COVID-19 Community Journal Club No. 13

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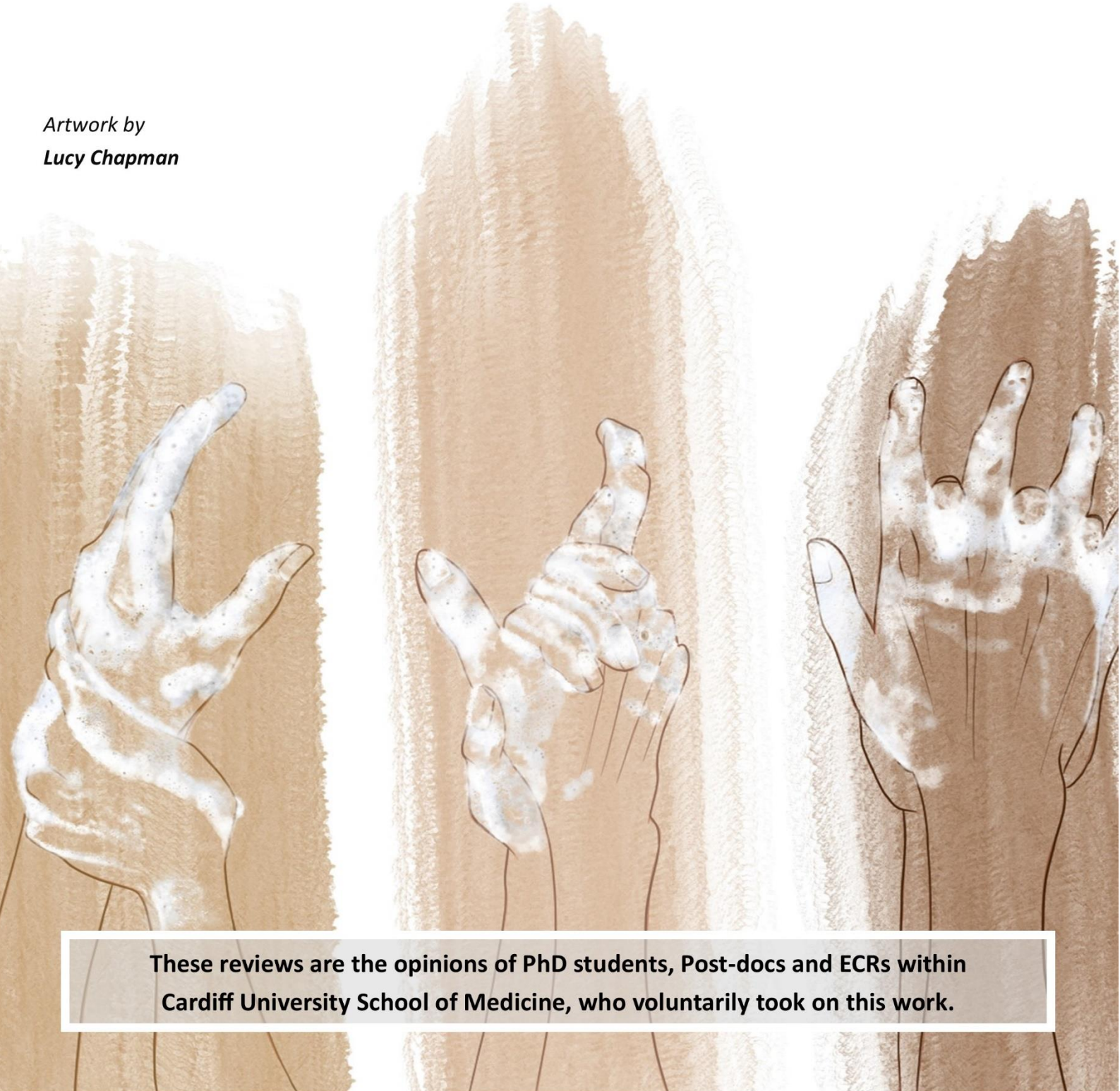
School of Medicine

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

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

Retracted Article: SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion

The above article, found at https://doi.org/10.1038/s41423-020-0498-4, was recently retracted by its authors (Wang, X. *et al*.) from Nature Cellular and Molecular Immunology for the following reasons:

"The authors should have used primary T cells instead of T-cell lines. In addition, there are concerns that the flow cytometry methodology applied here was flawed."

Such limitations were highlighted when the paper was reviewed in edition 2 of the Community Journal Club (page 47). This is not the first article on COVID-19 to be retracted from a peer-reviewed journal (see edition 9, page 7) and highlights the caution we must take when reviewing papers from this rapidly emerging field of science.

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News and Views

Translating Science on COVID-19 to Improve Clinical Care and Support the Public Health Response

del Rio, C. and Malani, P. 2020. *JAMA*

Link: https://doi.org/10.1001/jama.2020.9252

As we enter the 5th month of the COVID-19 pandemic and countries adapt to a new normal way of life, new challenges have arisen. This viewpoint explores such challenges. Currently, only remdesivir has been approved by the FDA for the **medical management** of COVID-19. Despite this, multiple therapeutics have entered clinical trials, including hyrdoxychloroquine as a preventative measure. Trials surrounding the therapeutic use of **convalescent plasma** are also ongoing.

A **viable vaccine** against SARS-CoV-2 is imperative to returning to normal life. Therefore, multiple studies have considered the unprecedented approach of undertaking controlled human infection challenges. Once a successful candidate is found, the next challenge will be the manufacturing of billions of doses. The authors state that it is unlikely that a COVID-19 vaccine will be readily available in less than 24 months.

To allow more substantial reopening of countries in the absence of a vaccine, many governments have considered using "**immunity passports**" and promoting "**herd immunity**". However, seroprevalance of SARS-COV-2 varies greatly between regions and whether individuals are protected from reinfection has not been determined. As 60% of the population is estimated to be required for herd immunity, it is likely that this approach without a vaccine will result in a great loss of life.

It is highly possible that clusters of outbreaks will continue over the coming months. Relaxing of social distancing rules poses a threat of a deadly, **second wave** of infection – similar to what occurred during the 1918 Spanish influenza pandemic. Moving forward, communities must have adequate testing capacity and a public health strategy (face masks, self-isolation) in place to reduce this threat. In the meantime, improving our understanding of who is at greatest risk and what is the best way to prevent severe disease is vital.

