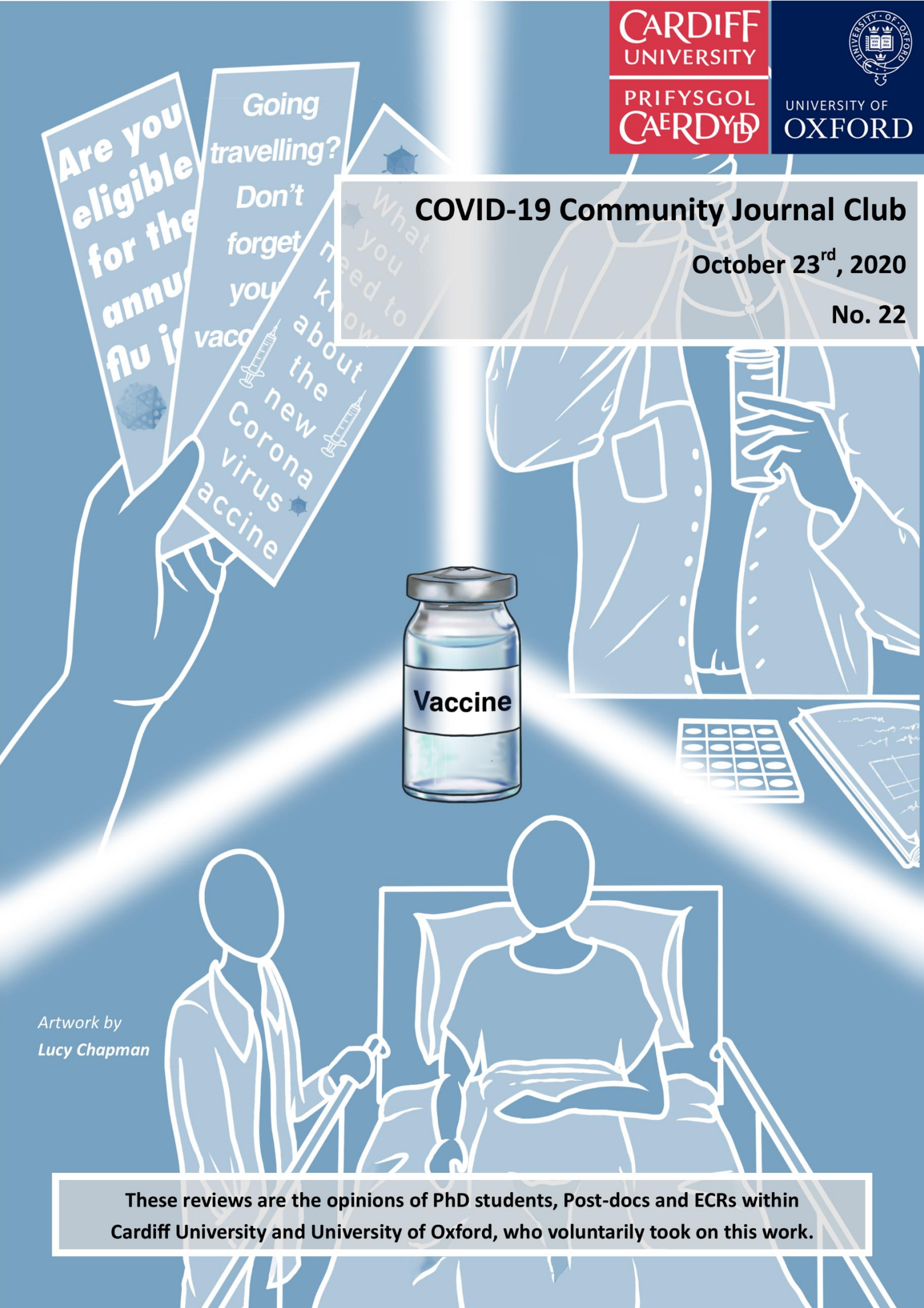

COVID-19 Community Journal Club

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Artwork by
Lucy Chapman

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

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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

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CAUTIONARY NOTE:
SOME REVIEWS ARE OF PRE-PRINTS POSTED ONLINE
(in *arXiv, bioRxiv, medRxiv and Research Square) BEFORE* PEER REVIEW.

