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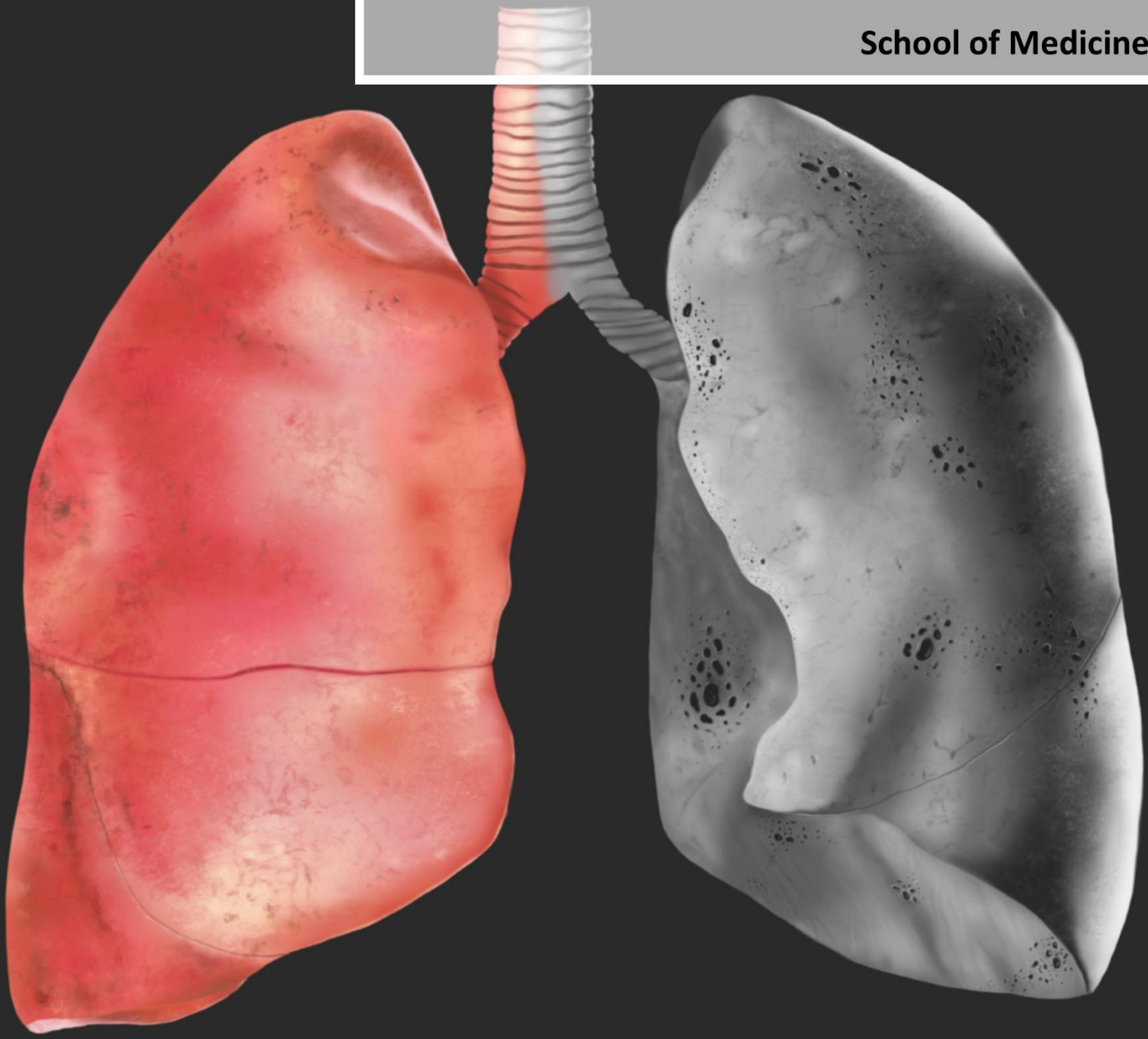

Artwork by
Lucy Chapman

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

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

Sources of Further Information

Statistical Reviews for Clinical Trials Testing Treatments for COVID-19
Conducted by the MRC-NIHR Trials Methodology Partnership
https://zenodo.org/communities/covid-19-tx-rct-stats-review/search?page=1&size=20

Coronavirus webinars from The British Society for Immunology

Controversy in immunity to COVID-19: do we want an immune response or not?
This session will be presented by former BSI President Professor Peter Openshaw and Dr Ryan Thwaites
Wednesday 27 May from 12:30 - 13:15 BST – *Recording available*
Learn more and register

Antiviral responses in COVID-19
This session will be presented by Professor Paul Klenerman
Wednesday 3 June from 12:30 - 13:15 BST – *Recording available*
Learn more and register

What can genomics tell us about SARS-CoV-2?
This session will be presented by Professor Judith Breuer
Wednesday 10 June from 15:30 - 16:15 BST - *Recording available*
Learn more and register

To register or to access recording of previous webinars go to the following webpage.
https://www.immunology.org/coronavirus/connect-coronavirus-webinars

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Speed Science: A cautionary note

The COVID-19 pandemic has resulted in a mass of academic papers being published on the novel virus. However, due to the speed at which these papers are being churned out they are lacking the usual external checks, scrutiny and validation which papers are normally subjected to pre-publication.

A high percentage of the papers being published are preprints. A knock-on effect of this is that the data presented may have been based on misleading and at times incorrect information. BioRxiv – a preprint server which allows information to be quickly and freely shared – has recently included a warning banner at the top of its website reminding users that these are 'preliminary reports' and that they 'should not be regarded as conclusive, guide clinical practice/health-related behaviour, or be reported in news media as established information'. A key example was an inflammatory paper which was published on BioRxiv that suggested similarities between COVID-19 and HIV. This work was swiftly retracted, but by the time it was taken down it had spread at lightning speed through the internet and been picked up by numerous news outlets.

There have been retractions of papers in big name journals; The New England journal of Medicine and The Lancet. Both of these papers have been reviewed in the digest – 'Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis' (see COVID-19 community journal club No. 7 and https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)31324-6/fulltext) and 'Cardiovascular Disease, Drug Therapy, and Mortality in Covid-19' (see COVID-19 community journal club No. 6 and https://www.nejm.org/doi/full/10.1056/NEJMc2021225).

While this breadth and volume of information currently being published on Coronavirus can be highly useful if it's correct, robust and repeatable science; misleading information can spread unnecessary panic (or lead researchers and clinicians down the wrong paths) at an already difficult time.

Related links:

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

https://www.statnews.com/2020/06/03/who-resuming-hydroxychloroquine-study-for-covid-19/

News and Views

Are SARS-CoV-2 seroprevalence estimates biased?

Takahashi, S. *et al*. 2020. *OSF Preprints*
Link: https://doi.org/10.31219/osf.io/y3fxt

Multiple serological assays exist to identify proportions of populations that have been infected with SARS-CoV-2. However, many of these assays have focused on ensuring near perfect assay specificity, which can be detrimental to assay sensitivity. Additionally, many of these assays have been validated with samples from severely infected patients, which may result in spectrum bias.