Mapping the T cell response to COVID-19

Li, J. *et al*. 2020. *Signal Transduction and Targeted Therapy*
Link: https://doi.org/10.1038/s41392-020-00228-1

Understanding T cell responses to SARS-CoV-2 will inform our understanding of protective immunity, disease pathogenesis and aid vaccine development. A study by Grifoni *et al*. analysed blood samples from 20 recovered adult COVID-19 patients and healthy controls, uncovering that:

- In response to epitope pools of spike and non-spike proteins, 100% of tested recovered patients produced a substantial CD4+ T cell response characterized by IL-2 and IFNγ and little/no IL-4, IL-5, IL-13 or IL-17α
- Approximately 50% of CD4+ T cell responses were directed towards spike protein, with the remainder being directed towards the remainder of the orfeome
- 70% of tested recovered patients displayed SARS-CoV-2 specific CD8+ T cell responses, the majority of which co-expressed IFNγ and granzyme B, with a substantial fraction of IFNγ+ cells also expressing TNF but not IL-10
- The magnitude of anti-RBD IgG and IgA titres correlated with spike-specific CD4+ T cell responses
- Using peptides pools representing individual SARS-Cov-2 polypeptides, 27%, 21% and 11% of CD4+ T cell responses were aimed at spike, M and N open-reading frames respectively; instead, 26% and 12% of the CD8+ T cell reactivity was aimed at the spike and N proteins respectively
- Importantly, between 40-60% of unexposed individuals possessed CD4+ T cells reactive to SARS-Cov-2

Despite limitations including small sample sizes, a focus on non-hospitalized COVID-19 patients and a lack of ‘common cold’ history from healthy controls, this study highlighted important targets for SARS-CoV-2 vaccine development and the distinct specificity patterns of T cells from COVID-19 patients.

Journal Reviews

Clinical

OpenSAFELY: factors associated with COVID-19 death in 17 million patients

Williamson, E.J. *et al*. 2020. *Nature*

Link: https://doi.org/10.1038/s41586-020-2521-4

Summary:

Data presented here was obtained from OpenSAFELY (https://opensafely.org/): a secure analytic program derived from fully pseudonymised primary electronic health care records in NHS England. Using records of 17,278,392 adults, Williamson *et al*. were able to determine particular factors that pose an increased risk to COVID-19 related death. Highlighted risks were being male, increased age, being of non-white ethnicity and living in deprivation, although the latter may be due to more social factors than clinical. OpenSAFELY will continue to rapidly add patient's records to the database; allowing more robust analysis to reveal more significant findings in the near future.

Main Findings:

- Of 17,278,392 adults included, 10,926 had COVID-19 related death.
- Greater age increases the risk of COVID-19 related death. Compared to 50-60 year old patients, those over 80 had a fully adjusted hazard ratio (HR) of 20.61. Risk is dramatically reduced in patients below 50 (HR 0.06-0.30).
- Risk was found to be greater in men than women (HR 1.59), when fully adjusted for age, sex and other clinical factors
- Non-white ethnicities and those living in deprived areas are at greater risk from SARS-CoV-2. Fully adjusted HRs were between 1.43-1.48 when considering ethnicity whilst those living in the most deprived areas have a HR of 1.80. Further analysis revealed that little of these increased risks are explained by typical clinical risk factors, inferring socio-economic factors are increasing COVID-19 risk.
- Increasing obesity (HR 1.92) and most common comorbidities (diabetes, severe asthma, pulmonary/cardiovascular disease) were associated with greater risk.
- Risk of COVID-19 is greatly increased when kidney function declines (HR 3.69)
- Increased risk with cancer was mostly seen in those with a recent diagnosis of a haematological malignancy (<1 year – HR 2.82).
- Current smoking had a fully adjusted HR of 0.89, however post-hoc analyses revealed that comorbidities could be consequences of smoking. A final model adjusted for demographic factors only (age, sex, deprivation and ethnicity) revealed a non-significant increase in HR for current smoking.

- A strong inverse association between hypertension and mortality in older individuals was also found was found, where those 70 and younger were at greater risk than those older than 70 ($p<0.001$).
- Sensitivity analysis revealed that increased hazard ratios have not been affected by social distancing policies in the UK ($p = 0.83$). The relationship between risk and deprivation appeared to be smaller.

Highlights:

- The largest cohort study conducted by any country to date – data available will continue to grow as more records are included.
- Determined risks among the general population rather than those with COVID-19.

Clinical Impact:

- Minimal – data found has already been highlighted in multiple other studies

Important Methodologies:

- Cohort study using national primary care electronic health record data linked to COVID-19 death data from the ONS

Limitations:

- Outcome definition included clinically suspected cases of COVID-19 may have incorrectly identified patients as having COVID-19.
- Although a large cohort, population may not be fully representative (only included 17% of GPs in London).
- Primary care records may contain missing or incomplete data (26% of records did not include ethnicity).

Multisystem Inflammatory Syndrome in U.S. Children and Adolescents

Feldstein, L.R. *et al*. 2020. *NEJM*

Link: https://doi.org/10.1056/NEJMoa2021680

Summary:

Feldstein *et al*. report emergence of a life-threatening hyperinflammatory syndrome 'temporally associated with SARS-CoV-2 infection' in children and adolescents across the United States. This multisystem inflammatory syndrome in children (MIS-C) involves damage to multiple organs in previously healthy individuals (<21 years of age). MIS-C is similar to Kawasaki disease. The study summarises the epidemiology and spectrum of clinical & pathological characteristics of 186 MIS-C patients across 26 states.

Main Findings:

- Overall, 70% of MIS-C patients were tested positive for SARS-CoV-2 by RT-PCR and/or antibody testing. The remaining 30% of MIS-C patients had an epidemiologic link to a COVID-19 positive person.
- The median onset of MIS-C symptom post COVID-19 was 25 days (range = 6-51 days).
- At least 4 organ systems were involved in 71% of MIS-C cases associated with COVID-19.
- Gastrointestinal (92%), cardiovascular (80%), hematologic (76%), mucocutaneous (74%) and respiratory (70%) were the most commonly affected organ systems in MIS-C patients.
- Patients with cardiovascular complications had elevated BNP levels (73%), experienced respiratory insufficiency/failure (59%), had increased levels of troponin (50%) and/or required vasoactive support (48%).
- 4+ biomarkers of inflammation were present in 92% of MIS-C patients (e.g. elevated erythrocyte sedimentation rate, increased levels of C-reactive protein, lymphocytopenia, neutrophilia etc.) (Figure 3).
- As of 20th May 2020, 70% of MIS-C patients had been discharged, 28% were still hospitalised and 2% passed away.

