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COVID-19 Community Journal Club No. 12

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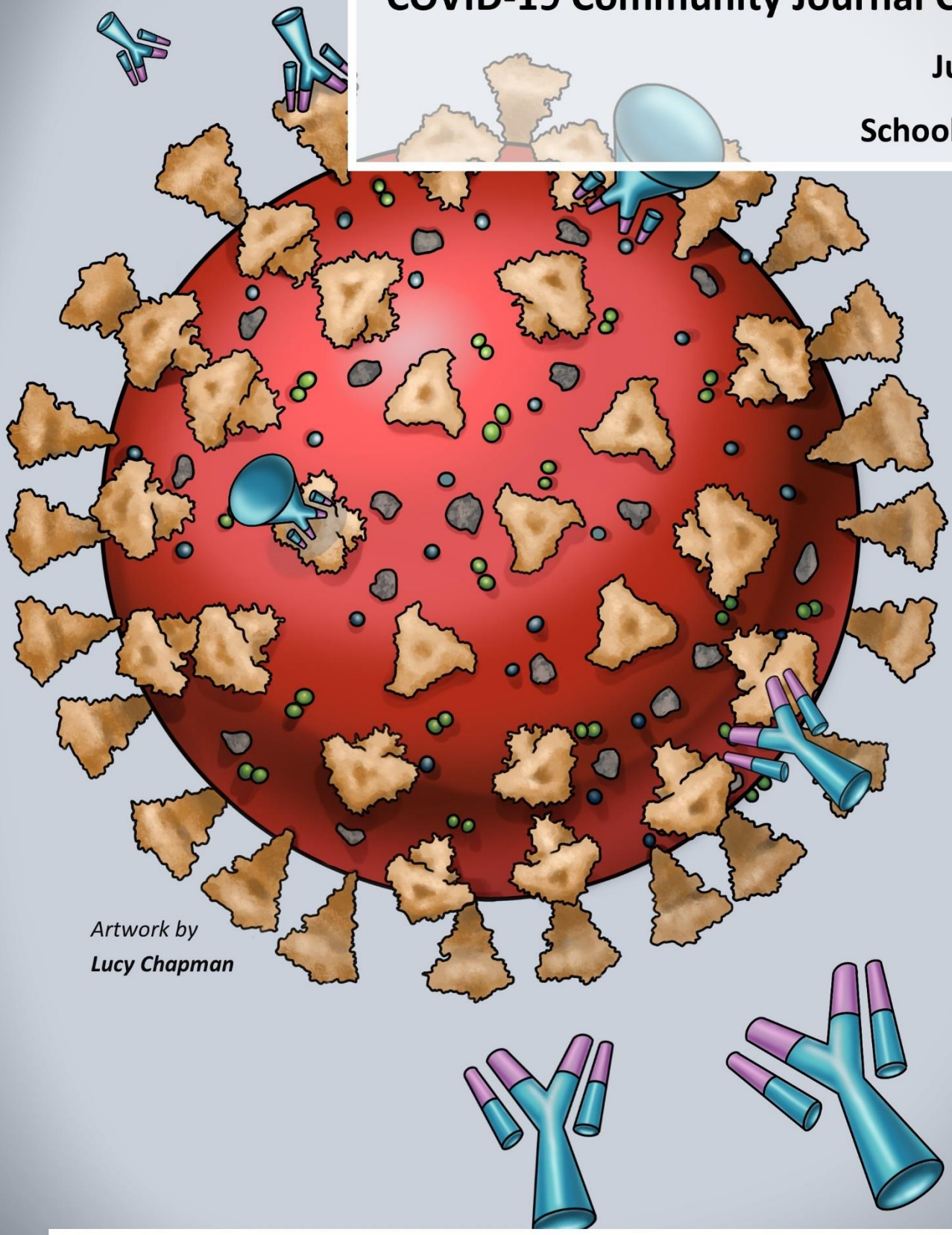

Artwork by
Lucy Chapman

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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Oliver Scourfield for compiling the digest.

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

All previous editions of the Community Journal Club can be found at:
https://www.cardiff.ac.uk/news/view/2260179-getting-to-grips-with-covid-19/_recache

‘Science has a racism problem. Scientists are problem solvers. Let’s get to it.’

(The Cell Editorial Team, 2020)

The Black Lives Matter movement has forced society worldwide to acknowledge its problem with racism, and several top scientific journals (e.g. *Cell, Science, NEJM, and Nature)* have highlighted once again that science is not immune to racism. These journals have reiterated ‘The message is clear: it is not enough to simply be not racist; one must work harder to be anti-racist.’ With this comes the understanding of both unconscious bias and white privilege – something we need to consider in every aspect our lives.

The first stage of treating a disease is diagnosis, and these journals have taken a good first step in bringing this to light and suggesting changes to their practices. *Nature Methods* have promised to ‘carefully consider inclusion when inviting researchers to contribute reviews or opinion pieces’ and to ‘feature more Black scientists and other under-represented minorities in our magazine content.’ In addition, they ‘will do more outreach, including giving talks about publishing and about editorial careers, to a more geographically diverse audience, and reach out to younger students at early career stages.’ *Cell* has laid out a plan that includes: Increasing representation of minorities, diversifying the advisory board and reviewer pool, listening to minorities, and educating themselves. They end with the message: ‘Science has a racism problem. Scientists are problem solvers. Let’s get to it.’

As scientists, we can do our own small part by being anti-racist – maintaining vigilance toward and calling out racism wherever we see it, taking courses aimed at identifying our own unconscious bias, and targeting our outreach and educational programs toward underrepresented groups and areas. Additionally, our research, especially clinical, should take into context other geographical areas and ethnicities to improve the scope of future treatments. Tackling racism and bias will increase diversity in science, empower current researchers and further increase the pool of creative and talented researchers, educators, and clinicians for a more egalitarian future.

Luke Davies, Freya Shephard, Linda Moet and Patricia Rodrigues (on behalf of the COVID-19 JC Community).

https://science.sciencemag.org/content/368/6496/1161?rss=1
https://www.cell.com/cell/fulltext/S0092-8674(20)30740-6
https://www.nejm.org/doi/full/10.1056/NEJMe2021693
https://www.nature.com/articles/s41592-020-0908-7

For an open letter from the Vice Chancellor of Cardiff University, Colin Riordan, please click here.

Highlighted Papers – Cardiff University Authors

Dr. Jonathan Hewitt
Division of Population Medicine, Department of Surgery

The effect of frailty on survival in patients with COVID-19 (COPE): a multicentre, European, observational cohort study

Professor David Price and Sian Llewellyn-Lacey
Division of Infection and Immunity, School of Medicine

Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19

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Focus on Antibody Diagnostics

This review is a collective update on the use of antibody tests to diagnose COVID-19 and references papers previously reviewed in the community journal club as well as those obtained from literature search.

CAUTIONARY NOTE:

SOME REVIEWS ARE OF PAPERS POSTED ONLINE (in *arXiv, bioRxiv and medRxiv)* BEFORE PEER REVIEW.

Highlighted Papers – Cardiff University Authors

The effect of frailty on survival in patients with COVID-19 (COPE): a multicentre, European, observational cohort study

Hewitt, J. *et al* 2020. *The Lancet Public Health*

Link: https://doi.org/10.1016/S2468-2667(20)30146-8

Summary:

Clinical assessments of frailty are currently utilised in care decisions for COVID-19 patients, although prevalence and outcomes have not been adequately evaluated. This study provides valuable insights into the impact of clinically-determined frailty for mortality and length of hospitalisation amongst a cohort of 1564 UK and Italian adult patients (median age 74 years) admitted to 11 hospital sites with COVID-19 between February 27th and April 28th 2020. Independent of age and defined comorbidities, higher clinical frailty scores (CFS) were associated with increased mortality rates and time to discharge. The findings will inform decisions concerning treatment stratifications and shielding guidelines.

Main Findings:

- 49.4% 1564 UK and Italian adult (> 18 years) patients admitted to or diagnosed within hospital with COVID-19 were clinically classified as frail (Clinical Frailty Scale 5-8); 1.7% were terminally-ill (CFS 9). In contrast, only 18.41% were categorised as fit (CFS 1-2).
- In-hospital mortality was 35.49% for CFS 5-8 COVID-19 patients compared with 10% of CFS 1-2 COVID-19 patients.
- Adjusted mortality hazard ratios (HR) compared with the fitter CFS 1-2 group were; 1.55 for CFS 3-4 (95% CI 1.00-2.41; p=0.052), 1.83 for CFS 5-6 (95% Confidence Intervals 1.15-2.91; p=0.011) and 2.39 for CFS 7-9 (95% CI 1.50-3.81; p<0.0002).
- COVID-19 patients in higher CFS frailty categories also experienced longer durations of hospital stay; >50% all CFS 1-4 patients but <30% all CFS 5-9 patients were discharged within 3 weeks, with twice as many CFS 5-9 patients censored at the date of death for this analysis.
- Adjusted time to discharge HR compared with CFS 1-2 COVID-19 patients were 0.94 for CFS 3-4 (95% CI 0.77-1.16; p=0.58), 0.7 for CFS 5-6 (95% CI 0.54-0.91; p=0.0084) and 0.66 for CFS 7-9 (95% CI 0.5=0.87; p=0.0035).
- Age, C-reactive protein (CRP) levels and renal function were each also associated with COVID-19 mortality risk.
- Adjusting for age and defined comorbidities (hypertension, diabetes, coronary artery disease, renal function, CRP levels, smoking status) did not alter frailty-associated risk, suggesting CFS represents an important independent variable.

Highlights:

- COVID-19 disease outcomes in adult patients admitted to UK hospitals are more robustly predicted by clinical frailty scale than age or comorbidities. Combinatorial assessment of these risk factors may better inform decisions concerning critical care.

Clinical Impact:

- Moderate.
- NICE guidelines currently recommend taking clinical frailty (assessed by CFS) into consideration for COVID-19 patients >65 years as part of a holistic appraisal of critical care need and intervention. This study supports the importance of frailty evaluation in critical care decisions and may provoke revisions of strategies for younger patients with higher CFS scores. Further studies are required to sponsor any substantial modifications of extant NICE recommendations.