Simulations performed by Takahashi *et al*. used 3 validation sets with different proportions of severe, mild, and asymptomatic infections. Set 1 was weighted due to severity prevalence assumed in the general population whilst set 3 contained 100% severe cases. They found that the true sensitivity in the general population would be 53.15%, irrespective of the validation set used. The study also modelled cross-sectional seroprevalence surveys performed at 60, 180, and 300 days into the pandemic and found that for any given value of true prevalence, measured prevalence decreased as time increased.

This suggests that, if applied for serosurveillance, many approved assays may underestimate the true seroprevalence in the population. To improve sensitivity, assays should be validated with samples that represent a range of disease severity, and also from different time-points since infection.

In silico veritas? Potential limitations for SARS-CoV-2 vaccine development based on T-cell epitope prediction

Silva-Arrieta, S. *et al*. 2020. *PLOS Pathogens*

Link: https://doi.org/10.1371/journal.ppat.1008607

Silva-Arrieta *et al*. opinion article describes the considerations and limitations of using *in silico* peptide prediction algorithms to design a vaccine for SARS-CoV-2. Whilst the authors acknowledge that there has been many improvements to prediction algorithms there are still considerations that must be taken when using these tools to design vaccines. This opinion article covers only HLA class I alleles. The considerations for using peptide prediction tools include:

1. **Variability of peptide epitope lengths** – Some prediction tools have an inherent bias towards 9-mer peptides. However, HLA class I alleles have different preferences of peptide length. For example, HLA-B8 shows a bias towards peptides less than 9 amino acids in length and HLA-B57 prefers 11-mers.

2. **T-cells can recognise different length epitopes in the same region** – using peptides of one particular length can limit the vaccine induced T-cell response. Allowing for epitopes length of 9 – 12 amino acids doubles the potential independent epitopes available to the T-cell pool. Taking this into account for vaccine design would ideally elicit polyclonal T-cell responses and help to prevent T-cell escape.

3. **Promiscuity of HLA class I binding and poorly defined epitope motifs** – studies have sought to define binding motifs for HLA class I alleles, however epitopes routinely subvert these motifs. Therefore when using prediction algorithms to design peptide vaccine epitopes the promiscuity of HLA class I binding must be taken into account in order to avoid missing potentially optimal peptide epitopes. Furthermore, prediction of epitopes for less well studied HLA class I molecules, such as HLA-E, can be challenging due to lack of understanding of binding motifs and the importance of peptide anchor residues.

4. **Antigen process and presentation preferences** – processing and presentation of peptides is not properly addressed by most epitope prediction tools. Therefore, predicted epitopes need functional validation to negate the risk that the predicted epitopes may in theory bind to HLA class I but not in practise.

5. **T-cell cross-reactivity and immunopathology** – it would be beneficial to design a vaccine that would allow for broad immunity to not just SARS-CoV-2 but to past and future coronaviruses. This could involve designing epitopes from conserved regions which is possible using prediction tools. Whilst T-cell cross-reactivity can be beneficial there can be associated immunopathology of a robust immune response. Therefore studies must assess the risk of eliciting such cells.

Journal Reviews

Highlighted Paper

SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract

Hou, Y. J. *et al*. 2020. *Cell*

Link: https://doi.org/10.1016/j.cell.2020.05.042

Summary:

The group has utilised a reverse genetics system to generate GFP and luciferase SARS-CoV-2 reporter viruses to help characterise virus tropisms, virus RNA transcription profiles, evaluate the expression profiles of ACE2 and TMPRSS2 and their impact on viral growth. They have shown that most ACE2 expressing cells are ciliated cells in the proximal airways and that the nasal epithelial has high SARS-CoV-2 infectivity with a gradient of infectivity reducing the further into the lung. They speculate that nasal surfaces may be the dominant initial site for infection due to the relatively high ACE2 expression in the nasal specimens and the high infectivity of the nasal surface epithelia. Due to the low level of ACE2 expression in the alveolar cells and the correlated poor infectivity, the role of direct transmission of infection to the lower lung surfaces by micro-aerosol inhalation is unclear. An alternative theory is that tracheal-produced virus accumulates in the oropharynx, via mucus clearance, and is subsequently aspirated into the deep lung – these aspirates also contain enzymes and inflammatory mediators that may condition alveolar cells for infection and this was affirmed by their autopsy studies. This study provides a SARS-CoV-2 infectious, full-length cDNA clone that replicates to normal WT levels allowing for robust *ex vivo* studies. With this clone, they were able to generate a high-throughput luciferase reporter SARS-CoV-2 assay for the evaluation of neutralising antibodies, which showed that SARS-CoV RBD-binding antibodies failed to neutralise SARS-CoV-2, but antibodies from convalescent COVID-19 patients demonstrated a successful neutralising response.

Main Findings:

- Using a full-length infection cDNA clone of SARS-CoV-2 clinical isolate, a wild type recombinant virus was produced, along with two reporter viruses containing a GFP or a GFP-fused nano-luciferase gene. All recombinant viruses, which had a silent mutation inserted to distinguish from circulating strains, replicated to titres equivalent to the clinical isolate and generated similar plaques.
- Trypsin did not trigger syncytium formation and demonstrated slighter lower virus titres than controls, whereas increased furin and TMPRSS2 expression increased viral

titres compared to WT cells showing that furin and TMPRSS2 enhance the replication efficiency/cytopathology of SARS-CoC-2 *in vitro.*

- Cross-reactive neutralising antibodies against SARS-CoV, MERS-CoV and Dengue virus were used to determine whether the recombinant viruses could be neutralised. None of the above viruses neutralised icSARS-CoV2-nLuc-GFP. However, mouse serum taken from BALB/c mice vaccinated and boosted with viral replicon particle encoding the SARS-CoV-2 gene was able to neutralise 99.4% of the icSARS-CoV2-nLuc-GFP virus after both prime and boost.
- Two out of 5 serum samples taken from the 2003 SARS-CoV outbreak were able to neutralise icSARS-CoV2-nLuc-GFP. Ten COVID-19 convalescent serum samples displayed variable neutralisation of icSARS-CoV2-nLuc-GFP.
- ACE2 and TMPRSS2 expression was shown to be higher in the nasal cavity with progressively reduced levels observed in the lower airway regions and minimal levels in the alveolar region. TMPRSS2 expression was overall higher in all regions than ACE2.
- Using RNA-ISH technique ACE2 expression was identified in ~20% of interrogated cells (vs ~5% by scRNAseq). The percentage of ciliated cells expressing ACE2 was higher in the nose than the bronchi.
- ACE2 and TMPRSS2 expression was significantly upregulated in the lungs of cystic fibrosis patients compared to normal controls. When looking at pathways to contribute to dysregulation of ACE2 expression, they found that IL1β upregulated ACE2 but not TMPRSS2, IFNβ increased ACE2 expression but decreased TMPRSS2 expression and IL-13 inhibited ACE2 expression.
- icSARS-CoV2-GFP replicated efficiently in several primary cells, with the highest titre values in the nasal surface epithelia, followed by the large airway (bronchi), lower airway (bronchiolar), type I and II-like pneumocytes. Viruses replicated more slowly in the bronchiolar than the bronchi.
- Ciliated cells were the main cell type to be infected – no correlation was noted between susceptibility to infection and ciliated cell percentages. MUC5B+ club cells and MUC5AC+ metaplastic goblet cells were not infected.
- When looking at SARS-CoV-2 infection in COVID-19 autopsy lungs, they found that ciliated cells within the superficial epithelia lining proximal airway surfaces (particularly the trachea) were infected; again, club and goblet cells were not infected. Submucosal glands in the large airway were not infected, but the alveolar cells, AT1 and AT2 cells were.