Highlights:

- MIS-C associated with SARS-CoV-2 infection can cause severe and life-threatening damage to multiple organs in predominately health children and adolescents.

Clinical Impact:

- Medium

Important Methodologies:

- Targeted surveillance for MIS-C in paediatric clinics across the United States.
- Stratification of clinical features of MIS-C based on SARS-CoV-2 infection status, age, ethnicity and other clinical illnesses (Table 2).

Limitations:

- Cases reported in the study are from participating hospitals only and do not represent overall cases in each state. Findings cannot be generalised beyond the study population.
- It was not possible to accurately determine the onset of SARS-CoV-2 infection.
- Long-term monitoring is required to assess potential long-term complications of MIS-C with COVID-19.

Antibodies and T cells

Structures of Human Antibodies Bound to SARSCoV-2 Spike Reveal Common Epitopes and Recurrent Features of Antibodies

Barnes, C.O. *et al*. 2020. *Cell*

Link: https://doi.org/10.1016/j.cell.2020.06.025

Summary:

The authors characterise the recognition of coronavirus spikes of polyclonal IgGs and Fabs from convalescent COVID-19 patients and showed that the different IgGs had different focuses on RBD epitopes, recognition and avidity. Increased binding and neutralisation was observed in IgGs compared to Fabs. Specificities of Fabs were examined using electron microscopy and indicated recognition of $S1^A$ and RBD epitopes on SARS-CoV-2 spike. The cryo-EM structure of a neutralising monoclonal Fab-spike complex revealed an epitope that blocks ACE2 receptor binding. Modelling suggests different potentials for inter-spike crosslinking by IgGs on viruses and these are not affected by spike mutations.

Main Findings:

- IgG and Fabs isolated from convalescent patients showed recognition of different coronavirus S proteins and RBDs, including SARS-CoV-2, SARS-CoV, MERS-CoV and common cold coronaviruses. Due to ELISA EC_{50} values and neutralisation potencies three patient samples were carried forward for further analysis (COV21, COV57 and COV107).
- When comparing IgGs to Fabs, most of the SARS-CoV-2 anti-S responses were reduced by at least 50% in the Fabs (except for COV57) and plasma IgGs are more potent neutralisers than plasma Fabs due to their bivalent architecture.
- Using nsEMPEM the authors map epitopes from Fabs onto the SARS-CoV-2 S protein, only Fabs from COV21 and COV57 showed stable binding. COV107 showed no evidence of binding.
- 3D reconstruction of SARS-CoV-2 S complexed with COV21 Fabs showed single extending density at the apex of the trimer corresponding to the Fab binding to a single RBD in an “up” position. This closely resembles a structure of S protein bound to a Fab from the S230 monoclonal antibody isolated from a SARS-CoV-infected individual, whose epitope overlaps with the binding site for the ACE2 receptor.
- 3D reconstruction of SARS-CoV-2 S complexed with COV57 Fabs showed that the density associated with one of the $S1^A$ subunits and pointed downward toward the viral membrane.
- Monoclonal antibodies isolated from patient COV107 were potently neutralising, the structure of one antibody, C105, bound to SARS-CoV-2 S protein was determined using the crystal structure of the unbound C105 Fab for fitting to the cryo-EM density. Two populations of the complex were found (1) an asymmetric S trimer with two “up”

RBDs, each complexed with a Fab and (2) a symmetric trimer with three RBSs in the "up" conformation, each complex with a Fab.
- Identified S mutations (D614G and other residues) are unlikely to affect these epitopes because the binding Fabs are distant from these, as long as the mutation does not cause a major conformational change.
- Modelling indicates that inter-spike crosslinking could occur for the COV21 epitope, but is unlikely to occur for the COV57 epitope due to the downward Fab orientations being unable to accommodate an Fc in a position that could join two Fabs.

Highlights:
- COVID-19 plasma IgGs can recognise SARS-CoV-2, SARS-CoV and MERS-CoV S proteins
- EM reconstructions of polyclonal Fab-S complexes reveal $S1^A$ and RBD epitopes
- Structure of neutralising Fab-S complex showed binding to "up" RBDs
- Structures define a recurrent VH3-53/VH3/66 derived anti-SARS-CoV-2 antibody class

Clinical Impact:
- Minimal

Important Methodologies:
- Cloning and expression of recombinant CoV proteins
- Purification of plasma IgGs and Fabs
- Negative stain electron microscopy (nsEM), Negative stain electron microscopy polyclonal epitope mapping (nsEMPEM) and cryo electron microscopy (cryo-EM)
- X-ray Crystallography

Novel SARS-CoV-2 specific antibody and neutralization assays reveal wide range of humoral immune response during COVID-19

Dogan, M. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.07.07.20148106

Summary:

Serologic antibody testing is crucial for measuring seroprevalence and understanding correlates of protection to SARS-CoV-2 infection. Dogan *et al*., describe two assays developed using plasma from COVID-19 patients with a range of disease severities. A bead-based fluorescence immunoassay to detect patient antibodies against SARS-CoV-2 spike receptor-binding domain (S-RBD) and nucleocapsid (N) proteins and a neutralisation assay to detect patient neutralising antibodies (nAbs) that block S-RBD binding to the host receptor ACE2.

Both assays may provide insights into the functional profiles of patient antibodies according to disease severity.

Main Findings:

- Severe COVID-19 patients (intensive care and deceased groups) had increased expression of S-RBD-IgG and N-IgG antibodies and decreased S-RBD-IgA compared to other hospitalised, convalescent donor or outpatient groups
- Convalescent donors had significantly decreased expression of S-RBD-IgG, S-RBD-IgA and N-IgG than intensive care or deceased groups raising the question as to whether convalescent plasma has clinical utility for treating severe COVID-19 patients
- 50% neutralisation titers (NT50) were 1000-fold higher in intensive care and deceased groups than hospitalised and convalescent groups
- Correlation of increased NT50 and expression of S-RBD-IgG, S-RBD-IgG1 and N-IgG during the acute phase in hospitalised, intensive care and deceased groups compared with convalescent and outpatient groups
- Correlation of increased NT50 and expression of S-RBD-IgG, S-RBD-IgA and N-IgG with increasing age during the acute phase
- Negative correlation between NT50 and expression of S-RBD-IgG, S-RBD-IgA and N-IgG from days 15-90 post-diagnosis suggesting a decline in antibody titer over time

Highlights:

- COVID-19 patients with increasing age and disease severity show increased anti-S, anti-N (IgG) and nAbs during the acute phase, with antibody titers declining over time.

Clinical Impact:

- Low.