Important Methodologies:

- Establishment of multicentre European observational cohort study; COVID-19 in Older PEople (COPE) study.
- Standardised tripartite diagnosis of clinical frailty and CFS assignment according to NICE guidelines.

Limitations / Comments:

- The variation in hospital cohort sizes (n=9-380) and death rates (11-44%) was substantial, suggesting additional confounding variables.
- Frailty assessments are reportedly not well-validated in patients <65 years or those with learning difficulties. This may circumvent accurate interpretation of risks associated with frailty in younger patients.
- Comparison of the COVID-19 risks associated with CFS with other diseases requiring hospital admission (e.g. influenza) would help ascertain any particular clinical value of this assessment in COVID-19 patients. Is frailty a risk factor for all hospital admissions?
- A graphical display of the data in Supplementary Table 1 would aid comprehension of the influence of frailty by age groups – particularly since the authors assert that frailty is not associated with any specific patient subgroups. The low incidence of frailty amongst the younger COVID-19 patients may necessarily limit extrapolations.
- Data for some patients is incomplete (e.g. smoking) and there is no information for key variables such as ethnicity and BMI.
- Previous metaanlyses (https://doi.org/10.1016/j.exger.2016.12.021) have revealed higher frailty scores for females in every age category, but lower mortality rates at any given age or frailty level. The current study indicates a greater impact of higher frailty scores for COVID-19 mortality and length of hospitalisation amongst females than males. This differential has not been addressed.
- Details concerning hospital treatment regimens and resources would be beneficial. It is unclear as to whether the risk of frailty derives from the hospital procedures (mechanical ventilation, proning, therapeutics) as well as the underlying infection. It

may be possible to segregate longitudinal data according to time of hospital admission relative to acquired knowledge about effective disease and patient treatments.

Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19

Sekine, T. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.06.29.174888

Comments from contributing author Professor David Price:

"Antibody tests underestimate population-level immunity against SARS-CoV-2. Antibody testing can occasionally fail to detect immune responses, e.g. after vaccination with HBV. This does not necessarily equate with a lack of memory B cell responses, but rather may indicate insufficient production to be detectable in the absence of immune re-challenge. An example strategy to assess population immunity based on T cell responses could make use of overlapping peptides and a simple readout such as ELISpot. Our data provide a comprehensive functional and phenotypic map of T cell immunity against SARS-CoV-2, including the novel finding that memory T cells exhibit features associated with immune protection in convalescent individuals previously infected with SARS-CoV-2. Applying the in-depth analysis detailed in this study to the UK population would require extensive research infrastructure. [This study was first posted to bioRxiv on 29th June 2020] Preprints could represent a whole new approach to publication in the future; the pandemic has prompted widespread uptake of this route to disseminate research."

Summary:

Sekine *et al.* performed in-depth T cell analysis on n=203 individuals, grouped into patients, recovered, exposed and healthy individuals. This study provides novel insight into the T cell response in non-critical COVID-19 patients and T cell dynamics post-SARS-CoV-2 infection.

Main Findings:

- $CD4^+$ and $CD8^+$ T cell numbers were reduced in AM and AS patients, in line with other studies (Diao *et al*., 2020, Liu et al., 2020).
- Preliminary analysis identified distinct activation/ cycling profiles in memory T cells ($CD95^+$ $CCR7^-$) of AS and AM patients, compared to MC and 2020 BD: $CD4^+$ T cells expressed CD38, CD69, Ki-67 and HLA-DR, in addition to these markers $CD8^+$ T cells expressed CD39, CTLA-4, LAG-3 and TIM-3.
- The activated patient $CD8^+$ T cells were further delineated into SARS-CoV-2 specific and CMV or EBV specific bystander $CD8^+$ T cells, using tetramers. Bystander $CD8^+$ T cells expressed CD38, potentially due to inflammation signalling, but did not have

increased expression of HLA-DR, PD-1 or Ki-67. This is the first study to use tetramers to detect SARS-CoV-2 specific T cells and distinguish other virus-specific T cells.

- Of the non-patient groups, the highest SARS-CoV-2 specific memory T cell responses were found in those who had experienced severe COVID-19 (SC), compared to MC, Exp, or 2020 BD, respectively. T cell responses were also found in 2019 BD, potentially suggesting cross-reactivity (determined by ELISpot).
- Memory T cells in convalescent individuals were polyfunctional. Both $CD4^+$ and $CD8^+$ T cells produced IFN-γ and TNF; $CD4^+$ T cells additionally produced IL-2, while $CD8^+$ T cells mobilised CD107a, indicative of degranulation. Further investigation of $CD4^+$ T cell functional polarization, based on activation markers CD69 and 4-1BB, showed skewing towards a Th1/ Th17 phenotype. The recall assay additionally demonstrated strong concurrent proliferative and cytokine (IFN-γ) responses in $CD8^+$ and $CD4^+$ T cells, with the $CD4^+$ T cells having a proportionally larger response.

Highlights:

- Tetramer staining of convalescent donors identified antigen specific $CD8^+$ T cells which had a stem-like, early differentiated memory phenotype ($CCR7^+$ $CD127^+$ $CD45RA^+$ $TCF\text{-}1^+$). The frequency of $CCR7^+$ and $CD45RA^+$ T cells positively correlated with symptom-free days post infection, while granzyme B^+ $CD8^+$ T cells were inversely correlated with symptom free days.
- SARS-CoV-2 specific T cell responses were present in seronegative convalescent, exposed and healthy donors.

Clinical Impact:

- Moderate-high. Robust T cell responses in convalescent individuals hold promise for development of vaccine induced immunity. Activated T cells suggests a lack of redundancy in the cellular response to SARS-CoV-2 and could mean population immunity is underestimated, however, this may also be due to sensitivity limits in serum testing.

Important Methodologies:

- Cohorts: Acute moderate (AM, n=10) and acute severe (AS, n=17) patients, convalescent individuals who had mild/ asymptomatic (MC, n=40) and severe (SC, n=26) disease, exposed family members (sharing a household with symptomatic MC or SC donors; Exp, n=30) including some asymptomatic individuals and healthy blood donors (BD), before and during the pandemic (blood collections in 2019 or 2020, n=25 and n=55 respectively).
- Phenotypic and functional T cell analysis was performed by flow cytometry. Recall proliferative response was measured by Cell Trace Violet dye dilution flow cytometry. Clustering of the phenotypes and cohort groups was carried out by principal component analysis and dimensional reduction.
- Antigen specific T cells were identified using tetramers recognising the 13 strongest binding peptides predicted (NetMHCpan4.2) from the SARS-CoV-2 proteome. Bystander T cell analysis was performed using EBV and CMV tetramers.

- Further functional responses were assessed using overlapping peptide pools incorporating the immunogenic domains of the Spike, nucleocapsid and membrane SARS-CoV-2 proteins and detection of IFN-γ by ELISpot.
- Antibody responses were detected by IgG chemiluminescent assays against the nucleocapsid, envelope and spike proteins.

Limitations:

- While overall study size was moderately large, the patient cohort sizes were relatively small.
- The study involves donors from Sweden (including some exposed by travel to Italy), which implemented minimal lockdown measures. Comparison of T cell responses in exposed or asymptomatic individuals in countries with different lockdown strategies is warranted.
- Tetramer analysis focused on HLA-A*02 and HLA-B*07, likely common alleles in the study population. It would however be useful to sample more HLA types representing the global distribution of the pandemic and to investigate T cell responses in patients of differing severity and recovered individuals who have HLA types shown to be more or less likely to present SARS-CoV-2 peptides (Nguyen, 2020).

News and Views

The biggest mystery: what it will take to trace the coronavirus source

Cyranoski, D. 2020. *Nature*

Link: https://doi.org/10.1038/d41586-020-01541-z

The origins of the novel SARS-CoV-2 has been one of the 'biggest puzzles' since the beginning of the COVID-19 pandemic. The precursor of SARS-CoV-2 is thought to have originated in bats. It has been reported that SARS-CoV-2 is most closely related to a group of coronaviruses found in horseshoe bats (*Rhinolophus*). However, none of the bat coronaviruses studied so far are 'similar enough' to SARS-CoV-2 to be the immediate progenitor. Consequently, it is highly likely that an intermediate host, although currently unknown, aided the virus to jump species boundaries and infect humans. In contrast, uncertainty around the emergence of SARS-CoV-2 has led to 'unsubstantiated speculation' that the virus accidentally escaped from a laboratory in Wuhan; the city where the outbreak first emerged.

The flow chart (https://media.nature.com/lw800/magazine-assets/d41586-020-01541-z/d41586-020-01541-z_18052298.jpg) summarises the steps required to trace the source of the viral outbreak.

Wuhan Institute of Virology (WIV) is well-known for its work on bat coronaviruses, including the group of coronaviruses closely related to SARS-CoV-2. Laboratories can be a possible source of outbreak if an employee accidentally contracts the virus from a sample/animal at the facility. No accidents have been reported at WIV and no incidents have been traced back to staff at WIV becoming ill. Similarly, although theoretically possible, it is highly improbable that SARS-CoV-2 emerged as a consequence of laboratory manipulation of a closely related coronavirus. Researchers around the world have carried out detailed analysis on the genome of SARS-CoV-2 and identified features that support the virus emerged naturally. Genetic data reveals that none of the available viruses could have served as a backbone to generate SARS-CoV-2 in the laboratory. Furthermore, although SARS-CoV-2 has been shown to successfully bind and infect human cells, computational analysis of its receptor binding domain (RBD) reveals that the virus-host interaction is not ideal and most likely evolved as a result of natural selection. Additionally, it has been reported that SARS-CoV-2 has naturally acquired a unique furin cleavage site to enable enhanced infection of human cells. Although it is very possible to insert such a site into a viral genome in a laboratory, a very similar site has been identified in a closely related coronavirus. Consequently, identification of similar RBD sequences and cleavage sites in closely related coronaviruses strongly suggest that SARS-CoV-2 acquired these specific features through natural processes. Nevertheless, unless a virus that is nearly identical to SARS-CoV-2 is found in a wild animal, it will be hard to establish the exact chain of transmission to humans and exclude laboratory as a source of outbreak.