Highlights:

- This study provides key reagents and strategies to identify type specific and highly conserved neutralising antibodies that can be assessed in the nasal cavity, blood and lower airways. Additionally, they demonstrate differences in ACE2 receptor expression and SARS-CoV-2 infectivity in the nose (high) vs the peripheral lung (low).

Clinical Impact:

- Minimal

Important Methodologies:

- Production of recombinant viruses
- Single cell-RNA *in situ* hybridisation
- Generation of SARS-Cov-2 S protein immunised mouse serum and monoclonal antibodies
- Neutralisation assays
- Whole mount immunostaining and imaging, Immunohistochemistry, qRT-PCR, Northern Blot

Limitations:

- More samples are needed to fully understand the kinetics magnitude and durability of the neutralising antibody response after a primary SARS-CoV-2 infection.

Antibodies – Responses and Testing

Neutralizing Antibodies Responses to SARS-CoV-2 in COVID-19 Inpatients and Convalescent Patients

Wang, X. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.04.15.20065623

Summary:

Wang *et al.* studied serum samples from 70 people with COVID-19. They found that all samples taken between day 10 and 60 post symptom onset contained neutralising antibodies. Diverse patterns of antibody titres over time were observed, however in general the peak antibody titres were found 31-40 days after symptom onset. Older people and those with severe disease had higher antibody titres.

Main Findings:

- All initial samples were positive for antibodies to SARS CoV-2 by both neutralising assay and ELISA. These were taken between 10 and 53 days after symptom onset.
- Neutralising and ELISA antibody geometric mean titres peaked at 31-40 days after symptom onset
- The proportion of patients with an effective neutralising titre at the most dilute threshold was largest at 31-40 days after symptom onset
- The proportion of patients with an antibody response detectable by ELISA at the most dilute threshold was also largest at 31-40 days after symptom onset
- However all of these responses appeared somewhat biphasic with an initial high titre (day 10-20, a gradual decline (day 21-30) and a second peak (day 31-40) and another decline (day 41-53)
- To resolve this 8 convalescent patients were tracked longitudinally. Four of these showed antibody titres that increased over time whilst four appeared to increase between day 10-20 and then declined. However all samples remained antibody positive (up to 60 days after symptom onset)
- Older patients (61-84 years) had significantly higher antibody titres than younger patients (31-45 years)
- After adjusting for other variables, people with severe disease had significantly higher antibody titres

Highlights:

- Confirmation by both neutralising assay and ELISA of antibody responses
- Suggests a slightly later peak than other studies
- Highlights need to follow patients longitudinally to further understand the memory response of the immune system

Clinical Impact:

- Neutralising antibody response can be expected to persist for at least weeks in most patients, suggesting a window in which to take convalescent plasma for protection or treatment of other people.

Limitations:

- Only one patient had asymptomatic infection and four had severe infection
- Not all patients were followed longitudinally which makes the pattern of antibody titre over time difficult to interpret

Detection of asymptomatic SARS-CoV-2 exposed individuals by a sensitive S-based ELISA

Rosendal, E. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.06.02.20120477

Summary:

The authors have developed a sensitive immunoassay (ELISA) for detection of SARS-CoV-2 spike protein antibodies that may facilitate assessment of exposure to infection and population immunity. Existing assays provide robust detection of clinical SARS-CoV-2 infection, but there remains legitimate concern surrounding the accurate diagnosis of asymptomatic cases. The newly-developed ELISA detects anti-SARS-CoV-2 immunoglobulin responses at earlier time points (<14 days after onset) in infection than when patient seroconversion is usually determined (15-20 days after onset) and outperforms other commercially-available ELISA kits. This assay may be of value for the recognition of weak and early immune responses to SARS-CoV-2 infection.

Main Findings:

- The development of a novel ELISA to detect anti-SARS-CoV-2 spike protein immunoglobulin in clinical samples at early stages post-infection is described.
- The assay employs recombinant trimeric spike proteins from the Wuhan Wu isolate as the capture antigen, combined with an alkaline phosphatase (AP)-conjugated secondary antibody that improves the sensitivity of the test for those mid-range samples that represent the weaker anti-spike protein responses.
- This optimised AP ELISA detected 20% (1/5) and 75% (9/12) qPCR-validated SARS-CoV-2 cases sampled 1-5 days or 6-14 days after disease onset, respectively.
- Specificity for SARS-CoV-2 infections was confirmed via functional neutralisation assays (viral plaque neutralisation and fluorescent inhibition) *in vitro.* No neutralisation was measured for samples testing positive for other coronaviruses and there was no cross-

reactivity of the ELISA with any other viral infections (alpha and beta coronaviruses, EBV, RSV, influenza, parainfluenza, metapneumovirus) within the control samples.

- The AP ELISA works with heat-inactivated patient serum (as required to minimise infection risk), so can be used for diagnostic clinical testing.
- This assay more efficiently detects immunoglobulin in samples collected 1-14 days post-disease onset than two commercially-available ELISA kits capturing SARS-CoV-2 nucleocapsid (Abbott Architect 1) and S1/S2 spike protein domain (LIAISON®) antibodies (63% versus 44% and 38% qPCR-validated cases, respectively).
- This assay also identified 7 unique samples (undetected by the other 2 commercial kits) from individuals with mild and asymptomatic infections, suggesting it has increased sensitivity for these challenging samples. However, validation was by their own spike competition assay and not by qPCR confirmation of infection.
- Longitudinal sampling revealed that SARS-CoV-2 immunoreactive antibodies in clinical samples can both increase and decrease over time (9-22 days), emphasising the importance of timely and repeated sampling of suspected cases.

Highlights:

- Development of improved ELISA assay for more sensitive clinical detection of anti-SARS-CoV-2 antibodies that may be present in early stage infections and asymptomatic cases.

Clinical Impact:

- Could be of moderate-high impact if externally-validated and proven effective in the reliable detection of specific SARS-CoV-2 infection amongst the population (including asymptomatic cases and those who low residual levels of immunoglobulin) as a means of tracking overall community exposure and immunity.

Important Methodologies:

- Novel SARS-CoV-2 Spike protein ELISA assay with improved sensitivity for the specific detection of patient antibody responses with 2 weeks of disease onset.
- Spike protein production and purification from plasmid expression systems.
- Spike competition (blocking) assays to determine specific reactivity.
- Virus and plaque neutralization assays using heat-treated patient sera.
- Fluorescent inhibition assays using heat-treated patient sera.