Important Methodologies:

- Antibody assay: biotinylated S-RBD or N proteins captured by streptavidin coated beads, incubated with patient plasma and stained with phycoerythrin (PE) conjugated anti-IgG, -IgG1, -IgG2, -IgG3, -IgG4, -IgA and -IgM antibodies.
- Generation of spike and SARS pseudo lentiviruses for measuring nAbs in patient plasma.

Limitations:

- Small sample size (n=87 subjects including 20 healthy blood donors).
- Healthy donors recruited before and during pandemic by negative PCR (potential for false negative results).
- Antibody assay shows cross-reactivity with SARS-CoV.
- Positive controls for assay were anti-S-RBD human IgG antibody and soluble ACE2-Fc – are these specific or are there other potential ligands for these targets?
- nAbs to S-RBD were from an unknown source.

Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection

Seow, J. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.07.09.20148429

Summary:

Seow *et al.* followed seroconversion and neutralising antibody (nAb) responses in 65 RT-qPCR confirmed Covid-19 patients for up to 94 days post onset of symptoms (POS). Disease severity enhanced the magnitude but not the kinetics of the nAb response. Findings showed that individuals with more severe infection, maintained high titres >60 days POS. However, those with less severe infection approached baseline titres over the follow-up period. This study highlights the similarity of SARS-CoV-2 infection with circulating seasonal coronaviruses in generating transient nAb responses. Findings to be considered for serological testing, protection against re-infection with SARS-CoV-2 and durability of vaccine protection.

Main Findings:

- IgM and IgA binding responses declined after 20-30 days POS, whereas IgG responses remained high in the majority of individuals, up to 94 days POS.
- nAb titres peaked on average at day 23 POS and decreased 2- to 23-fold during an 18-65 day follow up period.
- Individuals with high peak nAb ID_{50} (serum dilution that inhibits 50% infection), maintained Ab titres in the 1000-3500 range >60 days POS. In individuals with modest nAB titres following infection (100-300 range), titres became undetectable (ID_{50}<50) or were approaching baseline after ~50 days.
- Neutralisation ID_{50} values correlated well with IgG, IgM and IgA binding to all three antigens, S, RBD and N. S- and RBD- specific IgM and IgA had the capacity to facilitate neutralisation in the absence of measurable IgG, in acute infection.
- IgM and IgA responses against S at peak neutralisation were higher in the most severe disease group, with no significant difference for IgG to S. This shows a potential role for IgM and IgA in neutralisation.
- Examination of healthcare workers showed that some asymptomatic individuals could generate neutralisation titres >1000. As the mean peak ID_{50} was lower in these individuals, the decline in nAb titres towards baseline was more frequent compared to the patient cohort.

Highlights:

- Seroconversion was detected in >95% of cases and nAb appeared beyond 8 days POS
- Severe disease causes higher nAb response, but titres decline over time, similar to circulating seasonal coronaviruses associated with common colds.
- Asymptomatic individuals can generate high nAb titres (>1000).

- IgM and IgA have the capacity to facilitate neutralisation in the absence of measurable IgG, in acute infection.

Clinical Impact:

- Moderate - Waning of IgG response should be considered in seroprevalence studies in individual with unknown SARS-CoV-2 status. Also, IgA and IgM can be used as a marker of recent or acute SARS-CoV-2 infection – more relevant in hospital setting.

Important Methodologies:

- ELISA measuring IgG, IgM and IgA response against spike (S), the receptor binding domain (RBD) and nucleocapsid (N).
- Neutralisation assay using HIV-1 based virus particles, pseudotyped with SARS-CoV-2 S in a HeLa cell line stably expressing the ACE2 receptor.

Limitations:

- Categorised patients based on disease severity (need for supplemental oxygen). No information given on viral load or viral persistence.
- 77.2 % of cohort were male and no ethnicity information was collected – it would be interesting to see nAb differences based on sex and ethnicity.
- Further follow-up of cohorts with high nAb titres at the final time point needed.

Broad and strong memory CD4+ and CD8+ T cells induced by SARS-CoV-2 in UK convalescent COVID-19 patients

Peng, Y. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.06.05.134551

Summary:

T-cells from recovered COVID19 patients were reacted against a SARS-CoV-2 peptide library covering the proteome (except ORF-1). The level of IL-2 produced, and number of responding T-cell pools varied but most had a T-cell response. 39 peptides were recognized with some recognized by multiple patient's T-cells. Relative to mild cases, severe cases had a greater amount of antibody, IL-2 and number of T cell pools which responded. In severe cases more CD4 than CD8 T-cells produced IL-2, IFNγ and TNFα and were able to produce these in combination. Relative to other CD8 responding T-cells, nucleoprotein reactive T-cells had a greater proportion of responders that produced more than one cytokine.

Main Findings:

- By ELISpot, most patients had some T cell mediated response. The responses varied widely in number of T cell pools, level of IL-2 production and the component of SARS-CoV-2 to which T cells responded. No responses using healthy control samples were detected.
- Between mild and severe cases, the latter had a greater number of responding T cell pools and level of IL-2 produced.
- Positive correlations were observed between overall T-cell responses and end-point titres of antibodies against the spike, receptor binding and nucleoprotein domains indicating it is not one immune response or the other.
- Between mild and severe cases, the latter had more antibodies against spike, receptor binding and nucleoprotein domains.
- In T cell pools which responded to peptide, CD4 and CD8 T cells were evaluated for IL-2, IFNγ and TNFα production. In severe cases, more CD4 than CD8 T cells produced these cytokines.
- Of the cytokines assessed, a greater number of CD4 T cells were able to produce more than one cytokine relative to CD8 T cells.
- CD8 T cells which responded to SARS-CoV-2 nucleoprotein had a greater proportion of responders capable of producing more than one cytokine compared to CD8 T cells reacting to spike or envelope proteins.
- Very few CD4 T cells (in both mild and severe cases) were CD107a positive. The majority were CD8 T cells.
- 39 peptides from SARS-CoV-2 were identified (17, spike; 10, nucleoprotein; 6, membrane) with 6 immunodominant clusters (3 in the spike, 2 in the membrane protein and 1 in the nucleoprotein) and several were recognized by multiple patients. The best predicted epitope sequences are shown in table 2.
- SARS epitopes were tested and only 2 nucleoprotein and 1 spike protein elicited a response from SARS-CoV-2 patients.
- SARS-CoV-2 epitopes identified showed little homology to other common coronaviruses.

Highlights:

- Predicted epitopes from peptide reactive T cells are given here in the supplementary information and may prove useful for vaccine design for promoting both humoral and T cell immunity.

