2020.
Link: https://www.medrxiv.org/content/10.1101/2020.02.18.20024364v1.full.pdf

Summary (100 words max):
T cell counts (total, and in CD8 and CD4) decreased in patients with COVID-19 relative to healthy controls. This effect is greater in elderly patients (>60 years) relative to adults (20 – 60 years). The trend is more apparent in severe disease (and those who perished) relative to mild/moderate cases.

In positive patients admitted to the ICU, TNF-α, IL-10 and IL-6 were elevated relative to non-ICU patients. These levels fell and T cell numbers recovered when patients entered the decline phase. PD1 expression was elevated on ICU patient's CD8+ T cells and on all COVID-19 patient's CD4+ T cells.

Main findings:
- Total, CD8+ and CD4+ T cell counts are decreased in non-ICU COVID-19 patients and further in ICU patients.
- T cell counts are decreased the most in those >60 years of age which may explain greater lethality in this age bracket.
- T cell counts negatively correlate with disease severity.
- Elevated levels of Cytokines (TNF- α, IL6 and IL10) in ICU patients vs non-ICU patients which decrease during the decline phase. IFN-g also decreases during decline.
- T cell counts recover during decline phase.
- PD-1 is elevated in CD4 + T cells in COVID-19 patients regardless of ICU status whilst PD1 is elevated in CD8+ T cells only in ICU patients.

Highlights
- Lymphocyte counts could be used as a simple diagnostic to identify those who may require more medical support.

Limitations:
- The number of patients aged <20 is very low and T cell counts very spread so care should be taken when drawing conclusions about this group (fig 1. C.)
- Whilst the cytokine level data appears robust and trends clear between illness and decline phases (fig 2. A and C.), I'd question the correlation data. There are outliers in the IL10 graphs and the spread away from the line in IL6 and TNF- α data is large. The R values are closer to 0 than -1 or +1 in all graphs (fig 2. B).
- The longitudinal data on PD1 and Tim3 (no other data on Tim3) is based on a n=3 (fig 3. C.)

Clinical Impact: Moderate

Important Methodologies: Blood T cell count and characterisation (flow Cyt.)
- Cytokine levels (Method not in Methods section that I can see).

Multi-epitope vaccine design using an immunoinformatics approach for 2019 novel coronavirus in China (SARS-CoV-2)
Ye Fenget *et al*. (Unpublished, bioRxiv), 2020
Link: https://www.biorxiv.org/content/10.1101/2020.03.03.962332v1.full

Summary (100 words max):
Ye Fenget al. use an in-silico approach which utilizes the SARS-CoV-2 viral genome to identify antigenic B-cell epitopes and HLA restricted T-cell epitopes. B-cell epitopes with higher potential immunogenicity were identified in conjunction with adjacent T-cell epitopes to multi-epitope peptides vaccine candidate. Additionally, peptide sequences with multiple T-cell epitopes and high HLA binding-scores were selected. 30 peptide vaccine candidates were designed. Five peptides were located in the receptor binding domain (RBD), indicating they could induce neutralizing antibodies. In vitro and in vivo trials are required to validate the immunogenicity of these predicted peptides.

Main findings:
- 61 B-cell epitopes were predicted, of which only 19 were exposed on the surface. Of these, 17 were located in the spike protein that contained RBD and functioned in host cell binding
- 499 T-cell epitopes were predicted, of which 48 in the RBD region
- From the 19 B-cell epitopes and their 121 adjacent T-cell epitopes, 17 candidate peptides vaccine that contained both B-cell and T-cell epitopes were generated
- 13 T-cell epitopes-only vaccine peptides were also identified based on number of epitopes and high HLA binding score
- Two epitopes were predicted to possess 4~5 stable hydrogen bonds with their corresponding HLA allele, further indicative of their potential success as peptide vaccines

Highlights
- Uses an immunoinformatic pipeline to identify multi-epitope peptide sequences from SARS-CoV-2 that could be taken forward in peptide vaccines