Waiting for Certainty on Covid-19 Antibody Tests — At What Cost?

Weinstein, M.C. *et al*. 2020. *NEJM*

Link: https://doi.org/10.1056/NEJMp2017739

This paper discussed the use of serologic antibody testing to screen for possible immunity and to identify people who could return to the workplace, which was not approved by WHO. However, the authors think COVID-19 antibody test is a very good option, as we cannot act until we "guarantee" the accuracy of the immunity-certification process.

Main Ideas:

- WHO current guidance: "At this point in the pandemic, there is not enough evidence about the effectiveness of antibody-mediated immunity to guarantee the accuracy of an 'immunity passport' or 'risk-free certificate'.'' As there are many uncertainties in the antibody test.
- The authors think the antibody test is useful, the reasons include: caregivers make choices with less-than-perfect evidence all the time; Compared to many regulations issued by U.S. governors, which don't require evidence of immunity or prior infection in opening up workplaces.
- The author suggest us to weigh four pieces of information when assessing serologic testing as a basis for returning people to work:

    1. Prevalence: the presence of SARS-CoV-2 antibodies in the population.
    2. Test sensitivity and specificity.
    3. Whether antibodies confer immunity; if they do, what do we assume about the relationship between antibody level (titer) and the resultant degree and persistence of any immunity that is conferred?
    4. False positive cost and false negative cost.

- The bottom line is this: we have enough evidence and expert opinion to make an informed decision today. And we can put the monitoring systems in place to learn from that decision so that we can make even better choices tomorrow.
- Covid-19 antibody tests should be improved and reach certain level of accuracy.

A metabolic handbook for the COVID-19 pandemic

Ayres, J.S. 2020. *Nature metabolism*

Link: https://doi.org/10.1038/s42255-020-0237-2

This perspective article discusses the role of metabolism in COVID-19 pathogenesis, pathology, pathophysiology and host defence responses. The author goes in to detail regarding how COVID-19 disease progresses, how the metabolic health of an individual influences this and draws parallels between metabolic disorders and COVID-19 infections. The presence of metabolic risk factors such as obesity, diabetes and metabolic syndrome greatly increase an individual's susceptibility to COVID-19 infection in a variety of ways as well as resulting in a more severe disease course. The author also investigates metabolic health complications occurring in individuals that have recovered from severe or critical COVID-19 and discusses evidence that not only physical, but also mental effects are observed. There are several different methods currently employed to prevent and fight COVID-19 infections, these strategies and the effect that they are having on the population are discussed. These include avoidance defences such as hand washing and social distancing, resistance strategies such as destroying the virus by preventing viral entry or targeting the immune response and physiological defences, which block pathogenic responses and limit damage to tissues and organs. The main focus of this section is the way in which host metabolism can be targeted to promote physiological defence, limit susceptibility to damage and promote repair. The main aim of this article is to demonstrate the importance of metabolism in COVID-19 infection, with a focus on the risk posed by metabolic abnormalities and the suggestion to target metabolic strategies in order to promote disease tolerance.

Blood vessel injury may spur disease's fatal second phase

Matacic, C. 2020. *Science*

Link: https://doi.org/10.1126/science.368.6495.1039

Since the start of the pandemic several key studies have reported their observations related to the development of acute respiratory distress syndrome (ARDS), blood clots, organ damage and shock that develops typically a week after hospitalisation with Covid-19. In this news article Catherine Matacic, discusses the three step hypothesis scientists believe occurs in the second fatal phase of disease.

- **Step 1: Vascular Leakage**
  Following SARS-CoV-2 infection of the airways, endothelial cells become activated and damaged, resulting in vascular leakage into the airway space. This triggers an

inflammatory cascade; leukocytes infiltrate the airway spaces, release inflammatory cytokines, further damage to the endothelial cells occurs.

- **Step 2: Clotting**
  Damaged endothelium results in the underlying vessel membrane becoming exposed, triggering the clotting cascade. Clotting factors are released, platelets are recruited, and a clot forms. As these clots subsequently degrade, the levels of the biomarker, D-dimer, rocket, alerting clinicians to the danger the patient is in. Clotting spreads throughout the body and blocks the blood supply to vital organs

- **Step 3: Inflammation**
  In response to the widespread tissue damage associated with clotting, the patient's natural response is to recruit leukocytes, via the release of more inflammatory cytokines to help clear the tissue debris. In Covid-19 patients, however the release of cytokines spirals out of control, a 'cytokine storm', and patients develop septic shock that is typically fatal.

This emerging hypothesis of the role of endothelial cells in severe Covid-19 has resulted in a number of preliminary clinical trials. These studies aim to dampen or prevent the second fatal phase of disease from developing, by using existing treatments such as anti-clotting, anti-platelet, anti-inflammatory drugs and statins that are known to improve endothelial cell function.

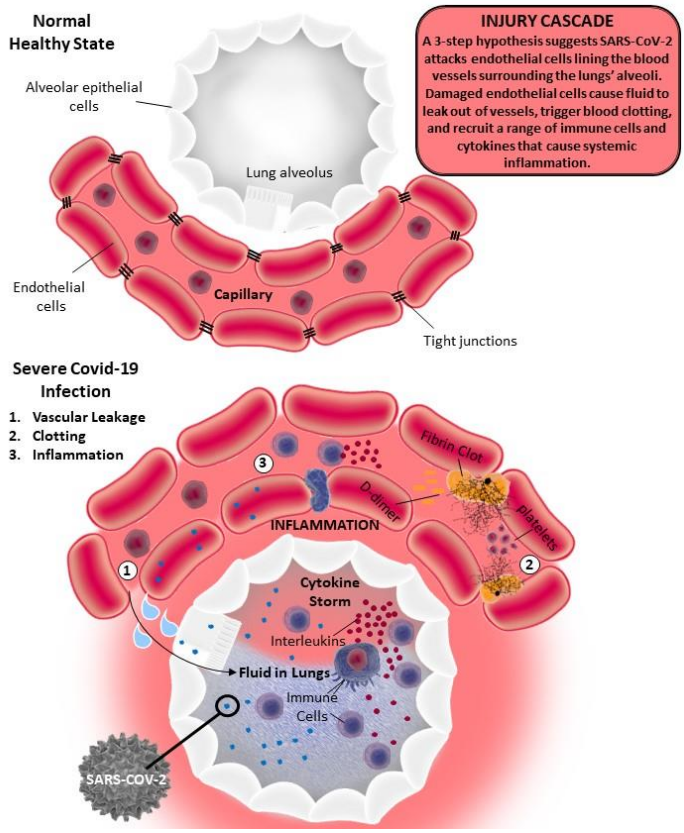

Figure adapted from Matacic et al. (2020).

Journal Reviews

Immune Responses: Clinical Implications and Possible Interventions

A consensus Covid-19 immune signature combines immuno-protection with discrete sepsis-like traits associated with poor prognosis

Laing, A.G. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.06.08.20125112

Summary:

Laing *et al.* identify a consensus peripheral blood immune signature in COVID-19 patients. The signature included a substantial increase in IP-10/CXCL10; B cell responses typical of virus infection; hyperactivation, proliferation and depletion of T cells; and monocyte and dendritic cell dampening more usually associated with sepsis. In addition, they demonstrated that CXCL10/IP10 over-expression, T cell cycling, and basophil and plasmacytoid dendritic cell reduction correlated with, and could be used to predict, COVID-19 progression.

Main findings:

- **Cytokines:** Increased IP10 levels amongst COVID-19 patients, levels were sustained and proportional to disease progression, discriminating severe from moderate and moderate from low. IP10 levels correlated with IFN-γ, IL-6, IL-10, IL8 (CXCL8)
- **B cells:** Very variable numbers of B cells. Significant reduction in CD5+ B cells, CD27+IgM+IgD+ B cells and naïve IgM+CD27neg B cells. Significantly increased CD38+CD27+ plasmablasts. B cells had reduced CD19 expression.
- **DCs, Basophils, Monocytes:** Severity-related depletions of plasmacytoid dendritic cells (pDC). CD11c+CD1c- DCs had active cell cycling and increased frequencies. CD1c+ DC had decreased HLA-DR expression, correlated to severity. Reduction in basophils. Increased CD16+CD14+ intermediate monocytes (MOIM), reduction of monocyte CD86 and HLA-DR.
- **T cell cytopenia:** T cytopenia across all disease categories. Decrease in CD4+ and particularly CD8+ T cells. TEM, TCM and naïve all decreased. CD8+ TEMRA decreased (but not CD4 TEMRA). Decrease, particularly in severe patients, of CD4+ Th17 cells. γδ T cells were very severely depleted particularly Vγ9Vδ2 cells.
- **T cell phenotype:** Increased frequency of activated HLA-DR+CD38+ T cells, particularly among CD8+ T cells in patients progressing to severe disease. Overexpression of HIF1 suggesting an adaptation to hypoxia or dysoxia. FASLG, GZM and PRF1 (encoding perforin) were all over-expressed. Overexpression of TNFRSF10B (encoding the TRAIL-receptor) and CASP3 suggested that CD8+ TEM were more prone to apoptosis. Chronic activation of CD8+ TEM cells with upregulation of genes PDCD1 (encoding PD-1), LAG3,

and CTLA4. However, TIGIT was downregulated (its ligand PVR may directly interact with SARS-CoV-2)

- **T cell proliferation:** Very high cycling of CD4+ and CD8+ TEM cells. 10-fold increases in the percentage of blood CD4+ and CD8+ T cells in either G1 or S- G2/M phases of the cycle. Over 10-fold increases of residual γδ cells in G1. Correlation of cycling CD4+ T cells with anti-SARS-CoV-2 IgG

Highlights:

- A consensus Covid-19 immune signature was identified in peripheral blood
- Describes sepsis-like innate immune cell dysregulation
- Identified markers that upon hospital admission correlated, often prognostically, with the severity of disease progression, including blood pDC and basophil depletion, increased IP10, and active cell-cycling, activation and depletion of CD8+ TEM

Clinical Impact:

- Measuring IP-10 (CLCL10) can be prognostic of disease severity.
- Authors suggest that their data support therapeutic strategies to boost T cell competence, e.g. by use of IL7, and IP10 antagonism to treat COVID-19

Important Methodologies:

- Well defined flow cytometry panels that could be easily adopted by other groups

Limitations:

- Somewhat confusing to have recovered patients grouped with the healthy controls

Blood parameters measured on admission as predictors of outcome for COVID-19; a prospective UK cohort study

Arnold, D.T. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.06.25.20137935

Summary:

Initial report of the UK DISCOVER trial, primary outcome to find clinical/blood biomarkers for COVID-19 that can predict disease severity.