Clinical Impact:

- None; implications for vaccine design strategy but not clinical practice.

Important Methodologies:

- *Ex vivo* stimulation of recovered, mild (n = 28) or severe (n = 14) COVID-19 patient's T cells relative to healthy controls (n = 19) in response to a peptide covering the SARS-CoV-2 proteome (excluding ORF-1).

- T-cell responses were assessed for intracellular cytokine production profiles and their HLA-restriction, i.e. ELISpot and multiparameter flow cytometry.

Limitations:

- Whilst outside the scope of the study, it would be interesting to see if the patient's antibodies reacted to distinct or common peptides from their T cells.

Next Generation Sequencing of T and B cell receptor repertoires from COVID-19 patients showed signatures associated with severity of disease

Schultheiß, C. *et al*. 2020. *Immunity*

Link: https://doi.org/10.1016/j.immuni.2020.06.024

Summary:

The authors conducted a comprehensive analysis of the adaptive immune response to SARS-CoV-2 in two cohorts of COVID-19 patients. The adaptive immune responses were assessed using next generation immunosequencing alongside antibody diagnostics, cytokine profiling and flow cytometry. Patient cohort one recovered from COVID-19 in the absence of any medical intervention whilst cohort two required hospitalisation from COVID-19. Assessment of the immune profile of patients with COVID-19 highlighted multiple points of dysregulation. Next generation sequencing of BCR showed higher somatic hypermutation in severe COVID-19 patients compared to patients from cohort one. The BCR repertoire was also found to be skewed towards IGHV3 usage. Furthermore, 31 potentially protective T-cell clonotype clusters were identified from COVID-19 patient repertoires. The T and B-cell clonotyping data formed the basis of an actively updated online repository.

Highlights:

- Interrogation of the immune profile of patients with active and the most severe COVID-19 showed leucocytosis, granulocytosis and T-cell lymphopenia compared to healthy controls
- 32/37 patients exposed to SARS-CoV-2 were positive for anti-SARS-CoV-2 IgA and IgG by ELISA whilst 35/37 were positive by a plasma neutralisation assay
- COVID patients demonstrated persistent cytokine deregulation including IL-10, IL-6, IL-8
- T-cell skewed towards a Th, Th17, Tfh and regulatory T-cell phenotype with an upregulation of inhibitory molecules such as BTLA, PD-1 and TIGIT – this pattern was not seen in memory or naïve T-cells
- High levels of CD40L in patients who recovered from COVID-19

- High somatic mutation, corresponding to a low percentage of naïve B-cells associated with severe COVID-19 patients who required ventilation or ECMO
- Low somatic mutation found in patients with less severe disease, indicating a larger pool of naïve B-cells
- BCR rearrangements converged towards IGHV3 usage
- Highly clonal T-cell repertoires were found in patients with active COVID-19
- Dominant T-cell response during active disease is predominantly made up of clusters with public T-cell clonotypes however upon recovery dominant T-cell clonotypes tended to be private
- Found 150 clusters of T-cell clonotypes with likely pathophysiological relevance, 31 of these are likely associated with protective immunity
- TCR repertoire of patients with mild COVID-19 was highly diverse

Clinical Impact:

- Low however understanding the B and T-cell repertoires could lead to a more targeted vaccine development strategy and help in the discovery of potent monoclonal antibodies. Furthermore, the T and B-cell responses could be used as a prognostic biomarker if the difference between protective and detrimental responses is understood.

Important Methodologies:

- Multiparametric flow cytometry for profiling of CD8/CD4 T-cell responses
- Next generation immunosequencing for T and B-cell repertoires
- GLIPH2 algorithm (grouping of lymphocyte interaction by paratope hotspots) – used to cluster TCR sequences based on presumed identical antigen recognition. This algorithm allowed the authors to identify the generation probability of the T-cell clonotypes which facilitated grouping of TCR sequences into high pGen and low pGen clusters, indication public or private TCRs sequences respectively.

Limitations:

- Difficult to obtain matched healthy donor or asymptomatic patient samples which are required to validate repertoire clusters
- Samples were only taken once or at a couple of time points, longitudinal analysis would further enhance identification of T and B-cell clonotype clusters
- The HLA type of patients should be taken into account when discussing the T-cell repertoires to further understand T-cell immunity to SARS-CoV-2 however a much larger cohort would be required to determine any associated between HLA type and disease severity

Immune Responses: Clinical Implications and Possible Interventions

Immunological and inflammatory profiles in mild and severe cases of COVID-19

Song, J-W. *et al*. 2020. *Nature Communications*
Link: https://doi.org/10.1038/s41467-020-17240-2

Summary:

Blood CD3+, CD4+ and CD8+ T cell and NK cells counts were lower and B-cells appeared at a higher frequency in 12 severe intensive care COVID-19 patients compared to 29 mild cases. T-cell profiling of blood from these patients and 6 healthy controls suggested CD8+ T-cells may be activated in disease, more so in severe cases. IL-6, TNF-α, IL-17A, IFN-γ, MCP-1 and IL-10, IL-4, IL-5 were increased in patients versus healthy controls.

Lung tissue from one severe patient who died showed infiltration of CD4+, CD8+ T-cells, macrophages and GZMB+ cells. Suggested that T-cell populations may be lower in peripheral blood due to infiltration in lungs.

Main Findings:

T-cell population	Sub-population marker	% in all Patients vs HC (P<0.05)	% in Severe vs Mild (P<0.05)	Correlation to time after disease onset
CD8+	$CD38^+HLA\text{-}DR^+$	Y ↑	Y ↑	Y, +
	CD38	Y ↑	N	N
	HLA-DR	Y- ↑ Severe only	N	Y, +
	PD-1	Y- ↑ Severe only	Y ↑	Y, +
	Tim-3	Y-↑ Severe only	Y ↑	Y, +
CD8+ Cytotoxic	$GZMB^+PRF^+GNLY^+$	Y ↑	Y ↑	
	$GZMB^+PRF^+$	Y ↑	N	
CD4+	$CD38^+HLA\text{-}DR^+$	Y ↑	N	
	CD38	N	N	
	HLA-DR	N	N	
	PD-1	N	N	N
	Tim-3	Y↑	N	Y, +
CD4+ Markers: CD27, CD45 RA and CCR7	Naïve	Y- ↑ Severe only	N	
	Circulating Memory	Y- ↑ Mild only	N	
	Transitional Memory	Y ↓	N	
	Effector Memory	N	N	
CD4+	Th1	Y- ↑ mild only	N	

Markers: CCR4, CXCR3 and CCR6	Th2 Th17 Th1Th17	Y- ↓ mild only Y- ↓ mild only Y- ↑mild only	N N Y ↓	
Regulatory	$CD127^{-}CD25^{+}$	unchanged		
Follicular	$PD\text{-}1^{+}CXCR5^{+}$	unchanged		

Highlights:

- T-cell counts are reduced in severe COVID-19 patients' blood, CD8+ T-cells appear more activated.