Clinical Impact:
- Minimal

Important Methodologies:
- Identification of B-cell epitopes with adjacent T-cell epitopes to form candidate multi-epitope peptides using in-house software iNeo-Design
- B-cell epitopes predicted using IEDB, Bepipred software, Kolaskar method and Emini tool
- T-cell epitopes predicted using netMHCpan and iNeo-Pred
- Interactions between T-cell epitopes and HLA molecules models using MDockPep

Limitations:
- All predictions were solely based on HLA alleles prevalent in the Chinese population
- Only in-silico – no in-vitro or in-vivo experiments

Highly pathogenic coronavirus N protein aggravates lung injury by MASP-2 mediated complement over-activation

Gao *et al.*/preprint on medRxiv/2020

Link: https://www.medrxiv.org/content/10.1101/2020.03.29.20041962v2.full.pdf

Summary (100 words max):

Gao et al. suggest a common mechanism by which pathogenic coronaviruses trigger over-activation of the complement cascade causing lung damage. The authors propose that nucleocapsid (N) proteins released by secretion from virally infected cells or after cell lysis dimerise and interact with MASP-2, a key serine protease in the lectin pathway of complement activation. This interaction induces MASP-2 auto-activation and binding to mannose binding lectin (MBL) activating the complement cascade causing tissue damage and inflammation.

Main findings:

- N proteins from highly pathogenic SARS-CoV, MERS-CoV and SARS-CoV2 share greater homology with each other than with other known coronaviruses
- N proteins from highly pathogenic but not from less pathogenic corona viruses enhance MASP-2 binding to MBL by directly interacting with MASP-2, inducing cleavage of complement proteins C4 and C2 as well as deposition of C4b and C3b fragments
- N proteins from highly pathogenic corona viruses increase complement dependent phagocytosis of E Coli by murine peritoneal macrophages
- Mice pre-infected with adenovirus expressing SARS-CoV/MERS-CoV N protein and challenged with LPS show higher fatality and severe lung damage compared to mice infected with empty adenoviruses. This effect is decreased by co-administration of anti-N or anti-MASP-2 antibodies or the use of MASP-2 knockout mice. This suggest a role for N protein in tissue injury caused by LPS released in secondary bacterial infections
- Immunohistochemistry on lung tissue from deceased COVID-19 patient show high staining with antibodies against MBS, MASP-2, C4a, C3 and C5b-9 of the complement cascade
- Significantly increased serum C5a levels are observed in severe COVID-19 patients compare to mild cases and healthy individuals
- In two COVID-19 patients treatment with recombinant anti-C5a antibody showed promising results

Highlights

- Clinical trial is in progress using recombinant anti-C5a antibody to treat severe cases of COVID-19. First results were reported on two severely affected patients where anti-C5a antibody administered IV for several days significantly reduced CRP, increased lymphocyte counts and was associated with disease deterioration.

Clinical Impact:

- Potentially high

Important Methodologies:

- *In vitro* MASP-2 autoactivation, C4 cleavage and complement deposition assay
- *In vitro* phagocytosis assay using E coli and murine peritoneal macrophages
- *In vivo* adenovirus injection and LPS challenge in mice
- Immunohistochemistry on human patient sample
- Sera collection from patients and C5a detection via ELISA
- Anti-C5a antibody therapy in patients as part of a phase II clinical trial

Limitations:

- Manuscript has not been peer reviewed yet

- Unclear from the article how many samples of lung tissue were stained for complement components, might be just one?

Immunopathological characteristics of coronavirus disease 2019 cases in Guangzhou, China
Yaling Shi *et al*. (Medrxiv), 2020
Link: https://www.medrxiv.org/content/10.1101/2020.03.12.20034736v1

Summary (100 words max):
Study analyses changes in various immune cell types and cytokines important for immune reactions and inflammation. Participants used formed three groups, those with mild or severe COVID-19 symptoms and a control. Data presented indicates patients with severe COVID-19 exhibited an overall decline of lymphocytes including CD4+ and CD8+ T cells, B cells, and NK cells. The levels of IL-2 and IL-6 relative to the length of hospital stay underwent a rise-decline pattern, probably reflecting the therapeutic effect. Highlights which immune cell types and markers are useful indicators of severity in COVID-19.