News and Views

All eyes on a hurdle race for a SARS-CoV-2 vaccine

Gaebler, C. and Nussenzweig, M.C. 2020. *Nature*
Link: https://doi.org/10.1038/d41586-020-02926-w

A previously unmet clinical need and technical advances have led to 180 potential SARS-CoV-2 vaccine candidates; 42 of which are currently undergoing evaluation in human clinical trials. Vaccines which are have "considerable design flexibility" have had a head start in the race to approval. One particular candidate, BNT162b1, is a lipid nanoparticle vaccine containing the mRNA of SARS-CoV-2 receptor binding domain. Pfizer and BioNTech have recently assessed BNT162b1 in phase I/II clinical trials (Mulligan et al. 2020, Sahin et al. 2020).

Various doses of BNT162b1 were given to healthy adults via a prime-boost regimen with a 3 week interval. Low-level anti-RBD antibody responses were identified, which were dose-dependent and increased 10-15 fold following the second immunisation. Promisingly, neutralising antibody titres were comparable to convalesced patients in all but the lowest dose. Higher dosage and booster also increased prevalence of common adverse reactions (injection site pain and headache), but has not clear impact on CD4/CD8 T-cell responses.

Another similar candidate, BNT162b2, was assessed in older adults (65-85), who are at most risk from SARS-CoV-2. This candidate uses the mRNA from full-length spike and demonstrated less systemic reactogenicity than BNT162b1. BNT162b2 is now progressing to phase II/III trials.

Many questions surrounding potential vaccine candidates remain. Aside from dosage and the inclusion of a booster, the most important question is how long vaccine-induced immunity lasts. Antibody levels, and neutralising capacity, reduced three weeks following the second dose, highlighting the importance of assessing longevity of vaccine candidates. Standardised tests to evaluate neutralising antibody and T-cell responses do not exist. The SARS-CoV-2 Neutralization Assay Concordance Survey is attempting to overcome this, enabling comparison of multiple vaccines.

While there is a global need for a successful vaccine candidate, it is important not to rush. This is even more important for the newer, RNA-based vaccines as extensive safety profiles of such candidates are not available. BNT162b1/2 are a final hurdle, a controlled phase III trial, from crossing the finishing line.

Antibodies and Convalescent Plasma

A human monoclonal antibody targeting a conserved pocket in the SARS-CoV-2 receptor-binding domain core

Fedry, J. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.09.30.318261

Summary:

In this study they describe the cry-EM structures of trimeric SARS-CoV and SARS-CoV-2 spike ectodomains in complex with the human monoclonal antibody 47D11 recently reported by the same group. The data presented shows that 47D11 is able to bind to the closed conformation of the receptor binding domain, distal to the ACE2 binding sit which is a conserved and mutationally constrained epitope on the SARS-CoV-2 RBD, which means that it could be used in combination with other ab that target the exposed receptor-binding motif. According to their data, the 47D11 could be used in combination with other ab that target the exposed receptor-binding motif. Also, 47D11 might be able to neutralize a wide range of future-emerging virus variants as its epitope seems to have a limited mutational space. These results expose a cryptic site of vulnerability on the SARS-CoV-2 RBD and provide a structural roadmap for the development of 47D11 as prophylactic or post-exposure therapy for COVID-19.

Research Highlights:

1. 47D11 specifically binds to the closed receptor binding domain (RBD) of the trimeric spike protein
2. 47D11 epitope is distinct from the ACE2 binding site.
3. 47D11 targets a conserved hydrophobic pocket in the RBD
4. Mutations in the RBD hydrophobic core have a detrimental effect on protein folding, compromising the tertiary structure of the RBD.
5. 47D11 epitope is highly conserved across circulating SARS-like viruses

Impact for COVID-19 research:

- This study shows the benefits of 47D11 ab as it binds to a conserved epitope in the RBD which also permits to use it in combination with other ab. Which makes 47D11 as a candidate for COVID-19 treatment.

Methodologies:

- Study Type: *in vitro, cryo-EM*
- Important cell lines/viral models used: *FreeStyle 293-F cells were used to expressed the recombinant proteins and 47D11. HEK-293T and VeroE6 were used for the neutralizations assay*

- Key Techniques: *Recombinant proteins and 47D11 were purified from the supernatant by protein-A spharose beads or streptactin beads purification. Neutralization assay and ELISA analysis. For the Cryo-EM sample preparation they used a 200kC Talos Arctiva equipped with a GAtan K2 Summit direct detector and Gatan Quantum energy filter operated in zero-loss mode with 20eV slit width. Single-particle analysis was performed in Relion v.3.1, MotionCor2, CTFFind4. For model building and analysis the software used were UCSF Chimera and Coot.*

Early transfusion of a large cohort of COVID-19 patients with high titer anti-SARS-CoV-2 spike protein IgG convalescent plasma confirms a signal of significantly decreased mortality

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

Link: https://doi.org/10.1101/2020.10.02.20206029

Summary:

In this retrospective cohort study Salazar *et al*. investigate the efficiency of convalescent plasma transfusion. 341 transfused patients were matched using propensity score based on risk factors with 594 controls. 0.6% of patients had serious adverse events. The plasma had, on average, an Ortho VITROS IgG anti receptor binding domain (RBD) signal/cutoff of 24, higher than the FDA required cutoff of 12. Transfused patients had significant lower mortality after 60 days, the improvement was higher in patients transfused with high anti-RBD titer. The optimal window for transfusion was 44h post-hospitalisation.