Clinical Impact:

- Low- these results need to be independently validated.

Important Methodologies:

- Lymphocyte detection kit (Tongshengshidai Biotechnology Co.)

Limitations:

- Why are the counts changed but not the frequency?
- Limited number of patients, especially with severe disease.
- Circulating monocytes do not appear to have been assessed.

Natural killer cell activation related to clinical outcome of COVID-19

Maucourant, C. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.07.07.20148478

Summary:

Maucourant *et al*. characterize host NK cell response towards SARS-CoV-2 infection in moderate and severe COVID-19 patients in the system circulation using high-dimensional flow cytometry, integration factor analysis and scRNAseq data. This study provides a comprehensive map of the NK cell response landscape in SARS-CoV-2 infected COVID-19 patients and yield insights into host response reactions towards viral and associated disease pathology. Also, reveals an activated NK cells phenotype with upregulated levels of effector molecules and chemokines, activating receptors and nutrient receptors. In addition, in this study, they also analyse publicly available scRNAseq data on BAL NK from COVID-19 patients to support and understand NK cells behaviour.

Main Findings:

- Peripheral blood CD56bright and CD56dim NK cells displayed an activated effector phenotype that is similar in nature in moderate and severe COVID-19 disease.
- NK cell response in acute COVID-19 occurred independently of inhibitory KIR expression and NK cell education status.
- Severe COVID-19 disease is associated with a high presence of adaptive NK cell expansions that display signs of proliferation and activation
- There are two different "immunotypes" in patients attributed to milder disease course or those contained for viremia, chronic underlying diseases, steroid treatment, mechanical ventilation and fatal outcome.
- NK activation pathways could be shared with other viral infections, connecting NK cell-specific phenotypic traits to components of the systemic inflammatory milieu related to COVID-19

Highlights:

- NK cell response towards SARS-CoV-2 infection and specific features unique to severe COVID-19 and hyperinflammation, providing a base for understanding the role of NK cells in patients with COVID-19.

Clinical Impact:

- Low

Important Methodologies:

- PBMC isolation using Ficoll gradient centrifugation. NK cell response and PBMC were analysed with a 28-color flow cytometry panel. Data analysis they used UMAP and PhenoGraph.
- KIR-ligand genotyping
- Serum protein quantification using proximity extension assay (PEA)
- Pre-processed scRNA-seq data obtained from a Liao, M *et al.* and read using Seurat. For gene enrichment they used PANTHER and MAST.

Limitations:

- Number of patient used in this study is low. 27 in hospital with COVID-19 disease (10 moderate and 17 severe). Age ratio from 18-78 years old whereas the controls used were from 50-59 years old.
- Patient recruited for this study were sampled 5-24 days after symptom debut

Early use of tocilizumab in the prevention of adult respiratory failure in SARS-CoV-2 infections and the utilization of interleukin-6 levels in the management

Antony, S.J. *et al*. 2020. *Journal of Medical Virology*

Link: https://doi.org/10.1002/jmv.26288

Summary:

The authors present a longitudinal retrospective study of 80 Texan SARS-CoV-2 patients treated with the IL-6 receptor monoclonal antibody, Tocilizumab (TCZ) within 24 hr hospitalisation, followed by the corticosteroid, methylprednisolone, treatment over 72 hr. Laboratory monitoring of IL-6, CRP, ferritin, LDH, D-dimer, procalcitonin, blood counts and metabolic parameters occurred at days 0, 3 and 6 post-treatment. Patient median IL-6 levels increased >1.6-fold post-treatment, whereas CRP, ferritin and LDH levels decreased. The authors suggest early use of TCZ may reduce the need for mechanical ventilation (MV). The proportion of patients requiring MV was 11.4% and the overall mortality was 8.9%.

Main Findings:

- Treatment of 80 SARS-CoV-2 patients with a single (4mg/kg/day) dose of TCZ IL-6 inhibitor, followed by 60mg methylprednisolone over 72 hr resulted in 11.4% requiring MV and 8.9% overall mortality.
- The treatment regime did not reduce patient median levels of soluble IL-6; day 0 = 342.50 pg/mL (78.25 -666.25); day 3 post-administration = 563 pg/mL (162 - 783); day 6 = 545 pg/mL (333.50 - 678.50).
- Patient median levels of CRP, ferritin and LDH did decrease with treatment over 6 days, although only CRP levels dropped to within a normal healthy range at 2mg/L (1-4; normal <8.0mg/L).
- 15% patients had bacterial coinfections and these patients had higher mortality (57.14% vs 42.86% patients without coinfections, $P = 0.008$)

Clinical Impact:

- Minimal based on limitations and the availability of more comprehensive studies, including: https://doi.org/10.1016/S2665-9913(20)30210-1

Important Methodologies:

- Nothing novel or bespoke.

Limitations:

- Very small study – only includes 80 hospitalised SARS-CoV-2 patients from 4 different sites in El Paso, Texas, evaluated between 1/2/2020 and 31/5/2020. Preceded by far more extensive studies, such as Guaraldi *et al*., Lancet Rheumatology from 24/06/2020 that sampled 544 Italian patients with severe COVID-19 (https://doi.org/10.1016/S2665-9913(20)30210-1).

- No control group included – all patients underwent the same TCZ + methylprednisolone treatment. It is not possible to say whether the treatments had any beneficial or detrimental impacts since there are no untreated patients to compare with. In contrast, the Guaraldi *et al*. paper describes 365 patients treated with standard of care and 179 patients treated with TCZ.
- Since the two therapeutics were applied as a single treatment regime, it is not possible to discern whether patient mortality is linked to either single agent or the combination. The title and premise is misleading since (given the elevated levels of IL-6 recorded post-TCZ treatment) it is not clear what contribution the corticosteroid has for patient outcome.
- The patient clinical and sociodemographic details are not linked to outcome and mortality and so it is not possible to evaluate the impact of comorbidities or individual serum measures for MV and survival. This severely weakens the paper.
- The figures in the data tables are inconsistent and inaccurate/incomplete, making it difficult to interpret or trust the data. Sometimes there are 79 patients when 80 is reported etc. and each of the 3 patient age bands seemingly contains >45% the cohort. Some corrections and clearer presentation are required.
- 15% are described as having bacterial coinfections, but the time that these arose is not discussed – were they pre- or post-hospitalisation and/or TCZ treatment? TCZ treatment is associated with immune compromise and so bacterial infections or reactivation of latent viruses can be fatal in treated patients. Without more information and longer term follow-up (this study only extended to 6 days post-treatment), it is not known what the true impact of the TCZ treatment is for these SARS-CoV-2 patients. Thus the treatment, itself, cannot be properly evaluated.