Main findings:
- Study shows that a comprehensive decrease of lymphocytes and elevation of IL-2 & IL-6 are reliable indicators of severe COVID-19.
- Comparisons and differences are made between mild and severe COVID-19 patients' immune signatures.
- INF-γ was not raised in either patient group – author suggests that while serum INF-γ remains unchanged, INF-γ in the lung tissue may be elevated but this requires further investigation.
- Blood conventional T cells, especially CD8+, are reduced in both patient groups.
- Lymphopenia is consistent with previous reports – useful indicator of severe COVID-19
- TNF-α and INF-γ are not reliable indicators of COVID-19 severity.
- Identifies changes in cytokine levels overtime. IL-2 & IL-6 are at relativity stable levels for the first two weeks of infection. On days 15-20, levels became elevated followed by a decline to normal levels. The author speculates that this suggest the aggravation of the disease and the following remission caused by therapies.

Highlights:
- Characterises levels of immune cells and cytokines in relation to levels of severity of COVID-19.

Clinical Impact:
- It would be useful to have reliable indicators of severity of disease to aid treatment pathways.

Important Methodologies:
- Characterization of immune cell subsets by flow cytometry.
- Uses standard COVID-19 test kit – no reference/indication of the reliability of the kit.

Limitations:
- Study recruited participants all from the same hospital and all of the same ethnicity.
- Control group participants were individuals with fever and negative for the SARS-CoV-2 test – these may have had any number of other underlying conditions which caused increases in cytokines and immune cells.

The role of IL-6 in monitoring severe case of coronavirus disease 2019
Tao Liu *et al*. (Medrxiv), 2020
Link:https://www.medrxiv.org/content/10.1101/2020.03.01.20029769v2#Sec3

Summary (100 words max):
Tao Liu et al. report baseline levels of laboratory parameters (peripheral blood cells, cytokines and biochemistry) and its changes after treatment in relation to the severity and outcome of COVID-19. Increased baseline IL-6, CRP, LDH and ferritin were closely related to disease severity. IL-6 dynamic change may associate with disease outcome and its response to treatment.
Main findings:

- More prominent level of D-dimer, ESR, LDH, CRP and ferritin in severe patients compared to non-severe ones.
- On admission, IL-6 but no other immunological parameters significantly increased in severe cases compared with non-severe cases. The baseline elevated IL-6 was positively correlated with other manifestations of severe COVID-19 (lung injury, the increase of CRP, LDH, ferritin, D-dimer).
- CRP, ferritin, LDH, IL-6 significantly decreased after recovery.
- The decrease in IL-6 after treatment was positively correlated with the improvement in chest CT images while patients with disease exacerbation (03 patients) had elevated IL-6 after treatment.

Highlights

- Suggest a potential role of plasma IL-6 as a maker for prognosis and monitoring in severe Covid-19 patients.

Clinical Impact:

- Minimal

Important Methodologies:

- Analysis of lymphocyte subset by flow cytometry
- Characterization of plasma cytokines by ELISA

Limitations:

- The time points of evaluation were not really clear (too general, only mentioned before and after treatment).
- Small sample size (especially in group of patients with disease exacerbation (n=3)) with simple statistical analysis.
- IL-6 and others (CRP, LDH, ferritin) were only measured at two time points (baseline and after treatment). Their dynamic changes during disease course is not investigated.

LY6E impairs coronavirus fusion and confers immune control of viral disease
Pfaender *et al*., 2020
Link: https://www.biorxiv.org/content/10.1101/2020.03.05.979260v1

Summary (100 words max):
Pfaender et al. identify host 'interferon-inducible lymphocyte antigen 6 complex, locus E' (LY6E) as a critical restriction factor against multiple coronaviruses (CoVs)*. Mice lacking *Ly6e* in haematopoietic cells (*Ly6e*$^{\Delta HSC}$) were highly susceptible to infection by the murine CoV; murine hepatitis virus (MHV). Multiple hepatic and splenic immune cell subsets were depleted in infected *Ly6e*$^{\Delta HSC}$ mice. Dynamic changes to transcriptional profiles occurred in organs of infected *Ly6e*$^{\Delta HSC}$ mice vs. functionally wild-type LY6E mice.