155 prospectively recruited COVID-19 patients were assessed for blood markers IL-6, suPAR, KL-6, Troponin, Ferritin, LDH, BNP, Procalcitonin, CRP and neutrophil/lymphocyte counts. Clinical NEWS scores and age were extracted from clinical records for comparison against disease severity. These markers were compared to clinical outcome using logistic regression

and Area Under the Curve (AUC) calculations, as well as specificity and sensitivity at Youden's index. IL-6 and suPAR performed best as biomarkers for disease severity.

Main Findings:

- 155 COVID-19 patients that had 28-day post diagnosis outcomes were assessed, comorbidities and ethnicity were recorded.
- 120 classed as non-severe, 35 classed as severe meaning they required intensive care, ventilation or died.
- 77% of COVID-19 diagnoses were SARS-CoV-2 PCR positive, the remainder tested negative but had clinical features.
- "Conventional" infection biomarkers C-reactive protein, neutrophil/lymphocyte ratio did not appear to be very predictive of disease severity (AUC 0.51 and 0.62 respectively).
- IL-6 (AUC 0.77) and soluble urokinase plasminogen activator receptor (suPAR, AUC 0.77) seemed to perform well as biomarkers.
- Clinical measures such as age and the National Early Warning Score (NEWS) also had high AUC scores (0.62 and 0.75 respectively).

Highlights:

- IL-6/suPAR levels at admission and NEWS clinical scoring may be predictive of COVID-19 disease severity.

Clinical Impact:

- Mid/High- IL-6 has also been proposed as a COVID-19 biomarker in Wuhan (102 patients) and Germany (89 patients) BUT needs further validation in larger cohorts.

Important Methodologies:

- DISCOVER cohort aimed to produce replicable data by using published guidelines.
- Analytic code available online https://github.com/gushamilton/discover_prediction/

Limitations:

- Relatively small sample number.
- 36 patients did not have stored blood for further tests e.g. IL-6 and suPAR.
- The outcome of "severe disease" included non-invasive ventilation, Intensive care admission and death.

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

Gomez Rial, J. *et al*. 2020. *medRxiv*

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

Summary:

The authors analysed plasma levels of SARS-CoV-2 patients suggesting that soluble monocyte activation markers are increased compared to healthy controls. They argue that monocyte activation is responsible for immunopathology.

Main Findings:

- Significantly higher sCD14 and sCD163 in patients (both ICU and non-ICU) compared to healthy controls, no difference between ICU/non-ICU patients
- sCD14 positively correlated with clinical laboratory parameters (LDH, CRP, PCT, Ferretin and IL-6) and negatively correlated with absolute lymphocyte counts in the non-ICU group
- sCD163 increased with time after hospital admission

Highlights:

- Monocyte activation markers are increased in SARS-CoV-2 patients compared to healthy controls

Clinical Impact:

- Limited, multiple clinical trials are already investigating the efficacy of tocilizumab treatment with interferes with monocyte function in SARS-CoV-2 infected patients. Data could support the use of specific monocyte targeting therapeutics but it's unclear at the moment whether monocytes actually drive pathology.

Important Methodologies:

- Analysis of 59 confirmed SARS-CoV-2 positive patients (22 with ICU admission requirement, 37 without) and healthy controls
- Measurement of serum sCD14 and sCD163 via ELISA

Limitations:

- 19/22 ICU admitted patients were treated with tocilizumab (IL-6 blocking) which interferes with monocyte function, would have been interesting to see comparisons of severe cases without tocilizumab treatment with non-ICU admitted patients
- A correlation does not demonstrate cause/effect, it is unclear whether monocyte activation precedes upregulated clinical markers and whether monocytes are the main drivers of pathology

Inhibition of Bruton tyrosine kinase in patients with severe COVID-19

Roschewski, M. *et al*. 2020. *Science Immunology*

Link: https://doi.org/10.1126/sciimmunol.abd0110

Summary:

Rochewski *et al*. carry out a small study to determine the effects of Acalabrutinab, a Bruton tyrosine kinase (BTK) inhibitor (used to regulate macrophage signalling and activation) in patients with severe COVID-19. BTK was administered off-label to 19 COVID-19 patients (11 on supplemental oxygenation and 8 on mechanical ventilation) over 10-14 days. Upon follow up the majority of oxygenated patient's oxygenation levels, C-reactive protein, interleukin-6 and lymphopenia normalised during treatment. More heterogeneous results were evident in the mechanically ventilated group complicated by the range of underlying conditions within this cohort. They go on to show a link between improved pulmonary function and decreased inflammation and an increase of BTK phosphorylation and IL-6 in CD14+ monocytes in infected patients suggesting BTK is specifically activated in severe COVID-19 cases.

Main Findings:

In the cohort requiring supplemental oxygenation:

- 73% (8 patients) no longer required oxygenation and had been discharged after 10-14 day treatment upon follow up.
- CRP returned to normal in 93% of patients and decreased in 9%, IL-6 normalised in 60%, and there was a 3- and 13- fold reduction in a further 2 patients.
- Changes in D-dimer and fibrinogen were variable and ALC increased in the majority of patients.

In the cohort requiring mechanical ventilation:

- Four out of eight (50%) were extubated (2 discharged).
- CRP normalised in 25% and reduced in 37%, IL-6 oscillated in 2 patients with interrated infections and ALC improved in 63% of patients.

- Evidence of an increase in phosphorylated BTK in CD14+ monocytes from patients with severe COVID-19 compared to healthy volunteer – BTK was specifically activated in monocytes from patients with COVID-19.
- A link was found between improved pulmonary function and decrease inflammation – inverse relationship between oxygen uptake efficacy and CRP levels.

Highlights:

- BTK is a likely to induce pathological inflammation in response to severe COVID-19.
- BTK inhibitor acalabrutinab improved the clinical outcome for COVID-19 patients requiring supplemental oxygenation and improved oxygenation in 50% of a mechanically ventilated cohort.

Clinical Impact:

- High – Study has led to a confirmatory international prospective randomised controlled clinical trial.

Important Methodologies:

- Analysis of phosphorylated BTK and IL-6 in whole blood using flow cytometry.

Limitations:

- Small cohort of 19 patients
- All received other treatments on top of Acalatrutinab and took a limited (10-14) days course of treatment
- Variable clinical response in intubated cohort

Terminal complement inhibition dampens the inflammation during COVID-19

Kulasekararaj, A.G. *et al.* 2020. *British Journal of Haematology*

Link: https://doi.org/10.1111/bjh.16916

Summary:

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare, acquired haematopoietic stem cell (HSC) disease characterised by intravascular haemolysis, increased thromboembolic risk and bone marrow failure. PNH erythrocytes are exquisitely sensitive to complement activation due to the lack of GPI-linked complement regulators, especially CD55 and CD59. Kulasekararaj et al. reports on the clinical course, degree of intravascular haemolysis and outcomes of COVID-19 in four patients with PNH, two established on terminal complement inhibitor and two treatment-naïve PNH patients. Although viral infections have been shown to induce haemolysis by activating complement, there has been no published report of COVID-19 in the context of PNH, and neither has the added benefit of therapeutic complement inhibition been examined thus far.

Main Findings:

- Inhibition of the complement pathway by targeting C5 may be an effective intervention in SARS-CoV-2.

Highlights:

- Complement plays a key role and is an integral component of the innate immune response to pathogens and its dysregulation or activation, either due to acquired

deficiency of complement regulatory proteins (i.e. PNH) or due to viral infection (i.e. SARS-CoV-2), can lead to significant tissue damage and importantly thrombosis due to endothelial damage.

- The four patients illustrate the presence of both conditions (PNH and COVID-19) concurrently and a differential response is seen in patients already on effective complement inhibition compared to patients not on C5 inhibition.
- Complement inhibition helps to control the intravascular haemolysis due to PNH, but also dampens the hyperinflammatory lung damage during COVID-19.

Clinical Impact:

- SARS-CoV-2 infection, like other virus infections such as influenza virus and respiratory syncytial virus, is likely to induce massive complement activation in this 'vulnerable' group and can lead to severe life-threatening complications and hospitalisation.
- Emerging evidence suggests that the activation of the complement system, even in the absence of PNH, is key in the pathogenesis of COVID-19-related lung injury and therefore C5 inhibition may be an effective therapeutic strategy in CoV-mediated disease.
- Trials (SOLID-C19, CORIMUNO19-ECU and ALXN1210-COV-305), are ongoing to test the efficacy of terminal complement inhibition in dampening the progression of complications and improve outcomes in patients with COVID-19·

Important Methodologies:

- A report on the clinical course, degree of intravascular haemolysis and outcomes of COVID-19 in four patients with PNH, two well-established on terminal complement inhibitor and two treatment-naïve PNH patients.

Limitations:

- Small sample size
- The adverse effect in patients not on C5 inhibitors may be circumstantial, as other known COVID-19 risk factors of mortality and morbidity, like older age, comorbidity, high body mass index (BMI) and male gender could have contributed to the worse outcome.