Vaccines

Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine candidate induces high neutralizing antibody titers in mice

McKay, P.F. *et al*. 2020. *Nature Communications*

Link: https://doi.org/10.1038/s41467-020-17409-9

Summary:

Development of a SARS-CoV-2 vaccine candidate using self-amplifying RNA (saRNA) encoding the SARS-CoV-2 spike (S) protein encapsulated within a lipid nanoparticle (LNP). Mice immunised with the vaccine presented with dose-dependent SARS-CoV-2 specific IgG antibody titres with high neutralising capacity and no antibody dependent enhancement (ADE). A Th1 characteristic immune response was seen in mice with high IFN-γ production upon re-stimulation of splenocytes with SARS-CoV-2 peptides. These data provide a promising outlook to a new vaccine candidate for the COVID-19 pandemic.

Main Findings:

- Mice were immunised twice, 1 month apart with self-amplifying RNA (saRNA) encoding the SARS-CoV-2 S protein (pre-fusion confirmation) encapsulated within a lipid nanoparticle (LNP). 6 weeks later, high titres of SARS-CoV-2 specific IgG antibodies were found in mouse sera in a dose-dependent manner.
- Even mice with low dose vaccine (0.01 μg) had higher titres of SARS-CoV-2 specific IgG compared to convalescent plasma from recovered COVID-19 patients.
- A Th1- biased antibody response was observed in vaccinated mice.
- Antibodies produced by mice were highly efficient at neutralising both pseudo- and wild-type SARS-CoV-2 *in vitro*. This efficiency increased dose-dependently.
- Mouse antibodies generated were more efficient at virus neutralisation assays than convalescent plasma from recovered COVID-19 patients.
- A positive correlation was found between titres of IgG antibodies and neutralising capacity in both vaccinated mice and recovered COVID-19 patients.
- No antibody-dependent enhancement (ADE) was found in vaccinated mice.
- A mild neutralising capacity of vaccinated mouse sera was found against SARS-CoV pseudo-typed virus, but not MERS-CoV or 229E-CoV.
- Re-stimulated splenocytes from vaccinated mice yielded high IFN-γ secretion as well as increases in cytokines such as TNF-α and IL-6.
- 4 hours post-vaccination, systemic cytokines were assessed in mice and higher doses had increased levels of IL-6 and IFN-β, among several others.

Highlights:

- Development of a scalable saRNA vaccine candidate against SARS-CoV-2.

- Immune responses in vaccinated mice were generally increased compared to recovered COVID-19 patients.

Clinical Impact:

- Moderate

Important Methodologies:

- Self-amplifying RNA vaccine.
- Pseudotype and wild-type SARS-CoV-2 neutralisation assay.

Limitations:

- COVID-19 patient severity was not accounted for when assessing sera.

Identification of SARS-CoV-2 Vaccine Epitopes Predicted to Induce Long-Term Population-Scale Immunity

Yarmarkovich, M. *et al*. 2020. *Cell Reports Medicine*

Link: https://doi.org/10.1016/j.xcrm.2020.100036

Summary:

Yarmarkovich *et al*. describe an 'immunogenicity map' for SARS-CoV-2, identifying 65 optimal 33mer sequences containing 9mer peptides predicted to induce $CD8^+$, $CD4^+$ T cell and B cell responses, at a population scale. The 33mers were also significantly enriched in immunogenic peptides common to SARS-CoV-1 which are already recorded in the IEDB.

Main Findings:

- 3524 9mer SARS-CoV-2 peptides were predicted to bind strongly to at least 1 HLA class I allele. One peptide, FVNEFYAYL, was predicted to bind to HLA class I alleles representing 90.2% coverage of the US population. 6098 peptides were not predicted to bind any HLA class I alleles, deemed 'immunologically silent.'
- A sequence length of 33mers was determined optimal; incorporating the most 9mers predicted to bind to HLA alleles representing maximal population coverage. The 33mer 'IAMSAFAMMFVKHKHAFLCLFLLPSLATVAYFN' contained peptides predicted to bind to HLA class I and HLA class II alleles representing 82.1% and 98.6% of the US population, respectively.
- Conservation analysis showed greatest cross-species divergence in the Spike protein (involved in host cell entry) and least divergence in the ORF1a gene (essential for viral replication).

- A 33mer peptide from the Spike protein, VGGNYNYLYRLFRKSNLKPFERDISTEIYQAGS, was found to have the highest combined linear and conformational predicted B cell epitope score.
- Sequences were further prioritised from functionally relevant regions of the Spike protein, including the receptor binding domain, a recently evolved furin cleavage site contributing to increased infectivity (Wrapp *et al*. 2020), a mutation site reported in a potentially dominant SARS-CoV-2 strain (Nerli, S., and Sgourakis, N.G., (2020) and Becerra-Flores, M., and Cardozo, T. (2020)) and regions recently suggested to increase binding to ACE2 (Shang *et al*. 2020). These are shown in a structural model of the SARS-CoV-2 spike protein.

Highlights:

- Optimised potential vaccine peptides were identified from an aggregate score of HLA binding and population coverage, conservation with other coronaviruses, dissimilarity to the human proteome and functional relevance in the Spike protein. This publication indicates ongoing work designing the 33mer vaccine vectors which will be made available for research testing.

Clinical Impact:

- Moderate- although requiring *in vitro* / *in vivo* validation, the authors suggest that the 33mer sequences could be produced as RNA/DNA vaccines. This approach is under investigation in SARS-CoV-2 trials (e.g. Moderna, mRNA vaccine (USA)).