**including Middle East respiratory syndrome coronavirus (MERS-CoV), Severe acute respiratory syndrome coronavirus (SARS-CoV) and the recent SARS-CoV-2.*

Main findings:
- The antiviral activity of LY6E is evolutionarily conserved and specific to CoVs.
- LY6E inhibited fusion of viral & cellular membranes and impaired CoV-mediated syncytia formation.
- Human lung adenocarcinoma cells with ablated *LY6E* expression were more susceptible to human CoV-229E.
- All immune cell populations were depleted in liver and spleen of *Ly6e*$^{\Delta HSC}$ mice expect for hepatic $CD8^+$ T cells and splenic $CD4^+$ and/or $CD8^+$ T cells.
- Ablation of *Ly6e* lead to loss of genes associated with type 1 IFN response, antigen presentation and diverse inflammatory/antiviral pathways in cells of infected *Ly6e*$^{\Delta HSC}$ mice.
- Ly6e directly protected primary B cells and dendritic cells from MHV infection.

Highlights:
- Lays foundation to advance understanding of ISG-mediated regulation of CoV *in vitro* & *in vivo* and possibly consider therapeutic approaches that mimic action of LY6E.

Clinical Impact: Minimal

Important methodologies:
- Identification of LY6E by Interferon-Stimulated Gene (ISG) Expression Screening.
- Characterization of immune cell subsets by flow cytometry.
- Transcriptomic approach to evaluate the effect of *Ly6e* ablation on global gene expression following CoV infection.

Limitations:
- The naturally occurring murine CoV MHV used in the study is very different to SARS-CoV-2 in that it causes hepatitis and encephalomyelitis in mice rather than respiratory complications as in COVID-19 disease. This could limit therapeutic potential of findings.
- Elucidation of molecular mechanisms underlying the protective effects of LY6E against CoV is required.

Structure, Function, and Antigenicity of the SARSCoV-2 Spike Glycoprotein
Walls *et al*. (Cell), 2020
Link: https://www.sciencedirect.com/science/article/pii/S0092867420302622
Title of paper: Structure, Function, and Antigenicity of the SARSCoV-2 Spike Glycoprotein

Summary (100 words max):
SARS-CoV-2 binds with high affinity to human angiotensin-converting enzyme 2 (ACE2) via the S2 subunit of the transmembrane spike glycoprotein, and uses it as an entry receptor to invade target cells. The SARS-CoV-2 spike glycoprotein has two distinct conformations and is surface exposed making it the target of neutralising antibodies. Identification of a furin cleavage site at the S1/S2 boundary of the SARS-CoV-2 spike glycoprotein that is cleaved during biosynthesis, is novel to SARS-CoV-2. SARS-CoV S mouse polyclonal sera potently inhibited entry into target cells of SARS-CoV-2 S pseudotyped viruses.