T cells

SARS-CoV-2 reactive T cells in uninfected individuals are likely expanded by beta-coronaviruses

Stervbo, U. *et al*. 2020. b*ioRxiv*

Link: https://doi.org/10.1101/2020.07.01.18274

Summary:

A relevance score based on sequence similarity between common pathogens for the European population and SARS-CoV-2 is used to identify 10 pathogens which may confer cross-protection and explain the broad clinical manifestation of COVID-19. 6-mer sequences within viruses of these pathogens have their Levenshtein distances from SARS-CoV-2 predicted epitopes compared and the endemic coronaviruses (OC43 and HKU1) have the highest degree of similarity which the authors' reason makes them the likely candidates for cross-protection. If the threshold is relaxed, VZV could confer cross-protection. The authors do not go on to test the candidate epitopes.

Main Findings:

- HLA-I top 10 ranked pathogens likely to confer cross-protection to SARS-CoV-2: two fungi (Candida tropicalis, Cryptococcus neoformans), one parasite (Trichomonas vaginalis), positive sense single stranded RNA viruses (endemic beta coronaviruses: **HKU1, OC43, 229E, NL63**), negative sense single stranded RNA virus (**Influenza B**), double stranded RNA virus (Rotavirus A, **RV**), double stranded DNA viruses (Human alphaherpesvirus 3, **VZV**).
- HLA-II top 10 ranked pathogens likely to confer cross-protection to SARS-CoV-2: same as above with gammaherpesvirus 4 (**EBV**) in place of Trichomonas vaginalis.
- OC43 and HKU1 had the highest number of epitopes identical to predicted SARS-CoV-2 epitopes. OC43 and KU1 had 211 and 195 for HLA-I, respectively. For HLA-II, OC43 and KU1 had 493 and 464, respectively.
- If the threshold was relaxed to allow 3 insertions, deletions, or exchanges in amino acid; VSV, OC43 and KU1 had 1292, 1189 and 1163 HLA-I matches respectively.

Highlights:

- The authors have demonstrated a method capable of predicting sequences to test *in-vitro* to confirm their findings.

Clinical Impact:

- Little to none.

Important Methodologies:

- Common pathogens in the European population identified, their protein sequences used to generate 6-mers which were compared to predict HLA-I and HLA-II SARS-Cov-2 binding epitopes and then ranked based on relevance.
- Calculated the Levenshtein distance (the minimum number of single-character edits required to change one word into the other) between predicted epitopes in the above viruses (in bold) and SARS-CoV-2 for HLA-I and HLA-II

Limitations:

- This paper contains solely *in-silico* analysis of similarity between sequences to suggest beta-corona viruses are the cause of cross-protective immunity to SARS-CoV-2. This would be greatly benefit from *in-vitro* studies to see if healthy, mild and severe patients T cells cross-react with proposed 6-mers although this is not trivial.
- Whilst 6, 7 and 8-mers are initially considered, only 6-mers are further investigated. The authors go on to state that HLA-II prediction is less accurate than HLA-I so within HLA-I is strange they only further investigate 6-mers as HLA-I ligands typically range from 8 – 12 amino acids (Gfeller et al., 2018).

Antibodies

Relative COVID-19 viral persistence and antibody kinetics

Huang, C-G. *et al*. 2020. m*edRxiv*

Link: https://doi.org/10.1101/2020.07.01.20143917

Summary:

Huang *et al*. followed viral load in throat swabs and antibody responses in serum of 15 RT-PCR confirmed COVID-19 patients in order to reveal factors for the different dynamics of COVID-19 antibody responses. The study showed that viral persistence rather than a high viral load caused strong antibody responses. Patients who cleared the virus quickly displayed lower antibody responses. The neutralization efficacy per unit antibody however is comparable between high and low antibody responses. This suggests that patients with high antibody levels produce a higher amount of inefficient antibodies.

Highlights:

1. A linear correlation of viral load and antibody response was observed in 7 out of 15 COVID-19 patients. A high viral load correlated with a high antibody response. The virus persisted significantly longer in these 7 patients compared to the remaining 8 patients that showed no clear correlation between viral load and antibody response.
2. The 7 patients with viral persistence and higher antibody levels were significantly older than the ones who cleared virus quickly.
3. Patients with longer persistence of virus produced significantly more antibodies than those who cleared the virus rapidly. However, the neutralisation titre was proportional to the amount of antibody in both groups.
4. In order to determine the neutralisation efficacy per antibody unit, the neutralisation titre was normalised by the amount of antibody. The neutralisation efficacy per antibody was comparable between patients with high and low antibody response. This indicates that high antibody responses contain a higher amount of inefficient antibodies.
5. 2 patients passed away (one young, one older). Both had high viral load and higher amount of antibodies in serum compared to survivors, but the neutralization efficacy per antibody was relatively poor.

Clinical Impact:

- Moderate, but should be taken seriously as it might have implications for treatment with convalescent serum. The authors state that there is a risk of some antibodies in convalescent serum not being protective or even being harmful due to ADE. Caution should be applied in timing of plasma collection.

Important Methodologies:

- Plaque reduction neutralization test
- Culturing Covid-19 virus strains from patients

Limitations:

- Small sample size
- Throat swabs might not be ideal for measuring viral load

Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody

Pinto, D. *et al.* 2020. *Nature*

Link: https://doi.org/10.1038/s41586-020-2349-y

Summary:

Pinto *et al*. describe several monoclonal antibodies (mAb) that target the S glycoprotein of SARS-CoV-2 which were identified from memory B cells of an individual who was infected with SARS-CoV in 2003. Demonstrated using cryo-electron microscopy and binding assays that one such mAb called S309 recognizes an epitope containing a glycan that is conserved within the *Sarbecovirus* subgenus, without competing with receptor attachment. S309 in combination with additional mAbs targeting distinct sites on the SARS-CoV-2 S^B domain shown to enhance SARS-CoV-2 neutralization. Such combinatory mAb therapy may be useful for both prophylaxis and treatment of disease.

Main Findings:

- Performed a memory B cell screening using peripheral blood mononuclear cells collected from a SARS-CoV infected patient
- Screening was done on blood drawn from same patient in 2004 (collected 19 mAbs) and in 2013 (collected 6 mAbs)
- 8/25 mAbs bound to CHO cells which express SARS-CoV-2 S glycoprotein or SARS-CoV S glycoprotein,
- Half-maximal effective concentration values ranged between 1.4 and 6,100 ng ml^{-1} (SARS-CoV-2) and 0.8 and 254 ng ml^{-1}(SARS-CoV)
- None of the mAbs bound to prefusion ectodomain trimers of the HCoV-OC43 or MERS-CoV S glycoproteins indicating a lack of cross-reactivity outside the *Sarbecovirus* subgenus
- The S309 IgG bound to the immobilized SARS-CoV-2 S^B domain and to the ectodomain trimer of the S glycoprotein with sub-picomolar and picomolar avidities
- The S309 Fab bound with nanomolar to sub-nanomolar affinities to both molecules

- Performed pseudovirus neutralization assays using a murine leukaemia virus (MLV) pseudotyping system
- S309 showed comparable neutralization potencies against both SARS-CoV and SARS-CoV-2 pseudoviruses
- S309 neutralized SARS-CoV–MLVs from isolates of the 3 phases of the 2002–2003 epidemic with half-maximal inhibitory concentration (IC50) values of between 120 and 180 ng ml^{-1}
- S309 partially neutralized the SARS-related coronavirus38 WIV-1 and the authentic SARS-CoV-2 (2019n-CoV/ USA_WA1/2020) with an IC50 of 79 ng ml^{-1}
- The S309 Fab fragment and a prefusion stabilized ectodomain trimer of SARS-CoV-2 S glycoprotein complex characterized using single-particle cryo-EM
- Resolved two structural states: a trimer with one S^B domain open (3.7 Å resolution), and a closed trimer (3.1 Å resolution) both with three S309 Fab
- Fab engages an epitope distinct from the receptor-binding motif and would not clash with ACE2 upon binding to S glycoprotein
- Only closed state structure analysed due to variability of the upward pointing S^B domain in open state
- S309 recognizes a proteoglycan epitope on the SARS-CoV-2 SB, distinct from the receptor-binding motif and is accessible in both the closed and open states of the S glycoprotein
- S309 paratope is composed of all 6 complementarity-determining region (CDR) loops, which bury a surface area of about 1,150 $Å^2$ at the interface with SB through electrostatic interactions and hydrophobic contacts
- 20-residue-long CDRH3 sits atop the SB helix accounts for about 50% of the buried surface area
- CDRH3 and CDRL2 sandwich the glycan of the SARS-CoV-2 S glycoprotein at position N343, through contacts with the core fucose moiety and to a lesser extent with the other saccharides within the glycan chain
- These interactions between S309 and the glycan bury an average surface of about 300 $Å^2$ and stabilize the N343 oligosaccharide
- S glycoprotein sequences of the 11,839 SARS-CoV-2 isolates reported to date indicates epitope residues are conserved in all but 4 isolates (such substitutions are not expected to affect recognition)
- Biolayer interferometry analysis of S309 Fab or IgG binding to the SARS-CoV-2 S^B domain or the ectodomain trimer of S glycoprotein revealed lack of competition between S309 and ACE2 for binding to the SARS-CoV-2 S glycoprotein
- Side-by-side infection of SARS-CoV-2–MLV in the presence of either S309 Fab or S309 IgG revealed comparable IC_{50} values (3.8 and 3.5nM) but only IgG achieved 100% neutralization
- observed efficient S309- and S306-mediated ADCC of SARS-CoV-2 S-glycoprotein-transfected cells, whereas the other mAbs tested showed limited or no activity
- Neutralization potency of S309 enhanced using non-competing S315 and S304 mAbs which target distinct sites on the S^b domain

Highlights:

- Structural data explain the S309 cross-reactivity between SARS-CoV-2 and SARS-CoV, as 17 out of 22 residues of the epitope are strictly conserved
- S309 could neutralize potentially all SARS-CoV-2 isolates known to be circulating to date, and possibly many other zoonotic *Sarbecoviruses*

Clinical Impact:

- Limited.