Important Methodologies:

- A mathematical model (Yarmarkovich *et al*., 2020) was used to score predicted binding of all possible 9mers from the 10 SARS-CoV-2 genes to 84 HLA class I alleles and identify an optimal k-mer. The optimal k-mers were analysed for HLA class II population coverage. Binding predictions were made using NetMHCpan 4.0 or NetMHCII 2.3, respectively.
- Clustal Omega was used to align and perform homology analysis on 727 protein sequences arising from the 10 SARS-CoV-2 genes of 15 related coronavirus species. The 33mers were scored for conservation as a function of inter-species and interhuman variation.
- Viral/ human sequence dissimilarity was scored based on individual amino acids (identical, similar classification or different polarity) and peptide-HLA binding pairs (also accounting for anchor residues and hydrophobicity).
- To identify B cell peptides, individual amino acid residues were scored using BepiPRED 2.0 and DiscoTope 2.0. Linear epitopes from the Spike, Matrix and Envelope proteins were scored, in addition to conformational epitopes from the Spike protein.

Limitations:

- Endogenous processing of the 33mer minigene vectors may not generate the predicted 9mer peptides, nor are 9mers the optimal peptide length for certain HLA class I alleles. Immunogenicity of the optimal 33mers or individual 9mers was not tested *in vitro* or *in vivo*.

- The cut-off for strong binding affinity is variable between HLA alleles, therefore, selecting <500 nM is likely to overestimate strong binding peptides.
- While prediction of conformational B cell epitopes is a novel aspect of the analysis, the authors note that 33mers may not re-fold into the correct conformations to be recognised by antibodies.
- Re-challenge responses were not tested; therefore, it is not possible to determine if the selected peptides confer long term immunity.

Phosphoproteomics

The Global Phosphorylation Landscape of SARS-CoV-2 Infection

Bouhaddou, M. *et al*. 2020. *Cell*

Link: https://doi.org/10.1016/j.cell.2020.06.034

Summary:

Bouhaddou *et al.* conduct an extensive analysis scrutinising the phosphorylation of host and viral proteins to uncover the host cell interaction with SARS-CoV-2. The phosphoproteomics analysis was conducted in Vero E6 in which they identify host cellular pathways involved in infection and interactions between viral and host cell proteins that aid infection. They go on to identify several anti-viral drugs/compounds by mapping to dysregulated kinases and demonstrate their anti-viral capabilities *in vitro.*

Main Findings:

- SARS-CoV-2 infection promotes casein kinase II (CK-2) and p38 MAPK activation, cytokine production and shuts down mitotic kinases and cell cycle arrest (p38 MAPK signalling, AKT, ERK signalling, Rho-GTPase, CK-2 cytoskeleton and cell cycle regulation).
- Down regulation of ROCK and PAK kinase activity and up regulation of CK-2 cytoskeletal targeting (potentially via an interaction with the viral N protein) enhances filopodial protrusions containing budding viral particles.
- 87 drugs/compounds were identified by global mapping to dysregulated kinases and pathways.
- Inhibition of p38, CK-2, CDK, AXL and PIKFYVE kinases demonstrated anti-viral efficacy.
- Anti-viral activity was evident for silmitasertib, gilteritnib, ARRY-797, MAPK13-IN-1, SB203580, ralimetnib, apilimod and dinaciclib.

Highlights:

- Infection promotes p38 MAPK cascade activation and the shutdown of mitotic processes.
- Infection enhances CK-2 filopodial protrusions to aid viral budding.
- Identify anti-viral activity for several FDA approved/in clinical testing/under pre-clinical development drugs/therapies.

Clinical Impact:

- Potential impact – Identification of potential anti-viral drugs for the treatment of COVID-19, although only preliminary data is presented in this study.

Important Methodologies:

- Proteomic analysis of Vero infected cells.
- Phosphopeptide enrichment/Mass spectrometry to identify phosphorylated proteins of interest.
- Spectral library generation
- Pharmacological profiling dose response analysis

Limitations:

- Use primate cells (Vero E6) to conduct phosphoproteomics screen, a human cell line would have been more relevant.
- Drug efficacy experiments carried out in vitro, albeit in a human lung cell line.

Virus Replication

Direct evidence of active SARS-CoV-2 replication in the intestine

Qian, Q. *et al*. 2020. *Clinical Infectious Diseases*

Link: https://doi.org/10.1093/cid/ciaa925

Summary

Qian Q *et al.* investigated the presence of virions and pathological changes in surgical rectal tissues of a clinically confirmed COVID-19 patient with rectal adenocarcinoma. Quantitative RT-PCR was performed on the rectal tissue specimens obtained from surgical resection, succus entericus and intestinal mucosa of ileostomy, and rectal mucosa during follow-up after recovery. Immunohistochemical analysis and immunofluorescence were carried out on rectal tissues to evaluate the distribution of SARS-CoV-2 antigen, and immune cell infiltrations. RNA of SARS-CoV-2 was detected in surgically resected rectal specimens, but not in samples collected on 37 days after discharge. Typical coronavirus virions in rectal tissue were observed under electron microscopy. Moreover, abundant lymphocytes and

Macrophages, some COVID positive, infiltrating the lamina propria.

Main Findings:

- Retrospective study on the clinically COVID positive patient's surgically removed tissue.
- SARS-CoV-2 nucleic acid detection was performed on the surgical specimens of rectal tissues, which was positive for SARS-CoV-2.
- Throat swab, rectal swab, terminal ileum mucosa, and succus entericus samples were collected on day 72 during follow-up were found to be SARS-CoV-2 negative.
- Direct evidence of SARS-CoV-2 infection and replication in the rectal tissues of surgical specimens was identified using electron microscopy, which visualized virions in ultrathin sections of rectal tissues
- Haematoxylin and eosin-stained rectal mucosa showed prominent lymphocytes and macrophages infiltrating the lamina propria without significant mucosal damage
- T-lymphocytes and macrophages were found to be more numerous than B lymphocytes in the lamina propria
- SARS-CoV-2 antigens were confirmed to be expressed on intestinal epithelial cells, lymphocytes and macrophages in the lamina propria

Highlights:

- First study to show SARS-CoV-2 had already replicated in the patient's rectum during the incubation period, with no obvious intestinal pathological damage
- Virus particles were found in intestinal epithelial cells, but IF and IHC showed that the viral components were mainly present in intestinal lymphocytes and macrophages, besides the intestinal epithelial cells.

- Virus spreads from the lungs to other non-pulmonary tissues probably by "virus-carrying" immune cells contain professional antigen-presenting cells such as macrophage, monocytes, and dendritic cells
- This study provides further evidence to support the fecal-oral transmission route of SARS-CoV-2, and the intestine represent a target organ of SARS-CoV-2

Clinical Impact:

- Minimal

Important Methodologies:

- qRT-PCR
- Electron microscopy
- H&E staining
- IHC & IF

Limitations:

- None