COVID-19 Community Journal Club

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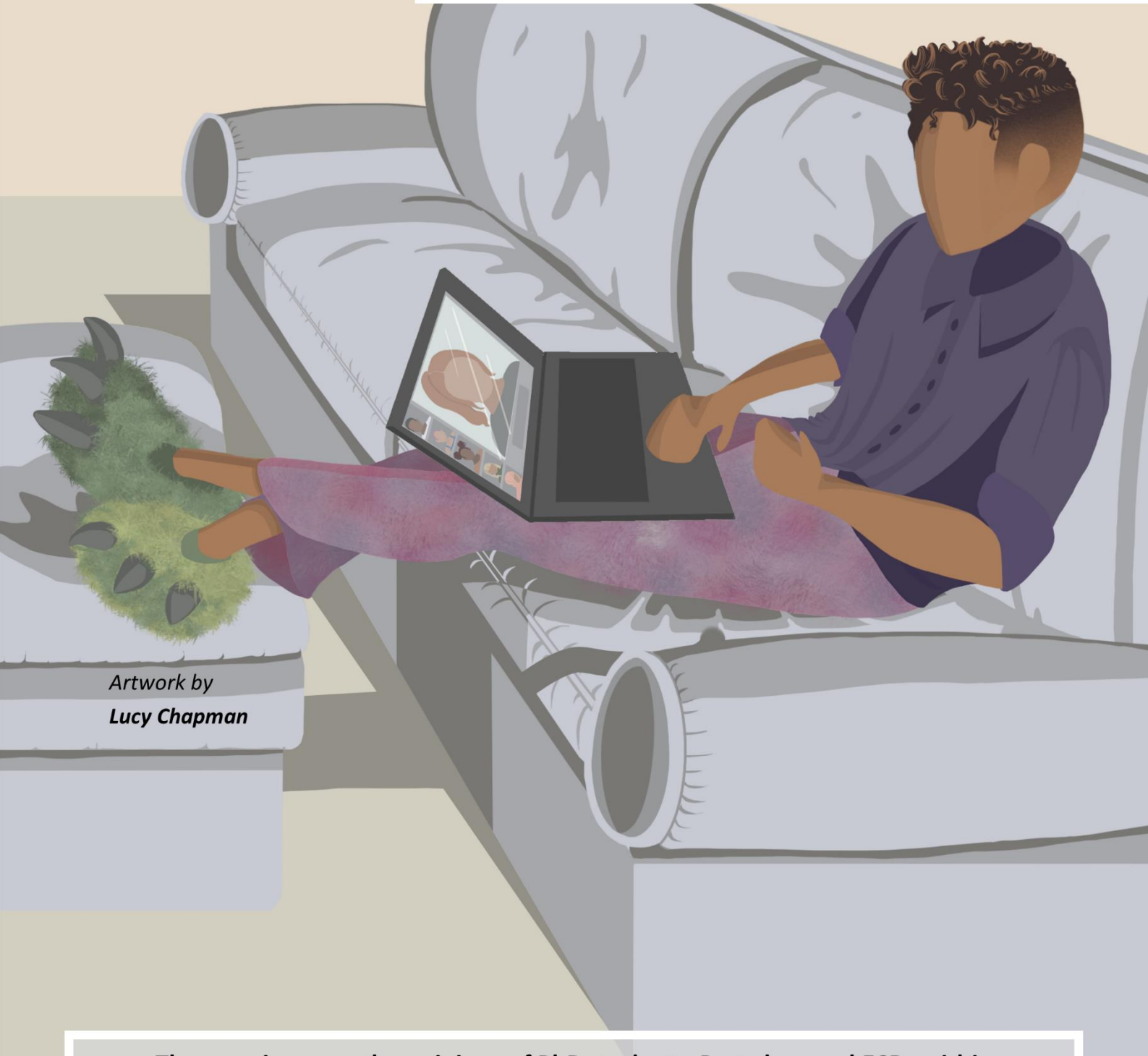

Artwork by
Lucy Chapman

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.

Antibodies and T cells

Transplacental Transfer of SARS-CoV-2 Antibodies

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

Link: https://doi.org/10.1101/2020.10.07.20207480

Summary:

The authors measured the levels of SARS-CoV-2 antibodies to the spike receptor binding domain in maternal serum and cord blood, finding efficient transfer of IgG antibodies in a large percentage of seropositive women with mild, moderate and severe COVID-19.