Main findings:
- Using BHK cells transiently transfected with hACE2, Angiotensin-converting enzyme 2 (ACE2) was determined as an entry receptor for SARS-CoV-2, with a similar affinity as SARS-CoV. This is in agreement with other papers.
- Sequence analysis of SARS-CoV-2 S showed a four amino acid residue insertion at the boundary between the S1 and S2 subunits compared with other SARS-CoV related S and this was conserved between the 144 SARS-CoV-2 samples sequenced to date. Western blot analysis showed that this site was cleaved during biosynthesis in HEK293T cells, but they show that this is not necessary for S mediated entry. It is hypothesised the expression of furin-like proteases could participate in expanding SARS-CoV-2 cell and tissue tropism and increasing its transmissibility and/or altering its pathogenicity.
- Previous work has identified 14 positions that are key for binding to ACE2 in SARS-CoV and analysis of the SARS-CoV-2 genome sequences showed that 8 out of the 14 are strictly conserved in SARS-CoV-2.
- A reconstruction of the closed SARS-CoV-2 S ectodomain trimer at 2.8 A˚ resolution (applying 3-fold symmetry) and an asymmetric reconstruction of the trimer with a single SB domain opened at 3.2 A˚ resolution was determined. The SARS-CoV-2 and SARS-CoV S subunits share 88% sequence identity, they are structurally conserved and can be superimposed with 1.2 A˚ root-mean-square deviation (rmsd) over 417 aligned Ca positions. The sequence and conformational conservation of the fusion peptide region observed across SARS-CoV-2 S and SARS-CoV S suggests that antibodies targeting this motif might cross-react and neutralize the two viruses as well as related coronaviruses.
- Previously showed that coronavirus S gylcoproteins are densely packed with heterogeneous N-linked glycans protruding from the trimer surface, which participate in S folding, affect priming by host proteases and might modulate antibody recognition. Same analysis was done for SARS-CoV-2, which has 22 N-linked glycosylation sequons per protomer, compared to 23 of SARS-CoV. 20 out of the 22 are conserved between the two viruses. S glycoprotein trimers found in highly pathogenic human coronaviruses appear to exist in partially opened states, while they remain largely closed in human coronaviruses associated with common colds.
- Due to both viruses using ACE2 for cell entry and that the S1 subunit is exposed at the viral surface, they hypothesised that exposure to one of the viruses would elicit cross-reactive and potentially neutralising antibodies against the other virus. Plasma was taken from mice immunised with a stable SARS-CoV to see if it could inhibit SARS-CoV-2 S-mediated entry – all sera tested inhibited transduction to ~10%.

Highlights

- Provides a structural framework to identify conserved and accessible epitopes across S glycoproteins that will support ongoing vaccine design efforts.

Clinical Impact:

- Minimal

Important Methodologies:

- Pseudovirus production and entry assays
- Biolayer interferometry
- CryoEM
- Immunisations with SARS-CoV

Limitations:

- Variability in SARS-CoV-2 structure between studies.
- Work was carried out on cell lines

SARS-CoV-2 Cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor
Hoffmann *et al*. (Cell 181, 1-10), 2020
Link: https://www.sciencedirect.com/science/article/pii/S0092867420302294

Summary (100 words max):

In this study they identify two molecules important for host cell entry, ACE2 and TMPRSS2 as SARS-CoV utilizes. Both of them could be targeted therapeutically to reduce host cell infection. TMPRSS2 inhibitor reduce viral entry of SARS-CoV-2 in human lung cells. Also, it shows that sera from convalescent SARS-patients efficiently inhibits SARS-CoV and partially cross-neutralizes SARS-CoV-2 pseudotype viral entry.

Main findings:
- SARS-2-S and SARS-S have similarities in entry receptors
- Most amino acid residues essential for ACE2 binding by SARS-S were conserved in SARS-2-S
- Ammonium chloride treatment strongly inhibited SARS-2-S driven entry into TMPRSS2$^-$ cells, suggesting CatB/L dependence
- SARS-2-S can use both CatB/L as well as TMPRSS2 for priming.
- TMPRSS2 inhibitor blocks SARS-CoV-2 infection of lung cells.
- Evidence that Ab raised against SARS-CoV will cross-neutralize SARS-CoV-2

Highlights
- Key insights into the first step of SARS-CoV infection, viral entry into cells, and defined potential targets for antiviral intervention

Clinical Impact:
- Medium

Important Methodologies:
- Amino acid sequence analysis to identify sequence similarities of receptor binding domain in SARS-CoV-2 and other coronaviruses
- Quantification of cell viability using CellTiter-Glo Luminiscent Cell (Promega)
- Analysis of SARS-2-S expression and particle incorporation by using chemolumisniscence
- Phylogenetic analysis using MEGA software whereas the reference sequence was obtained from the National Center for Biotechnology and GISAID