Limitations:

- The supplemental figure does not appear to be visible and so it is not possible to evaluate the data within it.
- The validated sample testing set contains only 30 SARS-CoV-2 qPCR-confirmed +ve cases, so further assessment of accuracy and reliability is required.
- The qPCR validation status of the blinded test dataset of 145 care home samples is not detailed so it is not possible to assess whether the increased sensitivity of the new ELISA over the commercially-available kits relates to an elevated false discovery rate or genuine identification of weakly-responsive infections.

- Cross-reactivity with SARS and MERS patient samples is not determined, although other beta and alpha coronavirus infections are represented in the negative controls.
- The application has been tested with patient serum and not plasma, so may not be suitable for all sampling modes.
- The assay will detect only anti-Spike protein responses and not any other antibodies (e.g. anti-nucleocapsid) that may be raised in SARS-CoV-2 patients. Since it is not yet clear what the prevailing immunoreactivity in asymptomatic individuals is, this is a noteworthy caveat.
- Although the authors declare immunoglobulin is more stable in the circulation over time than viral RNA detected by qPCR approaches, it is clear that their assay is time-dependent since 1/6 follow-up samples (9-22 days after initial sampling) dropped below the detection threshold. This suggests more limited value for tracking community-level exposure and immunity unless regular and comprehensive testing is conducted.
- This new assay is not too dissimilar from that published by Amanat F et al. (*Nat Med* (2020). https://doi.org/10.1038/s41591-020-0913-5), although the detection of AP signal at 405nm appears more potent than the original study using HRP-conjugated antibodies. It is not clear whether a comparable secondary antibody modification would similarly enhance the sensitivity of detection using the Amanat protocol.

A serological assay to detect SARS-CoV-2 antibodies in at-home collected finger-prick dried blood spots

Karp, D.G. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.05.29.20116004

Summary:

Karp et al analysed and validated an at-home finger-prick dried blood spot collection kit and the accompanying laboratory test eluting and measuring SARS-CoV-2 spike protein (S1) specific antibodies. The method demonstrated 100% sensitivity and specificity across 56 samples. This at-home test could be used as a complementary marker to boost the accuracy of RT-PCR testing, measure disease prevalence at population level and to identify possible donors of convalescent plasma. Furthermore, it could reduce the sample collection burden of stressed health care systems and the exposure of staff to SARS-CoV-2.

Highlights:

1. A total of 56 specimens were used for the study. 31 were confirmed (by RT-PCR or serological test) COVID-19 patients and 25 were from healthy donors. Participants received a dried blood spot testing kit via normal US postal service. After producing 2-5 dried blood spots, the kits were sent back to the laboratory for analysis via modified Antibody Detection Agglutination-PCR (ADAP) followed by RT-PCR.

2. Out of 31 COVID-19 patients, 31 tested positive. Out of 25 healthy participants, 25 tested negative. This result indicates 100% sensitivity and specificity of the test.
3. Shipping of test kits was carried out at room temperature. Several participants lived far away from the laboratory. There was no significant difference in RT-PCR signal distribution between samples received from within 100 miles or from over 1500 miles away.
4. Finger-prick blood could be diluted by extra-venous tissue fluid. To evaluate the impact of this, EDTA plasma samples of 4 COVID-19 patients were collected by standard phlebotomy and analysed in the same way as the finger-prick samples. Plasma signals were highly consistent and correlated to finger-prick samples collected at home.

Clinical Impact:

- Moderate. There are indications that this test could be useful in a clinical setting. Donors of convalescent serum could be identified like this and health care staff would not have to visit patients at home.

Important Methodologies:

- Antibody Detection Agglutination-PCR (ADAP)

Limitations:

- Sample size is relatively low. However, the study is still ongoing to increase the sample size.
- It is not 100% clear if all healthy donors were confirmed negative by RT-PCR or serological methods.

Clinical

Estimating excess 1-year mortality associated with the COVID-19 pandemic according to underlying conditions and age: a population-based cohort study

Banerjee, A. *et al*. 2020. *The Lancet*

Link: https://doi.org/10.1016/S0140-6736(20)30854-0

Summary:

Bnerjee et al. estimated the excess number of deaths over 1 year under different COVID-19 incidence scenarios based on varying levels of transmission suppression and differing mortality impacts based on different relative risks for the disease. Pre-COVID-19 1-Year baseline mortalities were first estimated. In a full suppression scenario, it was estimated there would be two excess deaths (vs baseline deaths) with a relative risk (RR) of 1.5, four (RR 2.0), and seven (RR of 3.0). In contrast, a do-nothing scenario would result in 146 996 excess deaths (RR of 1.5), 293 991 (RR of 2.0), and 587 982 (RR of 3.0).

Main Findings:

- Study included 3,862,012 individuals aged 30 years or older. 1,957,935 (50.7%) were women and 1,904,077 (49.3%) were men
- 3,331,280 (86.3%) were aged 70 years or younger (mean age 43·5 years [SD 11.7] in both sexes) and 530,732 (13.7%) were older than 70 years (mean age 78.1 years [6.1] in men and 80.2 years [7.0] in women
- 20% of the study population had at least one high-risk condition defined by Public Health England guidelines
- 13.7% were older than 70 years and 6.3% were aged 70 years or younger with at least one underlying condition
- Multimorbidity was common (10.1%), especially with cardiovascular disease, diabetes, chronic kidney disease, and COPD
- Mortality at 1 year was estimated at 4·46% (95% CI 4.41–4.51) among individuals at high risk
- 1-year mortality was 8·62% (8.32–8.92) for those with COPD, 7.84% (7·62–8·06) for those with chronic kidney disease, 6·37% (6·25–6·49) for those with cardiovascular disease, and 4·1% (4·0–4·2) for those with diabetes
- For those older than 70 years and male, 1-year mortality rates were 13·6% (12·9–14·3) for COPD, 11·5% (11·0–12·1) for chronic kidney disease, 10·4% (10·1–10·7) for cardiovascular disease, and 8·9% (8·5–9·4) for diabetes in men
- For women older than 70 1-year mortality rates were 12·3% (11·6–13·0) for COPD, 9·6% (9·3–10·0) for chronic kidney disease, 10·6% (10·3–10·9) for cardiovascular disease, and 9·8% (9·4–10·2) for diabetes in women
- At COVID-19 prevalence of 10% (mitigation) and 80% (do nothing), estimated the number of excess deaths to be 18 374 (mitigation) and 146 996 (do nothing) with an

RR of 1.5; 36 749 (mitigation) and 293 991 (do nothing) with an RR of 2·0; and 73 498 (mitigation) and 587 982 (do nothing) with an RR of 3·0

- Corresponding numbers of deaths at full suppression (0·001%) and partial suppression (1%) were two (full) and 1837 (partial) with an RR of 1·5; four (full) and 3675 (partial) with an RR of 2·0; and seven (full) and 7350 (partial) with an RR of 3·0
- Extrapolation to whole UK population estimated 376,001 deaths would occur with 8.43 million people in the high-risk group, of whom 5·77 million are older than
- 70 years of age, and 2·66 million have one or more underlying condition and are 70 years or younger
- Numbers of people at high-risk in other countries include 4.70 million in Canada, 8.31 million in France, 10.46 million in Germany, 7.51 million in Italy, 1.28 million in Sweden, and 41.76 million in the USA