Important Methodologies:

- Cryo-electron microscopy
- Biolayer interferometry
- Neutralization assay using murine leukaemia virus pseudotyping system

Limitations:

- No *in-vivo* studies performed to support use of S309 in prophylactic and/or post-exposure therapy to limit or treat severe disease

Seroprevalence of immunoglobulin M and G antibodies against SARS-CoV-2 in China

Xu, X. *et al*. 2020. *Nature Medicine*

Link: https://doi.org/10.1038/s41591-020-0949-6

Summary:

In this study, Xu *et al*. use an internally validated serological assay to evaluate levels of immunoglobulin M (IgM) and immunoglobulin G (IgG) against SARS-CoV-2 in the city of Wuhan and other regions of China. Seropositivity in Wuhan ranged from 3.2-3.8%, decreasing progressively further away from the epicentre of the outbreak. High seroprevalence was also observed in healthcare workers (3.3%) and in patients undergoing maintenance hemodialysis (1.8%). Studies such as this will contribute to establish cumulative seropositive prevalence rates for SARS-CoV-2, aid our epidemiological understanding of this outbreak and the contagiousness of SARS-CoV-2 and levels of immunity throughout vulnerable and general populations.

Main Findings:

- In Wuhan, the rates of seropositivity were 3.8% (2.6–5.4%, 95%CI) of 714 healthcare workers, 3.8% (2.2–6.3%, 95%CI) of 346 hotel staff members and 3.2% (1.6–6.4%, 95% CI) of 219 family members of the healthcare workers

- Moving west from Wuhan to two nearby cities (Jinzhou and Honghu), seroprevalence in 3,091 healthcare workers was estimated to be 1.3% (1.0–1.8%, 95% CI) and 3.6% (2.0–4.9%, 95%CI) in a cohort of 979 hemodialysis patients (with no present COVID-19 symptoms)
- Moving further west from Wuhan (Chongqing), seroprevalence of 3.1% (1.7–5.7%, 95%CI) and 3.8% (2.8–5.2%, 95%CI) were observed in cohorts of 319 healthcare workers and 993 hospital outpatients, whilst in a large cohort of 9,442 community residents in Chengdu, observed seropositivity was 0.58% (0.45–0.76%, 95%CI)
- Far south from Wuhan in the cities of Guangzhou and Foshan, observed seroprevalence rates were 2.8% (1.8–4.6%, 95% CI) in 563 hemodialysis patients, 1.2% (0.4–3.3%, 95%CI) in 260 healthcare workers and 1.4% (0.6–2.9%, 95%CI) in 442 factory workers.
- Prior assay validation in a cohort of 242 patients with RT-PCR confirmed COVID-19 showed an earlier and higher IgG response than IgM response to the same antigen, perhaps suggesting that using IgM as a marker of early acute phase response to SARS-COV-2 may not be as useful like it is in other viral diagnostics
- Individuals with either only IgM or IgG seropositivity were observed, highlighting the need to measure both during serologic surveys
- No differences were observed in seropositivity between genders, however, in their overall study cohort of 17,368 individuals, seropositivity was higher in individuals older than 65 years (2.0%) than those younger than 65 years (1.3%)

Highlights:

- Rates of seroprevalance decreased progressively as the distance to the epicenter of the outbreak in China increased
- From tested populations, seropositivity was higher in healthcare workers and patients visiting hospital for maintenance hemodialysis

Clinical Impact:

- Minimal

Important Methodologies:

- Assay validation using serum samples collected in June 2019 (pre-SARS-CoV-2) to establish assay specificity of 99.3% for IgG and 100% for IgM
- Seroprevalance tested across multiple cities

Limitations:

- Tested populations were not randomly sampled, introducing a potential sampling bias in estimates of seroprevalence
- Samples could have been collected outside the time frame of an antibody response, potentially producing false negatives and therefore underestimating the true prevalence rate of disease

- Assay assessed the presence of IgM and IgG antibodies specific for the spike protein and nucleoprotein; including other SARS-CoV-2 known to induce an antibody response could have strengthened their assay

Sensitive detection of SARS-CoV-2-specific-antibodies in dried blood spot samples

Morley, G.L. *et al*. 2020. m*edRxiv*

Link: https://doi.org/10.1101/2020.07.01.20144295

Summary:

Morley *et al.* shows the potential of dried blood spot (DBS) as a method for detecting antibodies against SARS-CoV-2. In this paper they describe the validation of DBS samples against matched serum in highly sensitive and specific SARS-CoV-2 enzyme linked immunosorbent assay (ELISA).

Main Findings:

- SARS-CoV-2-anti-spike glycoprotein antibodies can be eluted from DBS samples
- Responses between matched serum and DBS samples correlate strongly

Highlights:

- The use of DBS samples to overcome the necessity for venepuncture and the opportunity for wider population level sampling.

Clinical Impact:

- Moderate

Important Methodologies:

- Participants and sample collection. Total of 87 samples from 80 volunteers at the University Hospital Birmingham. They include 17 pre-August 2019 DBS samples to refine negative thresholds. Participants were healthy at the point of sampling. Laboratory analysis was blinded to PCR status.
- DBS antibody elution. For which they used the nephelometry automated COBAS 6000 (Roche, UK) to quantify the total IgG, IgA and IgM concentrations.
- SARS-CoV-2 ELISA. To measure IgG, IgA and IgM against soluble, stabilised, trimeric SARS-CoV-2 spike glycoprotein.

Limitations:

- The number of participants is low.

- Not certified by peer reviewed
- Data obtained from the experiments was grouped and the statistical analysis used was Spearman which assume data is ordinal.

Virology

Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases infectivity of the COVID-19 virus

Korber, B. *et al*. 2020. *Cell*

Link: https://doi.org/10.1016/j.cell.2020.06.043

Summary:

Authors utilize the pipeline to track SARS-CoV-2 mutations in the COVID-19 pandemic is based on regular updates from the GISAID SARS-CoV-2 sequence database which can be found on website cov.lanl.gov. These data are as of May 29, 2020. A SARS-CoV-2 variant carrying the Spike protein amino acid change D614G has become the most prevalent form in the global pandemic. Dynamic tracking of variant frequencies revealed a recurrent pattern of G614 increase at multiple geographic levels: national, regional and municipal. The shift occurred even in local epidemics where the original D614 form was well established prior to the introduction of the G614 variant. The consistency of this pattern was highly statistically significant, suggesting that the G614 variant may have a fitness advantage.

Main Findings:

- Spike D614G amino acid change is caused by an A-to-G nucleotide mutation at position 23,403 in the Wuhan reference strain.
- Early March 2020, G614 form was rare globally, but gaining prominence in Europe, and GISAID was also tracking the clade carrying the D614G substitution, designating it the “G clade”. D614G change is almost always accompanied by three other mutations.
- The transition from D614 to G614 occurred asynchronously throughout the world, beginning in Europe, followed by North America and Oceania, then Asia.
- G614 frequencies increased in all analysed 5/5 continents, 16/17 countries, (two-sided binomial p-value of 0.00027); 16/16 regions (p = 0.00003), and 11/12 counties and cities (p= 0.0063).
- Increase in G614 often continued after national stay-home-orders were implemented, and in some cases beyond the 2-week maximum incubation period.
- G614 is associated with potentially higher viral loads in COVID-19 patients but not with disease severity.
- G614 is associated with higher infectious titers of spike-pseudotyped virus such as single-cycle vesicular stomatitis virus (VSV) and lentiviral particles 9 displaying either D614 or G614 SARS-CoV2 Spike protein
- G614-bearing virions are not intrinsically more resistant to neutralization by convalescent sera.

Highlights:

- A SARS-CoV-2 variant with Spike G614 has replaced D614 as the dominant pandemic Form
- The consistent increase of G614 at regional levels may indicate a fitness advantage
- G614 is associated with lower RT PCR Ct's, suggestive of higher viral loads in patients
- The G614 variant grows to higher titers as pseudotyped virions

Clinical Impact:

- Minimal

Important Methodologies:

- PCR detection of SARS-CoV-2

Limitations:

- G614 variant shifts in any given geographic region could result from either founder effects or sampling biases
- Lack of association between G614 and hospitalization
- Laboratory evidence of increased fitness of the D614G mutation is based on two different pseudo virus models of infection
- Neutralization assays performed were based on sera from SARS-CoV-2 infected individuals with an unknown D614G status.

Vaccines

A universal design of betacoronavirus vaccines against COVID-19, MERS and SARS

Dai, L. *et al*. 2020. *Cell*

Link: https://doi.org/10.1016/j.cell.2020.06.035

Summary:

Dai *et al.* sought to identify immunogens as vaccine candidates for betacoronavirus family members SARS-CoV, SARS-CoV-2 and MERS. Previous studies have identified the receptor binding domain (RBD) as a candidate for vaccine design however RBD vaccines have demonstrated poor immunogenicity. To overcome poor outcomes, the authors used disulphide linked RBD dimers which enhanced the neutralising antibody titres compared to monomeric RBD. Furthermore, using a structure based approach, the RBD dimer designed as a tandem repeat single-chain (RBD-sc-dimer) which enhanced their stability and immunogenicity. RBD-sc-dimers were designed using the same pipeline for MERS, SARS-CoV-2 and SARS-CoV. The RBD-sc-dimer elicited high neutralising antibodies against SARS-CoV-2 in vaccinated mice. Finally, in a pilot study, large yields of RBD-sc-dimers were generated for MERS and SARS-CoV-2 using an industry standard CHO system. Suggesting clinical promise for vaccine design for the ongoing COVID-19 pandemic.

Highlights:

- Receptor binding domain (RBD) dimers are more immunogenic and confer enhanced protection to SARS challenge in mice than RBD monomers
- Authors used the crystal structure of the RBD to identify receptor binding motifs, a major target for neutralising antibodies
- A stable RBD dimer was then designed as a tandem repeat single-chain (RBD-sc-dimer) which retained vaccine potency
- Pipeline was used to design vaccine candidates for three betacoronaviruses MERS, SARS-CoV and SARS-CoV-2
- CoV RBD dimers were produced to high yields in a pilot study

Clinical Impact:

- Low – no human trials. However data provides an alternative vaccine target (aside from the spike protein). Furthermore, pipeline to design vaccines could be extended to include further betacoronaviruses, if they are found.