Research Highlights:

1. Convalescent plasma transplant of plasma with >1:1350 anti-RBD IgG improves overall mortality after 60 days
2. Convalescent plasma transplant caused serious adverse events in 0.6% of patients
3. The optimal window for transplant is 44h after hospitalization.
4. Convalescent plasma transplant does not improve mortality when anti-RBD IgG ratio is <1:1350,
5. Convalescent plasma transplant does not improve mortality when is administered after 72h of hospitalization or on intubated patients.

Impact for COVID-19 research:

- This study shows that convalescent plasma transplant is safe and effective when the anti-RBD IgG ratio is <1:1350.
- The ideal window for convalescent plasma administration is 44h after hospitalization.

Methodologies:

- Study Type: *clinical trial*
- Key Techniques: *Antibody titration*

Limitations:

- As addressed in the study, this is not a randomised control trial, but a retrospective cohort one. The patients are carefully matched, but some inflammation parameters have been excluded for lack of data and the retrospective nature means that some potential biases might have been overlooked.

T-cells

CD8+ T cell responses in convalescent COVID-19 individuals target epitopes from the entire SARS-CoV-2 proteome and show kinetics of early differentiation

Kared, H. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.10.08.330688

Summary:

This study presents in-depth combinatorial ex-vivo profiling of $CD8^+$ T cell specificity and phenotype in SARS-CoV-2 convalescent individuals. In convalescents, SARS-CoV-2 T cells recognise a broad range of epitopes from the SARS-CoV-2 proteome. The most prominent phenotypes of SARS-CoV-2 specific $CD8^+$ T cells were stem-cell memory (SCM) and transitional memory (TM2), which agrees with previous studies (Sekine et al. and Fan et al.). However, these phenotypes were distributed differently according to prevalence of the specific T cells; high prevalence $CD8^+$ T cells were enriched for TEMRA, EM and TM2 cells while low prevalence specificities were of a SCM and CM phenotype. Evaluation of the SARS-CoV-2-specific $CD8^+$ T cell response was cross-sectional and revealed with decrease in inflammation and sustainment of antibody neutralizing activity in the early recovery phase and an increase in the number of epitope responses over time.

Research Highlights:

1. While $CD8^+$ T cell responses to epitopes from the spike protein and ORF3a were the most common, $CD8^+$ T cells recognising nucleocapsid epitopes were most frequent. The highest number of epitopes were presented by HLA-A*01:01 and HLA-A*02:01 and the lowest by HLA-B*07:02. Of the peptide responses detected, 52/132 have not been previously described.
2. $CD8^+$ T cells for which positive epitope specificity was detected in a high prevalence of the study population were detected at higher frequencies than $CD8^+$ T cells which had less prevalent epitope specificities. While needing further investigation, this could suggest greater T cell expansion and persistence to certain dominant epitopes.
3. Higher frequency SARS-CoV-2 specific $CD8^+$ T cells, (as per point 2.) were negatively correlated with expression of early differentiated markers and positively correlated with late differentiation markers, indicating that the most prevalent epitope responses in the study population are demarcated by effective T cell differentiation.
4. SARS-CoV-2 specific $CD8^+$ T cells were less frequent than EBV and CMV specific $CD8^+$ T cells, but comparably frequent to Flu specific T cells. The SARS-CoV-2 T cells also had a distinct phenotype to these other virus specific $CD8^+$ T cells.
5. Neutralising antibody titers were negatively correlated with an early differentiation phenotype, effective humoral (antibody) and cellular (mature/ differentiated) responses occur complementary to each other.

Impact for COVID-19 research:

- Detection of novel SARS-CoV-2 epitopes, including those from less commonly investigated non-structural antigens, could inform vaccine development. The differentiated and memory phenotypes of epitope specific T cells, which were associated with higher prevalence and frequency in the convalescent individuals, could be an important indicator of the efficacy of a T cell response to SARS-CoV-2 which leads to recovery, and merits further investigation in different disease severity recovery cohorts.