Limitations:
- Only in vitro experiments they do not use any animal models.
- Missing some controls that could help to interpret the data.
- The number of sera from convalescent SARS patients to measure cross neutralize SARS-2-SDriven entry was only 3

Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV.
Xiuyuan Ou *et al*. (Nature Communications), 2020
Link: https://www.nature.com/articles/s41467-020-15562-9

Summary (100 words max):
Xiuyuan Ou et al. use a lentiviral pseudotype system to express SARS-CoV-2 S protein to confirm viral receptor usage, entry pathway and protease priming by SARS-CoV-2 in direct comparison to SARS-CoV. They confirm human angiotensin converting enzyme 2 (hACE2) is the receptor for SARS-CoV-2 and viral entry is achieved via endocytosis. Importantly they identify several potential drug targets for SARS-CoV-2. They go onto demonstrate a limited cross-netralization between convalescent sera from SARS and COVID19 patients.

Main findings:
- SARS-CoV-2 S protein utilises hACE2 receptor.
- SARS-CoV-2 enters 293/hACE2 cells via endocytosis
- PIKfyve, TPC2 and Cathespin L are critical for viral entry.
- SARS-CoV-2 S protein is less thermal stable than SARS-CoV S.
- Polyclonal anti-SARS S1 antibody (T62) inhibits viral entry of SARS-CoV S but not SARS-CoV-2 S pseudovirus.
- Patient sera from recovered SARS and COVID19 patients show limited cross-neutralization.

Highlights
- Identifies potential targets for drug development and vaccines against SARS-CoV-2.

Clinical Impact:
- Limited

Important Methodologies:
- Generation of pseudotyped SARS-CoV-2 S protein.
- Soluble hACE2 binding/inhibition assays
- SARS-CoV-2 virus entry inhibition assays (Inhibitors - Apilimod (1000 nm), YM201636 (10uM), Tetrandrine (3ug/ml), SID-26681509 (2um), E64D (30um).
- Cross-neutralistion assay with patient sera.

Limitations:
- Pseudotyped virus used in various cells lines.
- No in vivo models used.
- Limited patients sera analysed (1 SARS patient v 5 COVID19 patients).

Avoiding pitfalls in the pursuit of a COVID-19 vaccine
Lynne Peeples *et al.*, PNAS, 2020
Link: www.pnas.org/cgi/doi/10.1073/pnas.2005456117

Summary (100 words max):
Potential challenges in devising an effective COVID-19 vaccine exist. Firstly, immune enhancement can occur: where patients produce a defective response to the vaccine, increasing susceptibility to the natural virus. Antibody-dependent enhancement (ADE) can manifest, whereby the patient produces antibodies to aid the virus in its uptake. Cell-based enhancement results in a Th2 driven immunopathology. The latter is believed to be a higher risk for a COVID-19 vaccine. A vaccine based on SARS-COV-1 using the virus' receptor-binding domain, and an mRNA-based vaccine are both currently promising candidates for a COVID-19 vaccine in future.

Main findings:

- A COVID-19 vaccine could face issues in causing immune enhancement via antibody-dependent enhancement (ADE), whereby the patient produces antibodies to aid the virus in its uptake and infection in the host, or cell-based enhancement which results in a Th2 driven immunopathology, characteristic of allergic inflammation.
- ADE is an issue in vaccines for diseases such as Dengue virus, however, there is no evidence to suggest that ADE is occurring in COVID-19 patients. This opinion is based on the lack of real evidence for COVID-19 reinfections, and in some preliminary experiments showing that: 1. antibodies against original SARS infection can block entry of SARS-Cov-2; 2. rhesus macaques were not infected after a second exposure to the virus; and 3. this virus does not target macrophages.
- Cell-based enhancement is more likely to be an issue in coronavirus due to evidence of this reaction in mouse models of SARS-COV-1.
- A SARS-COV-1 vaccine using the spike protein of the virus produced a strong Th2 response in animal models. This was rectified when the spike protein was replaced with the receptor-binding domain of the virus.
- This SARS candidate vaccine developed two decades ago, is now being modified to work in COVID-19, by using receptor binding domains of the virus as an antigen.
- An mRNA vaccine candidate went into phase I clinical trials last month.