Highlights:

- Patients with conditions not on the vulnerable patient list may be at as high or greater mortality than those who are (eg. cardiovascular disease)
- 1-year mortality estimates extrapolated to other countries shown below (adapted from *Figure 1*)

	General population (millions)		**High-risk group (millions)**			**Baseline number of deaths in 1 year for high-risk group**
	Total	> 30 years	> 70 years	=< 70 years with underlying condition	Total at high risk	
Canada	37.06	23.44	3.22	1.48	4.70	209,732
England	55.98	35.41	4.87	2.24	7.10	316,805
Germany	65.50	41.43	5.69	2.62	8.31	370,681
Italy	59.20	37.45	5.15	2.37	7.51	335,028
Sweden	10.10	6.39	0.88	0.40	1.28	57,158
UK	66.44	42.03	5.77	2.66	8.43	376,001
USA	329.10	208.17	28.60	13.16	41.76	1,862,459

Clinical Impact:

- Reiterates importance of sustained stringent suppression measures as well sustained efforts to target those at highest risk because of underlying conditions with a range of preventive interventions

Important Methodologies:

- CALIBER as a data source; an open research platform with validated, reusable definitions of several hundred underlying conditions by linking health records from different data sources including primary care, hospital care and death registry

Limitations:

- Assumed the same impact (RR) of COVID-19 on excess mortality for each underlying condition, empirical data required to confirm this
- Relative risks will rise in a non-linear fashion with infection rates if health systems become overwhelmed by critically ill patients and require further modelling
- Possible that underestimated true 1-year mortality risks because physical distancing policies might themselves influence the background mortality risks
- Study was developed over a 72-h period before posting the results on March 22, 2020, the day before lockdown measures announced

Targeted Immunosuppression Distinguishes COVID-19 from Influenza in Moderate and Severe Disease

Mudd, P. A. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.05.28.20115667

Summary:

In an effort to understand the aetiology of respiratory failure in COVID-19, Mudd *et al*., 2020 report a comparative analysis of cellular and molecular profiles for 79 severe COVID-19 patients, 26 symptomatic influenza A/B patients and 8 healthy controls. Using a combination of single cell gene expression analyses and data-driven modular informatics, this study interrogates the expression profiles of circulating cell types, cytokines, gene expression and pathway analyses between groups and infer the contributions of cytokines in the development of COVID-19-related immunosuppression.

Main Findings:

- COVID-19 patients have reduced monocytes and HLA-DR expression on CD4+ T-cells but significantly more circulating early-antibody secreting B-cell plasmablasts in comparison with influenza or control subjects
- COVID-19 patients show two distinct cytokine profiles: 3/79 patients exhibited a classic cytokine storm; remaining patients showed significantly increased IL-6 and IL-8 but a decrease in expression of 28/35 cytokines promoting immunosuppression (GM-CSF, IFN-γ and IL-9) compared with influenza patients
- Lower levels of GCSF, IFN-γ, IL1-β, Eotaxin, HGF, IL1-Ra, IL-2R, IL-6, IL-8, IP10, MCP1, MIG, and VEGF were associated with testing positive for COVID-19
- IFN-γ and IFN-α response pathways were significantly down-regulated in COVID-19 for B cells, CD8+ T-cells, MCLPs, TRegs, PDCs, and monocyte/macrophage subsets extending to IFN-β and general type I IFN pathways across most subsets, particularly monocytes

- COVID-19 cells were significantly enriched for a number of pathways involved in cellular metabolism and proliferation
- Overall, higher inflammation is predictive of influenza infection whilst COVID-19 disease is generally characterised by reduced inflammation

Highlights:

- The identification of a metabolic stress response in COVID-19 patients potentially through IL-6 driven up-regulation of glucocorticoid production resulting in immune suppression highlights the need for further studies.

Clinical Impact:

- Moderate.

Important Methodologies:

- Single-cell RNAseq of patient peripheral blood mononuclear cells combined with data-driven modular informatics to determine unbiased cytokine response profiles between disease groups.

Limitations:

- Given the evidence of a potential link between IL-6 driven glucocorticoid signalling and immunosuppression in COVID-19 patients, stratification of all patients for weight may be informative.

Emergence of Low-density Inflammatory Neutrophils Correlates with Hypercoagulable State and Disease Severity in COVID-19 Patients

Morrissey, S. M. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.05.22.20106724

Summary:

Morrissey et al. identify emergence of a novel subset of neutrophils ($CD16^{int}CD44^{low}CD11b^{int}$) in peripheral blood of patients with severe COVID-19. These cells have a unique 'band-shaped nucleus' and were termed low-density inflammatory band cells (LDIBs). The study concludes that LDIBs contribute to COVID-19-associated coagulopathy (CAC) and could potentially be used as a clinical marker to monitor COVID-19 status and progression.

Main Findings:

- LDIBs were 'virtually absent' in healthy donors and could only be isolated from COVID-19 patients.
- Increases in LDIBs were associated with disease severity, intubation and mortality, whereas, decreases in this population trended with extubation and hospital discharge.
- Functional analysis revealed that LDIBs have phagocytic capacity, enhanced cytokine production and could spontaneously form neutrophil extracellular traps.
- Upon LPS-stimulation, LDIBs from severe COVID-19 patients produced significantly higher amounts of IL-6 and TNF-α compared to LDIBs isolated from moderate patients.
- Levels of D-dimer and ferritin (clinical markers of coagulation and systemic inflammation, respectively) were positively correlated with percentage of LDIBs in severe COVID-19 patients.

Highlights:

- Dynamic changes in LDIB neutrophil population correlates with disease status and progression.
- Excessive neutrophilia, particularly increase in LDIBs, contributes to the cytokine storm seen in COVID- 19 patients.

Clinical Impact:

- Medium/ High

Important Methodologies:

- Phenotypic characterisation of neutrophil subsets in patients throughout their clinical course of disease.

Limitations:

- Small patient & healthy donor cohort (n = 19; 7 severe, 6 moderate and 6 healthy).
- Participants were not age- and sex-matched (but had similar age mean/range).
- D-dimer and cytokine levels were not measured frequently in participants.
- Did not assess effects of any given medications on neutrophils and inflammatory mediators (six COVID-19 patients were receiving Hydroxychloroquine + Azithromycin treatment, which could have contributed to differences observed).

Increased serum levels of sCD14 and sCD163 indicate a preponderant role for monocytes in COVID-19 immunopathology

Gómez-Rial, J. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.06.02.20120295

Summary:

Suggested that COVID-19 pathology involves macrophage/monocytes given observations of monocyte-derived macrophages influx in damaged lungs and how blood monocytes correlate to patient outcome.

Measured monocyte/macrophage activation markers sCD14 and sCD163 by ELISA, both sCD14 and sCD163 higher in SARS-CoV-2 patients than controls. In non-ICU patients these markers correlated to several other clinical markers. Correlations were not observed in the ICU group proposed this was due to tocilizumab (IL-6 blocker) or corticoid treatment which interfere with monocytes.