Methodologies:

- Study Type: *ex vivo analysis of convalescent PBMC ($CD8^+$ T cells), antibodies and plasma (cytokines). This is a particular strength in comparison to peptide pool stimulation studies.*
- Key Techniques: *Mass cytometry combined analysis of $CD8^+$ T cell SARS-CoV-2 epitope specificity and phenotype, neutralizing antibody titer analysis, sandwich immunoassay of cytokines and chemokines from plasma. In silico correlation analysis of these experimental parameters.*

Limitations:

- Limitations noted by the authors include sample size (n= 30, divided into 3 groups of 10 individuals, based on IgG titers; high, medium and low, and only n=4 healthy controls sampled prior to the pandemic), HLA-I population coverage (6 HLA-I alleles representing 73% of the US population) and population representativeness; the sampling was geographically restricted (Washington and Baltimore, USA) and consisted of 80% (24/30) Caucasian participants.
- The authors also note that while the study sample incorporated a (cross sectional) range of post-disease resolution timepoints, longitudinal data enable stronger conclusions to be made. The minimum duration post-symptom resolution of 28 also does not capture the earliest recovery stages; other studies have reported distinct stages of T cell recovery 10-20 days after symptom onset (Mann et al., Rodriguez et al.)
- Since the study population included few individuals who had been hospitalized, this does not allow the comparison of the T cell phenotype and breadth of epitope responses in severe vs. milder COVID-19. Therefore, it is not known if the breadth of epitope response could be a determinant of better T cell control of SARS-CoV-2 infection. Additionally, since T cells were analysed only from peripheral blood, key epitope responses and phenotypes at the site of infection were not determined.
- Minimal evidence reported for clinical data and comorbidities of individuals. Therefore, it is not clear if any-existing conditions could contribute to the findings.

SARS-CoV-2 Uses CD4 to Infect T Helper Lymphocytes

Davanzo, G.G. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.25.20200329

Summary:

In this report they show the importance of CD4 in SARS-CoV-2 and how its interactions is necessary for infection of CD4+ T cells. A mechanism that resembles HIV infection. According to what has already been reported, SARS-CoV-2 is able to infect other cells with low levels of ACE2 (main receptor) what they report here is the alternative mechanism of SARS-CoV-2 to infect lymphocytes. This cells although they express low levels of ACE2 they do express CD4 which as they demonstrate it seems to bind to SARS-CoV-2 facilitating cell infection. Also, these finding would explain lymphocytopenia and dysregulated inflammatory response that has been reported in severe COVID-19 patients. When CD4+ T-cells infection seems to affect similar multiple housekeeping pathways that have been reported for HIV infected CD4+ T-cells. Considering their results and what has previously been reported, detecting IL-10 serum levels during the early stages of the disease might help predict COVID-19 outcome.

Research Highlights:

1. SARS-CoV-2 infection induces IL10 expression in CD4+T cells
2. CD4 stabilizes SARS-CoV-2 on the cell membrane until the virus encounters an ACE2 molecule to enter the cell
3. ACE2, TMPRSS2 and CD4 act in concert to allow the infection of CD4+ Tcells by SARS-CoV-2
4. IFNG and IL17A are upregulated in CD4+Tcells of patients with moderate illness

Impact for COVID-19 research:

- In this study they report a different mechanism for SARS-CoV-2 infection which would differ of the idea that only cells expressing ACE2 can be infected. Meaning that although if the levels of ACE2 are not high there is still the possibility of getting infected by Covid-19.

Methodologies:

- Study Type: *in vitro and in silico.*
- Important cell lines/viral models used: *Vero cells (CCL-81, ATCC) for SARS-CoV-2 propagation.*
- Key Techniques*: qPCR was used for virus titter. Whereas samples were extracted manually using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit. Other techniques were Plaque assays and co-immunoprecipitation using Pierce protein A/G magnetics beads immobilized with anti-CD4ab. For target selection the database used were PANTHER, P-HIPSTER and EnrichR. Also, In situ hybridization, immunoblotting, immunofluorescence and transmission electron microscopy was carried out.*

Limitations:

- Samples of patients considered for this study they all come from the Clinical Hospital of the University of Campinas. Would have been better if other hospitals participate in this study. Also human blood samples from severe patients were obtained from individuals admitted in a clinical trial. Although this might not affect to the results it should be considered.
- As it is described in the supplementary data for diagnosis, RT qPCR were performed in duplicates using TaqMan, would have been preferable to run triplicates instead.
- The number of participants is low. Covid-19 severe patients=17, moderate=10 and healthy donors=9.