Important Methodologies:

- Crystal structure based design of RBD dimers into a tandem repeat by linking through flexible end residues.
- RBD dimers were expressed in mammalian cells (HEK293T) as opposed to inset cells as mammalian expression has been shown to induce stronger neutralising antibodies
- Size and molecular weight of dimer produced was confirmed by gel filtration and ultracentrifugation
- Binding affinities to the appropriate receptor i.e. hCD26 (MERS) or hACE2 was determined by surface plasma resonance (SPR)

Limitations:

- Further work could confirm if the humoral response elicited is enough to provide protection against CoV challenge
- No analysis of cellular responses generated by the vaccine

Development of a Synthetic Poxvirus-Based SARS-CoV-2 Vaccine

Chiuppesi, F. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.07.01.183236

Summary:

A novel vaccine platform based on a three-plasmid system has been developed by Chiuppesi *et al.* to generate synthetic recombinant Modified Vaccinia Ankara (sMVA) vectors from chemically synthesised DNA. This attenuated poxvirus vector, sMVA, has been adapted to express SARS-CoV-2 spike (S) and nucleocapsid (N) proteins. This has created a novel vaccine candidate which, when administered to mice, results in robust antigen-specific humoral and cellular immune responses. There is also evidence of potent SARS-CoV-2 neutralising antibodies produced. These data suggest recombinant sMVA vectors are a promising vaccine platform to be used for SARS-CoV-2.

Main Findings:

- Balb/c mice were immunised twice with synthetic Modified Vaccinia Ankara (sMVA) single or double recombinant vaccine vectors containing spike (S) and/or nucleocapsid (N) antigens of SARS-CoV-2. All vaccinated mice generated high titres of antigen-specific antibodies after 1 immunisation, which increased upon the 2nd immunisation.
- S, receptor binding domain (RBD) and N-specific antibody responses generated in the vaccinated mice were higher than those found in human convalescent plasma.
- Similar responses to the vaccine vectors were found between Balb/c mice and C57BL/6 mice.

- Neutralising antibody (Nab) responses from immunised mice using pseudovirus assays, were comparable to mice treated with infectious SARS-CoV-2 virus.
- No antibody dependent enhancement (ADE) was observed in sera of immunised mice, using THP-1 monocytes *in vitro*.
- S and N-specific T-cell responses were generated in immunised mice.
- High cytokine secreting $CD8^+$ T-cells were found in all groups immunised with the S antigen, but not the N antigen.
- $CD4^+$ T-cells were found to produce mainly Th1-biased cytokines (TNF-α, IFN-γ).
- Similar SARS-CoV-2 antigen-specific humoral and cellular immune responses were seen in vaccine groups receiving sMVA-S and sMVA-N alone or in combination.

Highlights:

- Development of a unique, potential vaccine platform for SARS-CoV-2.

Clinical Impact:

- Moderate.

Important Methodologies:

- Generation of a three-plasmid system of the sMVA vaccine platform which was characterised both *in vitro* and *in vivo* to assess replication properties, growth kinetics, and immunogenicity.
- Generation of sMVA SARS-CoV-2 vaccine vectors using BAC recombination techniques in *E. coli*. Full length spike and nucleoprotein antigen sequences of SARS-CoV-2 were inserted. Antigen expression was characterised *in vitro* and antigen immunogenicity characterised *in vivo*.

Limitations:

- *In vitro* assay used to assess ADE of antibodies generated from immunised mice utilised the THP-1 monocytic cell line, which does not express the ACE2 target protein for viral entry.

SARS-CoV-2 spike glycoprotein vaccine candidate NVX-CoV2373 elicits immunogenicity in baboons and protection in mice

Tian, J-H. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.06.29.178509

Summary:

Tian *et al*. have designed a subunit vaccine NVX-CoV2373 that is based on the spike protein of SARS-CoV-2. This vaccine is thermostable in the prefusion conformation and specifically binds human ACE2 (hACE2) with a higher affinity than wild type spike protein. Addition of Matrix-M adjuvant to NVX-CoV2373 significantly increases specific IgG titres and the functionality (ACE2 blocking, neutralisation) of antibodies produced in both mice and olive baboons. Specifically, addition of adjuvant to NVX-CoV2373 promotes a multifunctional T cell response that is Th1 dominant. Responses were vastly improved with a second immunisation; with immunised mice protected from SARS-CoV-2 challenge.

Main Findings:

- Vaccine candidate NVX-CoV2373 particles were structurally like the cryoEM solved structure of the trimeric spike protein ectodomain.
- NVX-CoV2373 specifically bound to hACE2, with greater binding affinity than WT spike protein and other candidate BV2365 (IC_{50} = 18 ng/ml vs. 36-38 ng/ml).
- Immunisation of mice and adult olive baboons with NVX-CoV2373 plus Matrix-M adjuvant elevated levels of anti-S IgG titres 21-28 days post immunisation. IgG titres were significantly elevated following a second “booster” immunisation, that were detected up to 2 weeks later.
- The number of hACE2 blocking and virus neutralising antibodies were also increased following vaccination and greatly increased in those that received a second immunisation**.**
- Immunisation with NVX/Matrix-M protected mice from challenge of SARS-CoV-2; shown by reduced virus load and % weight loss 4 days post infection. Lung pathology as also significantly reduced at days 4 and 7 post challenge.
- In animals immunised with NVX without adjuvant, antibody titres and functionality were significantly lower or absent. This regimen was unable to protect mice from challenge.
- Inclusion of adjuvant significantly increased the number of $CD4^+$ multifunctional and Type 1 cytokine (IFN-γ, TNF-α, IL-2) secreting effector memory T cells from the spleens of mice and PBMCs of baboons.
- Type 2 cytokine secretion was slightly increased in mice but was too low to be detected in baboons, suggesting that Matrix-M promotes a Th1 dominant immune response.
- Adjuvant significantly increased the frequency of Tfh (CD4+ CXCR5+ PD1+) and germinal centre B cells (CD19+ GL7+ CD95+) in mice spleens.
- Vaccinated baboons had 7-fold higher anti-S IgG titres and 8-fold higher ACE2 blocking antibodies compared to convalescent serum of recovered COVID-19 patient.

Highlights:

- Results support the clinical development of NVX-CoV2373 vaccine
- Matrix-M adjuvant is significant in the immunogenicity of NXV-CoV2373

Important Methodologies:

- Creation of a subunit vaccine NXV-CoV2373 based on full length SARS-CoV-2 spike protein (WT) with 2 proline substitutions and mutations at a furin cleavage site.
- Vaccine structure and stability were assessed with various techniques including: Dynamic light scattering, differential scanning calorimetry and Transmission Electron Microscopy.
- Bio-layer interferometry (BLI) and ELISA to determine IgG titres and hACE2 binding
- ELISPOT and intracellular cytokine staining to evaluate functionality of T cells.

Limitations:

- Could've been interesting to see the T cell responses from lung tissue of mice.
- Small number of baboons in study (2-3 per group) – large differences can be seen between individuals in the data potentially skewing the results.

Focus on Antibody Diagnostics

Serologic antibody tests for SARS-CoV-2 diagnosis – where are we now?

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Abstract

Since the start of the SARS-CoV-2 pandemic, understanding the correlates of protection, in particular the antibody response post-infection, has been at the forefront of COVID-19 research. To date over 250 commercially available or in-house serologic antibody tests are either approved or currently under development to test the IgM, IgG and IgA responses to a range of different SARS-CoV-2 antigens. This review summarises some of the key studies published in the last six months focusing on the clinical utility of serologic antibody tests for individuals who were previously infected with SARS-CoV-2.

Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulting in the current coronavirus disease 2019 (COVID-19) pandemic has prompted an urgency to develop rapid, sensitive and specific diagnostic tests [1,2,3]. Infection with SARS-CoV-2 can be confirmed using nucleic acid real-time reverse transcriptase polymerase chain reaction (RT-PCR) to detect viral RNA routinely in upper (nasopharyngeal/oropharyngeal swabs, nasal aspirate, nasal wash or saliva) and lower (sputum or tracheal aspirate or bronchoalveolar lavage - BAL) respiratory tract samples. Whilst the RT-PCR test indicates whether a patient is currently infected with SARS-CoV-2 serologic antibody tests can show that a patient was previously infected with SARS-CoV-2. These serology tests can detect IgG, IgM and IgA antibodies in plasma and serum samples weeks after infection when the virus is no longer detected by RT-PCR. Platforms in use include enzyme linked immunosorbent assays (ELISAs), chemiluminescence immunoassays (CLIA) and laboratory-independent lateral flow assays consisting of disposable devices often used for point of care (POC) testing.

These serology assays may be useful for identifying convalescent patients that have recovered from COVID-19 and have high anti-SARS-CoV-2 titres suitable for convalescent serum donation [3]. It has also been proposed that serologic testing can identify who has been infected with SARS-CoV-2 and could provide “immune passports” allowing exposed individuals who have acquired antibody-mediated immunity to SARS-CoV-2 to return to work. However, mounting evidence shows that not all infected individuals go on to make a detectable antibody response and it is still unclear whether antibodies actually confer immunity and for how long [4]. Further research determining which patients do become antibody positive and the nature of this immune response are key to understanding protective immunity to SARS-CoV-2 and will be highly informative for vaccine development. For these serology assays to

be used for population-based screening they need to be both highly sensitive and specific in order to prevent misdiagnosis and misinformation [2].