Main Findings:

- 59 SARS-CoV2 patients were split into ICU (22) and non-ICU (37) and assessed alongside 20 aged-matched healthy controls.
- sCD14 and sCD163 were higher in SARS-CoV2 patients compared to controls but did not predict disease severity (i.e. ICU or non-ICU).
- Non-ICU patients' sCD14 correlated positively to IL-6, LDH, C-reactive protein, Procalcitonin and ferritin, clinical markers of inflammation/tissue damage.
- Correlations were not seen in ICU samples, suggested that tocilizumab/corticoid treatment interferes with monocytes/macrophages.
- sCD14 and sCD163 appeared to negatively correlate to lymphocyte number.
- Absolute monocyte numbers did not differ, again treatment in ICU group may have impacted these results.
- A positive correlation between sCD163 and delay between admission to sample collection suggested sCD163 as a potential indicator of disease progression.
- Overall results suggest monocyte/macrophage activation contributes to exacerbated immune response.

Highlights:

- Monocyte activation marks, sCD14 and sCD163, higher in SARS-CoV-2 patients and positively correlated with other clinical markers. Propose monocytes be targeted therapeutically.

Clinical Impact:

- Mid, suggest SCD163 may indicate disease progression but poorly correlated to other measures.

Important Methodologies:

- ELISA for sCD14 and sCD163.

Limitations:

- Not yet peer reviewed.
- Low patient numbers tested.
- Interference of tocilizumab/corticoid treatment in ICU group may have impacted results.
- Disparity between figure and text regarding sCD163 negative correlation to lymphocyte number.

An inflammatory cytokine signature helps predict COVID-19 severity and death

Del Valle, D. M. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.05.28.20115758

Summary:

Del Valle et al followed 1,484 patients with suspected or confirmed COVID-19 at the Mount Sinai hospital from admission to the day of discharge or death. They measured serum IL-6, IL-8, TNF-a, and IL-1b levels upon admission and correlated these results with clinical and laboratory markers of disease severity and with disease outcome. They found that elevated IL-6 and TNF-a serum levels at presentation were strong predictors of disease severity and survival independently of standard clinical biomarkers of disease severity, including laboratory and clinical factors. These results suggest that multiplex cytokine profiling should be used to stratify patients and guide resource allocation and prospective interventional studies.

Main Findings:

- IL-6, IL-8 and TNF alpha elevated in COVID-19 serum compared to healthy donor serum or plasma from CAR-T cell treated patients with no CRS.
- Men had higher levels of IL-6 and IL-6, IL-8 and TNF alpha increased with age.
- Age and chronic kidney disease were significantly associated with increased risk of death.
- IL-6, IL-8 and TNF alpha significantly associated with worse outcome regardless of demographics and co-morbidities.
- Treatment with corticosteroids and Remdesvir showed a reduction in IL-6 overtime but did not effect TNF alpha levels.
- COVID-19 patients had sustained cytokine levels over days and weeks.

Highlights:

- High serum IL-6, IL-8 and TNF alpha at hospitalisation are predictors of patient survival.
- IL-6 and TNF alpha serum levels are independent predictors of severity and death.

Clinical Impact:

- High – IL-6 and TNF alpha levels should be considered in management and treatment of COVID-19 patients.

Important Methodologies:

- ELLA cytokine test – rapid cytokine detection system based on four parallel singleplex microfluidics ELISA assays.

Limitations:

- Sample size available.

Mouse Model

A Mouse Model of SARS-CoV-2 Infection and Pathogenesis

Sun, S-H. *et al*. 2020. *Cell Host & Microbe*

Link: https://doi.org/10.1016/j.chom.2020.05.020

Summary:

Sun *et al*. generated a mouse model expressing human ACE2 (hACE2) by using CRISPR/Cas9 knockin technology. In comparison with wild-type C57BL/6 mice, both young and aged hACE2 mice sustained high viral loads in lung, trachea, and brain upon intranasal infection. Although no fatalities, interstitial pneumonia and elevated cytokines were seen in SARS-CoV-2 infected-aged hACE2 mice. Interestingly, intragastric inoculation of SARS-CoV-2 was seen to cause productive infection and lead to pulmonary pathological changes in hACE2 mice. This animal model could be a useful tool for studying SARS-CoV-2 transmission and pathogenesis and evaluating COVID-19 vaccines and therapeutics.

Main Findings:

- An hACE2 Humanized Mouse Was Established humanized ACE2 mouse by using CRISPR/Cas9 knockin technology.
- Robust viral RNA replication was seen in lung, trachea, and brain tissues from both young and aged hACE2 mice, whereas no detectable viral RNA was seen in spleen, kidney, liver, intestine, and serum. High levels of viral RNAs in faeces of aged hACE2 mice.
- The hACE2 mice are highly susceptible to intranasal infection of SARS-CoV-2 in comparison with WT mice, with sustained robust viral RNA replication in lung Clara cells as well as in trachea and brain.
- The results demonstrated that intragastric administration of SARS-CoV-2 could also establish productive infection and lead to pulmonary pathological changes in hACE2 mice.

Highlights:

- Establishment of Covid-19 animal model with a stable humanized ACE2 to study the dynamics of viral infection along with organ specific issues.

Clinical Impact:

- Minimal

Important Methodologies:

- CRISPR/Cas9 knockin
- RT-PCR

- Western blotting
- Immunohistochemistry
- Multiplex Immunofluorescent Assay

Limitations:

- None

Transmission

Full genome viral sequences inform patterns of SARS-CoV-2 spread into and within Israel

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

Link: https://doi.org/10.1101/2020.05.21.20104521

Summary:

Miller et al. sequenced 212 SARS-CoV-2 samples in Israel in order to trace the origins and spread of the virus. A phylogenetic analysis revealed multiple introductions into Israel, followed by local transmission, being travellers from the U.S. the ones who had a greater contribution to the spread. The implementation of social distancing measures allowed the basic reproduction number of the virus to drop two-thirds from its initial value (~2.0-2.6). A comparison between reported and model-estimated case numbers showed that between 1-10% of infected individuals resulting in 80% of secondary infections. These findings emphasize the efficient virus transmission between and within countries, as well as the effectiveness of social distance measures.

Main Findings:

- 224 unique single nucleotide variants were identified – non-synonymous variant D614G found in the spike protein was amongst the most abundantly detected variants.
- 5 different genomic deletions were found - #3 spans 10 nucleotides and likely prevents ORF7a translation, #4 occurred at the end of ORF8 and causes the replacement of the last amino acid for 5 additional amino acids.
- Multiple introductions of the virus in Israel from the U.S. (70%) and Europe (U.K., Belgium, France)
- Phylodynamic inference based on the susceptible-exposed-infected-recovered (SEIR) epidemiological model revealed that 1-9% of infected individuals were responsible for 80% of secondary infections.