Diagnostics

Direct detection of SARS-CoV-2 using CRISPR-Cas13a and a mobile phone

Fozouni, P. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.28.20201947

Summary:

Fozouni P. *et al* report a CRISPR-Cas13a-based mobile phone assay which can be used for the detection of SARS-CoV-2 RNA from nasal swabs. Astoundingly, their system demonstrates a sensitivity of approximately 200 copies/µl in 30 minutes, being also able to establish whether a clinical sample is positive within 5 minutes. Importantly, their assay was quantitative, allowing quantification of viral loads during SARS-CoV-2 screening in an accurate, quick, portable and low-cost fashion.

Research Highlights:

1. From a set of CRISPR RNA (crRNA), their chose crRNA 2 and 4 resulted in an increased detection limit of 1,000 copies/µl of target RNA, with the limit of detection of full-length viral copes being 270 copies/µl
2. Their Cas13a system was highly specific, detecting no signal above background when tested against respiratory viruses, including alphacoronavirus HCoV-NL63 and betacoronaviruses HCoV-OC43 and Middle East respiratory syndrome coronavirus (MERS-CoV)
3. The addition of a third crRNA, (crRNA 21) specific for the viral E gene enhanced detection to as few as 31 copies/µl
4. Their triple-crRNA Cas13a assay correctly identified 5 SARS-CoV-2 patient samples as positive, with signal correlating to the quantity of viral RNA in the sample, highlighting the quantitative nature of the assay
5. A mobile-phone based fluorescence microscope was able to measure the signal generated by the Cas13a assay, showing a sensitivity of upto 200 copies/µl in 30 minutes but also being able to detect a positive sample with high viral loads within 5 minutes; this is compared to the current average wait time for qPCR test results of 4.1 days in the United States

Impact for COVID-19 research:

- Their time-sensitive Cas13a-based assay using multiple crRNAs to different targets would be safeguarded from viral mutations, providing a direct quantification of viral RNA in samples that could be used as a quick, portable and low-cost method for diagnosis and monitoring of patient infection

Methodologies:

- Study Type: *In-Vitro*
- CRISPR-Cas details: *Cas13a homolog from Leptotrichla buccalls with crRNAs corresponding to primer sets released by the Centre for Disease Control and Prevention and those released from Wuhan by Zhu et al., 2020*
- Key Techniques: *An in-vitro transcribed target RNA system; fluorescent Cas13a nuclease assays; a mobile-phone fluorescent microscope*

Limitations:

- Given the potential of this detection assay, it would have strengthened the usability of the assay if more patient samples had been screened

A haemagglutination test for rapid detection of antibodies to SARS-CoV-2

Townsend, A. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.10.02.20205831

Summary:

Many current serological assays will not be universally available; particularly due to their expense. Townsend *et al*. have produced a Haemagglutination Test (HAT), at 0.27 pence per test well, to detect antibodies which bind the receptor binding domain of SARS-CoV-2. Such long-established tests are simple to use and scale up. The HAT test developed has a high specificity (99%) and sensitivity (90%) rate for the detection in those with PCR-diagnosed COVID-19. This test can detect rising antibody titres during early hospital admission and can hopefully be applied as a point of care test (POCT) during hospitalisation of patients.

Research Highlights:

1. Using 98 samples from donors diagnosed by RT-PCR at least 28 days prior, and 199 negative pre-COVID-19 samples, HAT obtained an average 90% sensitivity and 99% specificity. However, these percentages were inferior to an established chemiluminescence assay (100% for both).
2. In the hospital setting, HAT was superior to the Siemens Atellica Chemiluminescence test (sensitivity: 86% vs. 74%) to detect RBD-binding antibodies from samples obtained from PCR-diagnosed patients within the first 5 days of hospital admission, which included a range of severity. Specificity was 100%. A strong correlation ($P<0.001$) existed between both tests.
3. HAT was able to detect as rise in agglutination titre in 68% of 25 donors between days 1, 3 and 5 of hospital admission.

4. Preliminary analysis suggest lyophilised HAT has the potential to function as a POCT on capillary blood obtained from finger-prick.

Impact for COVID-19 research:

- Serological assays are needed for detection of seroconversion following infection or vaccination and also epidemiological surveys as the pandemic progresses. This inexpensive test may be of benefit to the lower-income countries.
- Although different designs, preliminary results are comparable to those from Kruse *et al*. 2020

Methodologies:

- Study Type: *in vitro*
- Important cell lines/viral models used: *Sera from COVID-19 patients and pre-COVID donors*
- Key Techniques: *RBD linked to single domain antibody IH4 to produce fusion protein IH4-RBD-6H to use for test, Established test using various RBD/spike binding antibodies and ACE2-Fc, Indirect ELISA*.