Current literature evaluating SARS-CoV-2 serology assays

The Cochrane Systematic Review of 57 publications up to the 27 April 2020 [5] reported their assessment of the accuracy of 25 commercially available and numerous in-house diagnostic assays in identifying SARS-CoV-2 individuals in the community and hospital setting (see Table 1). The review raises concerns over the interpretation of serologic antibody tests as some studies only included COVID-19 cases, none considered asymptomatic individuals and most included individuals based on RT-PCR results. Potential bias due to multi-centre study designs, lack of blinding of index tests, reference standards, lack of details of participant numbers, characteristics and inclusion of hospital patients that likely represent only severe disease were all contributing factors leading to inaccurate reporting of serological tests. Moreover, for studies that did not stratify data for time post-symptom onset (days 0-35), the Cochrane Review observed heterogeneity for IgA, IgG, IgM sensitivity. There was a lack of studies for day 35 post-symptom onset, so it was not possible to estimate the sensitivity of tests after 5 weeks and this review did not include the Roche or Abbott serology tests which are approved for use by Public Health England.

The following studies analysed antibody responses to SARS-CoV-2 in detail and consistently found that tests were insensitive early in infection (days 1-7 after symptom onset) but more sensitive later on (days 14 onwards). Detection of IgG responses seem to be more consistent than IgM and detection rates increase when both are combined. Whilst fewer studies looked at IgA responses these were generally more sensitive and detection rates improved later in infection (optimal results were obtained days 14 onwards). Details of results from each study are described below.

In a study by Hu *et al*., detection of SARS-CoV-2-specific IgG and IgM antibodies in 211 confirmed COVID-19 patients showed the concentration of IgM and IgG peaked at days 19-21 after onset of symptoms [6]. IgM antibodies were detected in 73.58% of cases on day 13-15 and IgG in 97.87% on day 16-18 (two patients did not develop IgG or IgM antibodies during the course of disease). There was a higher concentration of IgG in critically ill patients in comparison to those with mild to moderate disease (although this was not significant) and there was no difference in IgM levels.

In a larger study of 285 COVID-19 patients, antibody responses to SARS-CoV-2 were positive in all patients for IgG within 19 days of onset of symptoms [7]. IgM antibodies peaked in 94.1% cases at approximately 20-22 days after symptom onset and IgG levels peaked in 100% of cases at approximately 17-19 days after onset of symptoms. The median time period for IgG and IgM seroconversion was day 13 after onset of symptoms. Peak IgG levels varied widely (more than 20-fold) across patients. This study also evaluated cross-reactivity with SARS-CoV; whilst no cross-reactivity with the S1 subunit of the SARS-CoV spike antigen, cross-reactivity with nucleocapsid (N) antigens was shown. This study also looked at asymptomatic contacts

and showed that in some instances, asymptomatic patients who tested negative by standard molecular methods, actually tested positive by serological testing.

Cross-reactivity was also reported in studies of the sensitivity and specificity of commercial IgA and IgG S1 (spike) based ELISA tests [8]. Patients previously infected with HCoV-OC43 (seasonal human coronavirus) were cross reactive in both commercial IgG and IgA S1 kits, therefore contributing to false-positive results. However, in ELISA assays developed in-house none of the serum samples from endemic human coronaviruses were reactive to SARS-CoV-2 S1, although patient sera from SARS-CoV patients were cross-reactive. The authors point out that due to the low prevalence of SARS-CoV this is unlikely to pose a significant problem.

Another study evaluated several commercial assays which targeted S, receptor binding domain (RBD) protein and compared the results with those from a plaque reduction neutralization assay (PRNT 50) [9]. The rationale being that patients with neutralizing antibodies to RBD are more likely be protected. The Wantai assay using RBD as coating antigen is highly sensitive for detecting total immunoglobulin. They found that the DiaSorin Liaison (IgG) and Euroimmun (IgG/A) assay measured long-lived IgG and/or memory IgA antibodies, providing good quantitative relationships between antibody measurement and protection and the Orient gene rapid antibody test showed high specificity that could be useful for population screening in laboratory settings.

The efficacy of 10 lateral flow and 2 ELISA tests using a panel of 130 samples from 80 SARS-CoV-2 individuals and 108 pre-COVID-19 specimens was tested [10]; sensitivity, specificity and cross-reactivity was assessed. No consistent cross-reactivity was observed in this study and sensitivity increased with time (81.8-100% of PCR positive samples were serologically positive >20 days after the onset of symptoms). Higher positivity in lateral flow assays was found in patients admitted to intensive care. Test specificity ranged from 84.3-100% in COVID-19 negative samples.

In a study of 173 COVID-19 patients which measured total antibody, IgM and IgG using recombinant nucleoprotein antigen in an ELISA test, antibodies were detected in <40% of patients in the first 7 days of illness and then increased to 100% (total Ab), 94.3% (IgM) and 79.8% (IgG) by 15 days after onset; no significant difference in seroconversion was seen between critical and non-critical patients [11].

A study comparing antibodies to SARS-CoV-2 S and N protein by ELISA in 214 patients with laboratory confirmed SARS-CoV-2 and 100 healthy blood donor controls found the S-based ELISA was significantly more sensitive than the N-based ELISA for detecting IgM, IgG or pooled antibodies [12]. Consistent with other studies sensitivity was low at days 0-5 and 6-10 post onset (<60%), higher (80-88.9%) at days 11-15 post onset of symptoms and more than 90% at later stages of the disease. Sensitivity of IgM and IgG detection in the S-based ELISA was low at days 0-5 and 6-10 post onset (<60%). No positive result was found in either N- and S-based IgM and IgG ELISAs in the healthy blood donor samples indicating good specificity.

In an attempt to improve detection of weak, early immune responses to SARS-CoV-2 infection or asymptomatic cases a sensitive immunoassay (ELISA) using recombinant trimeric S protein as the capture antigen, combined with an alkaline phosphatase (AP)-conjugated secondary antibody was developed [13]. This optimised AP ELISA detected 20% and 75% qPCR-validated SARS-CoV-2 cases sampled 1-5 days or 6-14 days after disease onset, respectively and no cross-reactivity was detected in control samples from patients with other viral infections (alpha and beta coronaviruses, Epstein-Barr virus, Respiratory syncytial virus, influenza, parainfluenza, metapneumovirus). This assay also identified samples from individuals with mild and asymptomatic infections, suggesting it also has increased sensitivity for these challenging samples.

Whilst most studies have detected IgM and IgG antibodies in patients with SARS-CoV-2 a recent study using an RBD CLIA has shown the benefit of examining the IgA response [14]. At 4–10 days after symptom onset, the IgA assay exhibited the highest detection rate as 88.2% compared to 76.4% and 64.7% for IgM and IgG respectively. The authors also show IgA levels were significantly higher in severe cases than mild or moderate cases indicating that IgA could be used as diagnostic marker of severe COVID-19 disease.

Table 1. Analysis of commercial antibody tests according to the Cochrane Systematic Review – Diagnostic.

IgG		IgM		IgG/IgM	
Beijing Beier Bioengineering	CGIA, CLIA, ELISA	Artron Laboratories IgM/IgG	CGIA	Acro Biotech - IgG/IgM	CGIA
Beijing Hotgen	ELISA	Autobio Diagnostics IgM/IgG	CGIA	Artron Laboratories IgM/IgG	CGIA
Beijing Wantai	ELISA, CGIA	Beijing Hotgen	ELISA	Autobio Diagnostics IgM/IgG	CGIA
Bioscience Co (Chongqing)	CLIA	Beijing Hotgen	CGIA	Beijing Hotgen	ELISA
Darui Biotech	ELISA	Beijing Wantai	ELISA	Bioscience Co (Chongqing)	CLIA
EUROIMMUN	ELISA	Beijing Wantai	CGIA	CTK Biotech OnSite IgG/IgM	CGIA
EUROIMMUN Anti-SARS-Cov	IIFT	Bioscience Co (Chongqing)	CLIA	Dynamiker Biotechnology IgG/IgM	CGIA
EUROIMMUN Beta	ELISA	CTK Biotech OnSite IgG/IgM	CGIA	Hangzhou Alltest - IgG/IgM	CGIA
Hangzhou Alltest - IgG/IgM	CGIA	Darui Biotech	ELISA	Shenzhen YHLO	CLIA
Innovita Biological - Ab test (IgM/IgG)	CGIA	Dynamiker Biotechnology IgG/IgM	CGIA	Vivachek - VivaDiag IgM/IgG	CGIA
Shenzhen YHLO	CLIA	EUROIMMUN	ELISA	Zhuhai Livzon	CGIA
Snibe Diagnostic - MAGLUMI	CLIA	EUROIMMUN Anti-SARS-Cov	IIFT	Zhuhai Livzon	ELISA
Vivachek - VivaDiag IgM/IgG	CGIA	Hangzhou Alltest - IgG/IgM	CGIA		
Zhuhai Livzon	CGIA	Shenzhen YHLO	CLIA		
Zhuhai Livzon	ELISA	Vivachek - VivaDiag IgM/IgG	CGIA		
		Xiamen InnodDx Biotech	CLIA		
		Zhuhai Livzon	CGIA		
		Zhuhai Livzon	ELISA		

CGIA: colloidal gold immunoassay; **CLIA:** chemiluminescence immunoassay; **ELISA:** enzyme-linked immunosorbent assay; **FIA:** fluorescence immunoassay; **IIFT:** indirect immunofluorescence assay; **LFA:** lateral flow assay.

Concluding remarks

The urgent need for accurate serologic antibody testing in response to the ongoing SARS-CoV-2 pandemic has been met with mixed success. There are a number of commercially available serologic antibody tests, but the effectiveness of these tests is still uncertain. All assays with potential for clinical diagnostic utility should undergo stringent validation and are comprehensively described by the Cochrane Systematic Review. Of those presented herein, various tests measuring IgG, IgM and IgA and IgG/IgM combinations have helped establish the kinetic profiles of anti-SARS-CoV-2 antibodies but also highlight the disparity in levels of sensitivity and specificity for accurate diagnosis and cross-reactivity with SARS-CoV. That said, some tests have identified asymptomatic individuals that previously tested negative using the RT-PCR test. This highlights the importance of understanding the serological response in order to track the spread of the virus and identify the correlates of protection.

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