Highlights:

- Superspreading events are a main feature of SARS-CoV-2 spread, suggesting that focused measures to reduce contacts of select individuals/social events could dramatically mitigate viral spread

Clinical Impact:

- Minimal

Important Methodologies:

- Use of genomic data to effectively track the spread of an emerging virus using phylogenetic and phylodynamic approaches that have been developed to study viral outbreaks

Limitations:

- Preprint has yet to be peer-reviewed

Prevalence of SARS-CoV-2 Infection in Residents of a Large Homeless Shelter in Boston

Baggett, T. P. *et al*. 2020. *JAMA*

Link: https://doi.org/10.1001/jama.2020.6887

Summary:

The authors present the findings from a 'Boston Healthcare for Homeless Program' (BHCHP) and partners initiative. This consisted of screening on-the-door with tracking and tracing of positive individuals. They identified a single large homeless shelter responsible for an increasing number of COVID-19 cases and residents were recruited for assessment which included self-reporting of symptoms, body temperature measurements and qPCR testing of nasopharyngeal swabs for SARS-CoV-2. 11.5 % of participants reported no symptoms and 36 % were positive for SARS-CoV-2. Of the SARS-CoV-2 positive participants, 87.8 % were asymptomatic.

Main Findings:

- 1 % report fever, 8.1 % reported cough, 0.7 % reported shortness of breath, 5.9 % reported other symptoms (incl. 1.5 % nasal and sinus symptoms, 1.2 % diarrhoea). 88.5 % of residents reported no symptoms (11.5 % reported symptoms).
- 36 % were positive by PCR test for SARS-CoV-2. 84.4 % of these were men, although there were more male participants (71.6 %).
- Among SARS-CoV-2 positive participants, cough, shortness of breath and fever were uncommon.

Highlights:

- 87.8 % of participants who were positive for SARS-CoV-2 were asymptomatic.
- All data is presented clearly in table 1 which can be independently analysed.

Clinical Impact:

- Moderate, this could have an impact on track and trace practises and makes the case for regular testing for asymptomatic individuals.

Important Methodologies:

- 408 residents not previously diagnosed with COVID-19 were assessed, this included self-reporting age, sex, race, ethnicity, presence of cough, shortness of breath with the option to report any further symptoms. Oral thermometers were used to assess body temperature with fever defined as 37.8 ºC. SARS-COV-2 PCR of nasopharyngeal specimens was used to determine participant infection.

Limitations:

- The authors state the percentage of participants who are male, and the percentage of participants infected who were male. It would be more useful to state, the percentage of SARS-CoV-2 positive individuals *within* each gender. This would remove the bias of less females being recruited to the study. However, this can be calculated from the data in table 1 i.e. whilst 84.4 % of positive participants were male, 42.5 % of males recruited were positive and 19.1 % of recruited females were positive.
- Whilst not a limitation, it is interesting to note a study in the Lancet (Treibel et al, 2020; review in COVID19 Journal club 7) found 7.1 % of NHS healthcare workers were positive and asymptomatic at the peak of the epidemic in the UK.

Introductions and early spread of SARS-CoV-2 in the New York City area

Gonzalez-Reiche, A. S. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.04.08.20056929

Summary:

Gonzalez-Reiche et al. present the results obtained by a phylogenetic analysis of SARS-CoV-2 isolates obtained from 84 patients seeking care at the Mount Sinai Health System between February 29, 2020 and March 18, 2020, together with 2,363 sequences deposited in GISAID up to April 1, 2020.

Main Findings:

- NYC-SARS-CoV-2 epidemic has been mainly sourced from untracked transmission between the US and Europe

Highlights:

- If we consider New York City as an international hub, the data here shown provides not only a snapshot of the diversity of the disease-causing SARS-CoV-2 at a global level but also informs on the dynamics of the pandemic at the local level.

Clinical Impact:

- Minimal

Important Methodologies:

- Collection of nasopharyngeal swab samples that tested positive for SARS-CoV-2. Tests were done by using Roche LightCycler 480 II and the confirmatory testing and viral load determination by qRT-PCR
- Whole-genome amplification and sequencing. Sample preparation for sequencing using the Artic Consortium protocol and designed tiling primers. Sequencing by Illumina and PacBio.
- Phylogenetic analysis. Global background sequences were downloaded from GISAID EpiCoV database. The alignment was done with MAFTT v7.455 and the ML tree was inspected in TempEst for outliers. Bayesian phylodynamic inferences were done for each of the major clades that contained putative NYC introductions.

Limitations:

- Small number of global isolates from cases identified in the first weeks of March 2020
- Absence of detailed epidemiological data on travel history and contacts.

Vaccines

Landscape and Selection of Vaccine Epitopes in SARS-CoV-2

Smith, C. C. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.06.04.135004

Summary:

Smith and colleagues propose that an optimal SARS-CoV-2 vaccine should stimulate $CD4^+$ and $CD8^+$ T cells and B cells, to induce a Th1 response, cytotoxicity/ immune memory and neutralising antibodies, respectively. Accordingly, they describe large scale prediction and criteria-based selection of combined T cell and B cell epitopes from SARS-CoV-2.

Main Findings:

- *In silico* analysis identified 2486 and 3138 unique HLA-I and HLA-II ligands, respectively. There were 7344 'nested' ligands, for which an HLA-I sequence was embedded within an HLA-II sequence. The number of ligands was proportional to protein length.
- Ligand binding affinity correlated better with tetramer positivity for HLA-II ligands ($CD4^+$ epitopes), compared to HLA-I ligands/ $CD8^+$ epitopes.
- The final set of T cell epitopes consisted of 292 $CD4^+$, 616 $CD8^+$ and 423 nested epitopes.
- Of 58 linear epitopes from n=5 studies, 19 regions of the Spike protein fit the linear sequence accessibility criteria for B cell epitope identification. After further filtering, 3 candidate epitope regions, 18, 4 and 4 amino acids in length, were identified. Structural analysis showed the 18mer region is only accessible when the RBD is in an open confirmation.
- The T cell epitope discovery results were collected into an optimised set of 22 27mer sequences with the best population coverage and ease of peptide manufacturing. Adding the B cell epitopes to this set reduced population coverage of $CD4^+$ epitopes to a greater degree than the $CD8^+$ epitopes, indicating less sequence overlap between B cell and $CD4^+$ T cell epitopes.
- scRNAseq analysis of immunomodulatory receptor expression in $ACE2^+$ cells identified potential targets e.g. upregulation of VISTA, Galectin 9, for combination therapy.

Highlights:

- A novel, multi-layer criteria-directed pipeline which identifies an optimal set of $CD4^+$, $CD8^+$ and B cell epitopes for future vaccine development, with additional consideration of adjuvant design and manufacturing.

Clinical Impact:

- Moderate - the comprehensive method for identifying 'multi-tasking' (overlapping) T and B cell potential epitopes from SARS-CoV-2, suitable for testing in mouse models, could accelerate vaccine testing.