Limitations:

1. As stated in the discussion, the levels of sensitivity and specificity are too low for the assay to be recommended by the UK MHRA or Infectious Disease Society of American
2. Authors concede that superiority of HAT to Siemens test could be due to the fact that early in disease pathology, the IgM which dominates is particularly effective at crosslinking the IH4-RBD labelled RBCs, so may be disguising differences.
3. Considering the authors view their test during early hospitalisation, there is very little data on POCT.

Virology

SARS-CoV-2 Infection Depends on Cellular Heparan Sulfate and ACE2

Clausen, T.M. *et al*. 2020. *Cell*

Link: https://doi.org/10.1016/j.cell.2020.09.033

Summary:

Clausen *et al.* identify cellular heparan sulfate as an important co-factor for SARS-CoV-2 infection. Heparan sulfate was shown to interact with receptor binding domain (RBD) of the SARS-CoV-2 spike glycoprotein. Docking studies revealed heparan sulfate binding site is located adjacent to the binding site of angiotensin-converting enzyme 2 (ACE2). The study suggests that heparan sulfate mediates SARS-CoV-2 binding to ACE2, enhancing viral attachment and infection.

Research Highlights:

1. SARS-CoV-2 spike protein interacts with heparin/heparan sulfate through RBD. It can bind to heparin/heparan sulfate and ACE2 simultaneously.
2. SARS-CoV-2 is co-dependent on cellular heparan sulfate and ACE2 for cell binding.
3. Variation in heparan sulfate structure across different cell types/tissues may affect spike-cell binding, contributing to SARS-CoV-2 tissue tropism. For example, lung heparan sulfate was more potent compared to heparan sulfate isolated from liver or kidney.
4. Mutations in genes involved in biosynthesis of heparan sulfate (*EXT1* and *NDST1*) significantly reduced SARS-CoV-2 infection in Hep3B cells.
5. Treatment with unfractionated heparin (UFH) significantly inhibited SARS-CoV-2 infection of cells.

Impact for COVID-19 research:

- Modulation of heparan sulfate or heparin-based therapies offer potential therapeutic options against COVID-19.

Methodologies:

- Key Techniques: *Molecular modeling, analytical heparin-sepharose chromatorgraphy, Infection of primary human bronchial epithelial cells at air-liquid interface*

Limitations:

- Additional functional assays are required to optimize the use of metabolic inhibitors of heparan sulfate and/or validate the anti-viral effects of exogenous heparin (e.g. mice models, organoids etc.).

Plasma ACE2 activity is persistently elevated following SARS-CoV-2 infection: implications for COVID-19 pathogenesis and consequences

Patel, S.K. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.10.06.20207514

Summary:

This paper demonstrates for the first time that plasma ACE2 activity is elevated following COVID-19 infection and that it remains elevated to at least 114 days post infection. Blood samples were taken at a median of 35-, 63- and 114-days post infection and plasma ACE2 activity remained consistently high in almost all subjects.

Research Highlights:

1. Plasma ACE2 activity at 30-38 days post infection was 97-fold higher in recovered SARS-CoV-2 patients compared to controls (5.8 vs 0.06 pmol/min/ml, $p<0.0001$)
2. Significant increase in plasma ACE2 activity with disease severity (11.2 severe vs 5.4 mild pmol/min/ml, $p=0.033$)
3. Men had significantly higher plasma ACE2 activity levels compared to women (9.2 vs 2.1 pmol/min/ml, $p<0.0001$)
4. Plasma ACE2 activity remained persistently elevated over time until 114 days post infection (the last time point sampled)

Impact for COVID-19 research:

- Plasma ACE2 activity is increased in severe COVID-19 patients, this may allow identification of at-risk groups of prolonged illness

Methodologies:

- Study Type: *Prospective Observational Study*

Limitations:

- Serial samples were only available in 23 patients, a larger study is required for validation

Immunopathology

Inborn errors of type I IFN immunity in patients with life-threatening COVID-19

Zhang, Q. *et al*. 2020. *Science*

Link: https://doi.org/10.1126/science.abd4570

Summary:

Mutations affecting the Type I interferon (IFN) response may account for some of 659 severe COVID-19 cases (life-threatening pneumonia) not attributed to epidemiological risk factors. Sequenced 13 loci known to affect influenza susceptibility. *In vitro* patient SV40-fibroblasts with deleterious TLR3, IRF7 or IFNAR1 mutations had greater susceptibility to SARS-CoV-2 infection. Serum from 10 of the severe COVID-19 patients with these mutations had reduced IFNα. Identified a small proportion of severe COVID-19 patients (3.5%) have mutations diminishing the Type-I IFN response may benefit from type-I IFN administration.