Highlights:

- ADE in COVID-19 looks unlikely, whereas Th2 immunopathology in COVID-19 needs further research.
- There are multiple promising vaccine candidates in trials for COVID-19. They suggest that some vaccines that were developed for the original SARS virus could work for this virus after adjustments to adapt to COVID-19.
- Experts agree that clinical trials of vaccines for COVID-19 should include a careful assessment of possible immune complications before releasing the vaccine to the public.
- It is important to develop these vaccines, even if the spread is stopped, since ecological disruption increases the odds of other coronavirus jumping to humans.
-

Clinical Impact:

- Important for vaccine development.

Important Methodologies:

- N/A (news article).

Limitations:

- Lack of patient data available to understand immune enhancement in COVID-19.
- Article based on the opinion of the interviewed researchers.
- Lack of references.

The SARS-CoV-2 Vaccine Pipeline: an Overview
Chen *et al.* (Curr Trop Med Rep), 2020
Link: https://link.springer.com/article/10.1007/s40475-020-00201-6

Summary (100 words max):
The key features for a suitable SARS-Cov-2 vaccine are 1) minimal undesired immunopotentiation 2) suitable for adult healthcare workers 3) suitable for adults >60 years of age or with underlying diabetes or hypertension 4) suitable for stockpiling.
Chen *et al.* assembled a brief overview of current SARS-CoV-2 vaccine development approaches; strategies such as whole virus, subunit and nucleic acid vaccines are being explored by a variety of pharmaceutical and academic bodies.

Main findings:

- **Major hurdle remains undesired immunopotentiation** in the form of oeosinophilic infiltration or increased infectivity following challenge infections after immunizations with whole virus vaccines or even complete spike protein (S-protein) vaccines.
- A new vaccine may not be available in time for the current pandemic – looking at the trend in coronavirus pandemic outbreak every decade, the vaccine needs to be suitable for stockpiling.
- **The coronavirus binds to the ACE2 receptors found in the human lung via the S-protein**
- **SARS-CoV-2 exhibits ~89% nucleotide similarity to SARS-like coronaviruses.**

- Johnson & Johnson – whole virus vaccine – Janssen's AdVac® adenoviral vector manufacturing in PER.C6®
- University of Hong Kong – whole virus vaccine – live influenza vaccine expressing SARS-CoV-2 proteins
- Codagenix – whole virus vaccine – "codon deoptimization" technology to attenuate viruses
- **Advantage to whole virus vaccine is their ability to stimulate toll-like receptors (TLRs) including TLR3, TLR 7/8 and TLR9**

- University of Queensland – subunit vaccine – synthesizing viral surface proteins
- Novavax – subunit vaccine – immunogenic virus-like nanoparticles based on recombinant expression of the S protein
- Clover Biopharmaceuticals – subunit vaccine – trimerized SARS-CoV-2 S-protein using Trimer-Tag® technology
- Texas Children's Hospital Center for Vaccine Development – subunit vaccine – vaccine comprised only of the receptor-binding domain (RBD) of the SARS-CoV-2 S-protein
- **SARS-CoV RBD vaccine elicits high levels of protective immunity on the homologous virus challenge, plus is able to minimize host immunopotentiation**

- Inovio Pharmaceuticals – DNA vaccine
- Moderna Therapeutics and Curevac – RNA vaccine
- Although successful in 1993, findings not translated to humans

Highlights

- Lists potential candidates for SARS-CoV-2 vaccines including live viruses, recombinant protein subunits and nucleic acids, however additional manufacturing steps and formal toxicology testing would be needed.

- **RBD vaccinations seem to be the most efficient and safest approach**

Clinical Impact:

- Moderate

Important Methodologies:

- N/A

Limitations:

- Whilst the paper gives nice brief overview of current vaccine development, it remains that it is very brief with no in-depth recounting of development strategies.
- Despite promising results from the RBD vaccine, it is limited in that this is the only vaccine of its kind listed in this review.