Important Methodologies:

- HLA-I, HLA-II and murine MHC (H2-b/d) ligand predictions were made *in silico* using NetMHCpan 4.0, NetMHCflurry 1.6.0 (HLA-I) and NetMHCIIpan 3.2 and 4.0, and individual affinity thresholds were applied to HLA-I and HLA-II predictions.
- T cell epitopes were derived from the ligand data by comparing binding affinity and elution data from the predictive tools and intrinsic sequence features (amino acid characteristics) with viral tetramer binding data from the IEDB, to give an immunogenicity score. Epitopes were further filtered by entropy and protein expression levels.
- B cell epitopes on the Spike protein were identified from previously described *in vivo* responses: studies that analysed convalescent sera or neutralising antibodies. Further criteria applied were linear, accessible sequences, not glycosylated, low polymorphism, sequentially proximal to functional regions and > 4 amino acids.
- Sequence polymorphism (entropy), was calculated by sequence alignment of 79 SARS-CoV-2 genome sequences and applying a formula which accounted for which the number of possible amino acids and the probability of each amino acid being in each position.

Limitations:

- T cell frequency and responses *in vivo* to the predicted epitopes were not experimentally tested.
- B cell epitopes were identified from only 5 previous studies, novel epitopes were not investigated. Additionally, 3D epitopes were not considered since the study aimed to identify concurrent B and T cell epitopes. Therefore, only a small number of B cell epitopes fit the criteria to add to the set of proposed vaccine sequences.
- The exclusion of polymorphic sequences, intended to maximise vaccine responses in the population, could eliminate immunogenic mutated epitopes that occur due to selective pressure.

How Efficacious Must a COVID-19 Coronavirus Vaccine be to Prevent or Stop an Epidemic by Itself

Bartsch, S. M. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.05.29.20117184

Summary:

Bartsch and colleagues have developed a computational model of SARS-CoV-2 spread, in the absence of control measures, and assessed the impact of vaccination in the USA in terms of both health and economic outcomes. The model allows comparison between different R_0 rates, vaccine efficacy and coverage and the time-point at which vaccination occurs. The authors conclude that to prevent or extinguish a SARS-CoV-2 pandemic, in the absence of other measures (social distancing etc.), vaccination would need to occur within 90 days of the start of the epidemic and have an efficacy of at least 70% and cover at least 60% of the population.

Main Findings:

- The model covers a 2.5-year time frame from epidemic start and depicts how vaccination may both reduce the number of cases and spread the case load over a longer period of time. *
- Models pandemic outcomes for a number of variables; vaccine efficacy (20% -100%), population coverage (50% - 100%), timing of vaccination from epidemic start (0 - 180 days), a R_0 of either 2.5 or 3.5. The model also examines two vaccination outcomes; preventing infection and preventing symptomatic disease (i.e. viral shedding).

Highlights:

- Suggests that vaccine efficacy will need to be at least 70% to control a pandemic and the later vaccination starts the higher the vaccine efficacy will need to be.

Clinical Impact:

- If the model is adaptable to the profiles of vaccines currently in development this model could be useful for estimating the coverage needed in different populations.

Important Methodologies:

- Computational model. Unclear if this model is available to other users to enter their own parameters

Limitations:

- Makes a number of assumptions i.e. vaccine protection is immediate
- * Epidemic curve figures are poor quality and unintelligible for some of the data.
- Authors compare different coverage rates for different vaccine efficacies. Data would have been clearer if the same variables were included for each comparison.

Virus Detection and Infectivity

Clearance and Persistence of SARS-CoV-2 RNA in COVID-19 patients

Carmo, A. *et al*. 2020. *Journal of Medical Virology*

Link: https://doi.org/10.1002/jmv.26103

Summary:

Better understanding of SARS-CoV-2 viral clearance is needed to refine isolation periods and permit patient discharge from hospital. Retrospectively, Carmo *et al*. evaluated the number of days that COVID-19 patients need before SARS-CoV-2 levels in the upper respiratory tract become undetectable from both nasopharyngeal and oropharyngeal swabs. Subgrouping patients and correlating their findings with serum IgG levels, they found that the majority of COVID-19 patients test positive for over two weeks. In addition, viral RNA persistence may better associate with a weaker immune response rather than disease severity.

Main Findings:

- 210 patients with detectable SARS-CoV-2 RNA were divided into two groups – those who displayed two consecutive negative swab results (55.2%) and those who remained positive (44.8%).
- **Two Consecutive negative results** – 69.8% of these patients needed more than 20 days to achieve their first negative test.
- Most of these patients were discharged with mild illness (58.5%) while 39.8% were inpatients with moderate to severe illness. The typically younger discharged patients took longer to achieve their first negative result than inpatients, who were older (26.3 days vs. 22.5 days, $p = 0.027$).
- **Remained positive** – 32.55 days passed between the first and last positive tests. Women remained positive for longer than men (34.2 days vs. 28.7 days).
- 24.4% of these patients had received a negative result at least once, although this associated with a longer duration of overall positive result ($p<0.001$).
- 61.2% of these patients were discharged whilst 38.3% were inpatients. Discharged patients remained positive for a significantly longer duration than inpatients (35.38 vs. 28 days, $p<0.001$).
- **Serum IgG levels** were found in 73.4% of 79 patients that achieved two negative results and 67.9% of 84 that remained positive. These values contain patients who were also IgM positive.
- In both groups, IgG levels were significantly lower in patients that were discharged.

Highlights:

- Revealed that the majority of COVID-19 patients have detectable viral levels for over 20 days. Patients remain positive even after seroconversion.

- May guide those who lack testing resources. Re-testing may only be needed after the 20th day from the first positive result.

Clinical Impact:

- Low

Important Methodologies:

- RT-PCR to detect SARS-CoV-2 RNA in either nasopharyngeal or oropharyngeal swabs.
- Chemiluminescent analytical system to detect serum IgM and IgG levels.

Limitations:

- Limited study design – small final cohort of patients from a single ICU unit in Portugal.
- RT-PCR levels doesn't evaluate the infectious capacity SARS-CoV-2 – may not aid in isolation periods.

Predicting infectious SARS-CoV-2 from diagnostic samples

Bullard, J. *et al*. 2020. *Clinical Infectious Diseases*

Link: https://doi.org/10.1093/cid/ciaa638

Summary:

RT-PCR (E gene) cannot tell the presence of infectious virus and this paper aimed to determine RT-PCR cycle threshold (Ct) values and symptom onset to test (STT) which can indicate COVID-19 infectivity. After analysing 90 samples, the authors found COVID-19 Vero cell infectivity was only observed for RT-PCR Ct < 24 and STT < 8 days. Infectivity of patients with Ct >24 and duration of symptoms >8 days were low.

Highlights:

- COVID-19 infectivity (as defined by growth in cell culture) is significantly reduced when RT-PCR Ct values are greater than 24 and the durations of symptoms are longer than 8 days.
- This result could be used to predict the period of maximal transmission risk. This paper supported clinical criteria for discontinuing isolation of hospitalized COVID-19 cases: 14 days from symptom onset or 72 hours symptom free, as RT-PCR positivity persists beyond infectivity.

Clinical Impact:

- Moderate

Important Methodologies:

- Retrospective cross-sectional study
- Tissue Culture Infectious Dose 50% (TCID50) Assay

Limitations:

- Only focused on a single gene target of COVID-19
- Could have recall bias of symptom onset.
- Patients' cases and assay have variations.
- Small sample size.