Research Highlights:

1. PCA analysis suggested the individuals with predicted loss-of-function mutations (LOF) were enriched in the severe COVID-19 patient group, compared to 534 asymptomatic/mild patients and 1000 genome project controls.
2. 24 variants were loss-of expression/function or hypofunctional in a IFN-β-luciferase reporter assay. Variants were in *TLR3*, *UNC93B1*, *TICAM1*, *TBK1*, *IRF3*, *IRF7*, *IFNAR1* or *IFNAR2* genes.
3. IFNα2 responses in patients with IRF7 deficiencies were reduced in PHA-driven T cell blasts (PHAT cells, n=3) and plasmacytoid dendritic cells (pDCs, n=1).
4. Observed a higher SARS-CoV-2 infection level after 24 hours (MOI 0.5) in $TLR3^{-/-}$ $IRF7^{-/-}$ and $IFNAR1^{-/-}$ patient derived SV40-Fib cells co-expressing ACE2 and TMPRSS2 than in healthy donors. Infection was reduced in wild-type IRF7 or IFNAR1 "rescued" patient cells.
5. Measured 13 types of IFNα in blood of the 10 of 23 severe COVID-19 patients with loss-of-function mutations, found IFNα was lower in this group (<1pg/mL) than previously published cohorts.
6. Additionally identified 29 patients that had auto-antibodies against Type I IFNs but did not have LOF mutations.

Impact for COVID-19 research:

- This research supports others, that a Type I IFN response is important in COVID-19.
- Highlights that risk of developing severe COVID-19 pathology includes underlying genetic mutations in addition to epidemiological factors (age, gender, co-morbidities).

- Identifies a subset (3.5% in this study) of patients with severe COVID-19 that could potentially benefit from Type I IFN treatment.

Methodologies:

- Study Type: *Cohort Study*
- Important cell lines/viral models used:
  - *Patient generated SV40-Fibroblasts expressing ACE2 and TMPRSS2.*
  - *SARS-CoV-2 strain UDS-WA1/2020 used.*
- Key Techniques:
  - *Whole genome and exome sequencing*
  - *In vitro SARS-CoV-2 infection model in patient SV40 fibroblasts*
  - *Single-molecular array (Simoa) IFNα digital ELISA*

Limitations:

- Only explains a small proportion of severe COVID-19 cases.
- Very small number of patient samples available for IFNα, PHAT cell and pDC analyses.
- Limited focus on loci that are linked to influenza virus immunity.

Functional immunoparalysis characterized by elevated Interleukin-10 and Interleukin-10-to-Lymphocyte Count Ratio is associated with severe disease and poor outcomes in coronavirus disease 2019 (COVID-19)

Henry, B.M. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.28.20203398

Summary:

Henry *et al*. investigate serum levels of the anti-inflammatory cytokine IL-10 at the point of first contact with hospital. High levels of IL-10 (and IL-10:Lymphocyte count ratio) are associated with worse outcomes over the next 30 days. The findings suggest that in response to the cytokine storm, levels of IL-10 are elevated in an attempt to compensate. High levels of IL-10 then cause "functional immunoparalysis" which in turn leads to increased risk of bacterial co-infections. Thus immunoparalysis driven by IL-10 should be considered in addition to hyperactivation and exhaustion of immune cells, with important implications for treatment protocols.

Research Highlights:

1. High levels of IL-10 and IL-10:Lymphocyte count ratio associated with:
    - more severe disease (a one-unit increase in IL-10 was associated with 42% increased odds of severe COVID-19 (p=0.031), whilst a one-unit increase IL-10/lymphocyte ratio was also associated with 32% increase in odds of severe COVID-19 (p=0.013)).
    - increased incidence of secondary bacterial infections
    - increased incidence of severe acute kidney injuries
2. Immunoparalysis is similar to compensatory anti-inflammatory response syndrome (CARS) seen in sepsis
3. IL-10 levels correlate with IL-6

Impact for COVID-19 research:

- This study shifts the paradigm of hyperactivation/exhaustion/lymphopenia as drivers of severe disease to include immune paralysis driven by high levels of IL-10 and is an area that deserves further study
- Disease management: the authors suggest that stratifying patients by serum IL-10 at admission may enable those at highest risk of bacterial co-infection to be identified and started on prophylactic antibiotics, although this will require a clinical trial to confirm

Methodologies:

- Study Type: *cohort study*

Limitations:

- Cohorts are of medium size and those with severe disease are significantly older than those with mild/moderate disease. However, this does not detract from the overall findings of the paper.

Long-Term Immunity

Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients

Isho, B. *et al*. 2020. *Science Immunology*

Link: https://doi.org/10.1126/sciimmunol.abe5511

Summary:

The authors examine blood and saliva of current and convalescent COVID-19 patients to determine the stability of neutralising antibodies targeting SARS-CoV-2 spike protein and RBD over 3 months after infection. They observe durable IgG antibody responses targeting spike and RBD in both saliva and serum, but declines in serum and saliva IgM and IgA.

Research Highlights:

1. Serum IgG antibodies against spike protein peak 16-30 days post symptom onset and are sustained over 115 days (spike trimer) and 65 days (RBD)
2. Serum IgM and IgA levels to both, spike trimer and RBD peak at the same time but decrease more rapidly
3. Similarly, acute COVID-19 patients have increased saliva IgG, IgA and IgM antibodies to spike and RBD antigens. IgG antibodies remain stable for 3 months, while IgA and IgM do not persist past day 60 post symptom onset
4. For all antigen-antibody combinations positive correlations are observed between saliva and serum, with correlations for IgG and IgM higher than for IgA

Impact for COVID-19 research:

- Medium, saliva could be used as an easier option to diagnose both present and past COVID-19 infection

Methodologies:

- Study Type: *In vitro*
- Key Techniques: *automated ELISA*

Limitations:

- It would be interesting to follow the patients for longer to determine how long the IgG response will be stable for.
- It is unclear to which extend IgG, IgM and IgA contribute to neutralisation and whether the sole presence of IgG after 3 months can prevent reinfection

Temporal Analysis of COVID-19 Convalescent Plasma Donations Reveals Significant Decrease in Neutralizing Capacity Over Time

Girardin, R.C. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.10.04.20206011

Summary:

Convalescent plasma (CP) therapy has been used as a treatment for SARS-CoV-2 infection for many patients during the COVID-19 pandemic, however, the efficacy is not yet fully established. The virus neutralising capacity of 196 COVID-19 CP donors was measured using 981 samples collected over 112 days. The plaque reduction neutralisation test (PRNT) was utilised to assess viral reduction by the samples. 79.5% of samples met the FDA neutralising antibody (nAb) cut off (1:80) with a 50% PRNT ($PRNT_{50}$) outcome at first donation, whilst only 48.8% met this value 85 days or more after first donation. These findings can inform clinicians on optimum conditions for CP therapy for COVID-19 and aid in understanding the long-term antibody responses in recovered patients.

Research Highlights:

1. Male CP donors and those who were older had a greater neutralising capacity than females and those who were younger (under 48).
2. 25.8% of donors with a plaque reduction neutralisation test (PRNT) over 90 ($PRNT_{90}$) and 73% over 50 ($PRNT_{50}$) had a neutralising antibody (nAb) titer of $\geq$1:80, whilst 9.5% $PRNT_{90}$ and 51.4% $PRNT_{50}$ had a nAb titer of $\geq$1:160.
3. The number of samples reaching the minimum $\geq$1:80 and recommended $\geq$1:160 nAb titres diminished over time. The largest decreases in $PRNT_{50}$ titres happened $\geq$43 days from first donation.
4. The accuracy of the OVSARS2IgG surrogate neutralisation test to determine high quality CP was analysed by comparing to live virus nAb titres and PRNT levels. 100% of the samples that reached the FDA cut off ratio of 12 had 100% specificity and positive predictive value for $PRNT_{50}$ and nAb titres $\geq$1:20 and $\geq$1:80. Whilst a cut off ratio of 18.45 had improved specificity and positive predictive value for samples with nAb titres $\geq$1:160.

Impact for COVID-19 research:

- Help clinicians define optimum time frame for CP collection.
- Understanding the long-term antibody response to COVID-19 may help inform clinicians and scientists about re-infection risk.

Methodologies:

- Study Type: Cohort study with *in vitro* analysis
- Important cell lines/viral models used: *Vero E6 cells*
- Key Techniques: *Plaque reduction neutralisation test (PRNT), Ortho VITROS SARS-CoV-2 IgG test (OVSARSIgG).*

Limitations:

- Low donor number and cohort not specified.
- Timings of the first donation were not specified- those with a later first CP donation may have lower neutralisation titres.