Research Highlights:

1. 54% of seropositive women tested positive for SARS-CoV-2 via nasopharyngeal PCR (NP-PCR) during pregnancy
2. 87% of newborns to seropositive women were seropositive, IgG levels in cord blood positive correlated with maternal IgG, no infants had detectable IgM levels
3. Of 11 seronegative infants to seropositive mothers, 5 mothers were seropositive for IgM only and in the remaining 6 cases maternal IgG levels were significantly lower compared to seropositive mother/newborn dyads
4. Higher maternal antibody levels were associated with higher cord antibody levels but there was no correlation between antibody levels and disease severity
5. No newborns to mothers that were contagious at the time of delivery were NP-PCR positive

Impact for COVID-19 research:

- Moderate: potentially helpful for neonatal management and vaccine development/vaccination guidelines for pregnant women

Methodologies:

- Study Type: *In vitro*
  Key Techniques: *maternal and neonatal cord blood serum collection during birth, ELISA for IgG and IgM antibodies*

Limitations:

- The authors did not distinguish between neutralising and non-neutralising antibodies
- It is unclear how long the transferred antibodies will persist in babies and whether they can protect against SARS-CoV-2 infection
- The sample size is quite low, with only 83 mother/newborn dyads

Predicting COVID-19 Severity with a Specific Nucleocapsid Antibody plus Disease Risk Factor Score

Sen, S. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.10.15.341743

Summary:

Using Phase ELISA and coronavirus antigen microarray (COVAM), Sen *et al*. used 86 COVID-19 patients to identify a 21-residue epitope which associated with more severe disease. This epitope was mapped to SARS-CoV-2 nucleocapsid and was termed Ep9. Patients with anti-Ep9 antibodies demonstrated evidence of antibody-dependent enhancement (ADE), with elevated IL-6 levels and an early IgG response. It is possible that identification of anti-Ep9 antibodies can help stratify COVID-19 patients and also aid in diagnostics; anti-Ep9 antibodies can be detected within 5 days post-symptom onset.

Research Highlights:

1. Anti-Ep9 antibodies were found in 27% of patients' plasma, with peak titres observed between days 4-9 post symptom onset.
2. Ep9 epitope is cross-reactive with SARS-CoV-2: there is a 90% amino acid similarity between SARS-CoV-2 and SARS-CoV-2. However, antibodies to Ep9 are highly specific for β-coronaviruses, so can be utilised for diagnostics.
3. Patients with anti-Ep9 antibodies are 3.17x more likely to demonstrate severe COVID-19 symptoms than those without, and this correlates with comorbidities. Length of hospitalisation and duration of symptoms are also prolonged with presence of anti-Ep9 antibodies.
4. A strong correlation between IL-6 and aspartate aminotransferase (AST) was only seen in patients with anti-Ep9 antibodies. Coupled with the early IgG seroconversion of these antibodies, it is possible anti-Ep9 antibodies are causing ADE in these patients.

Impact for COVID-19 research:

- This paper highlights that the presence of antibodies against the 21 residue Ep9 epitope correlates with severe COVID-19. The presence of such in patients may help stratify patients earlier in disease progression.
- Highlights the importance of investigating epitopes distant from the spike protein of SARS-CoV-2. Authors provide all epitopes screened in the supplementary figures.

Methodologies:

- Study Type: *in vitro, in silico*
- Key Techniques: *Phase display ELISA with patient plasma, serum coronavirus antigen microarray*

Limitations:

- By the author's own admission, this paper only uses the serum of 86 patients who are mostly of Hispanic descent. A more diverse population pool would strengthen this finding.
- Poor distribution of patients by disease severity in study.

Prevalence of antibodies to SARS-CoV-2 in healthy blood donors in New York

Kamath, K. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.10.19.20215368

Summary:

This paper investigated seropositivity in the blood by re-analysing data for SARS-CoV-2 associated epitopes in addition to adding a new panel of 1559 healthy donors. The Serum Epitope Repertoire Analysis (SERA) is highly specific and sensitive and enables discovery and semi-quantitative detection of 52 antibody epitopes with high resolution that can be mapped to eliciting antigens and organisms. This data showed that as time progressed from March to July the percentage of seropositive healthy donors increased from 0% to 11.6%.

Research Highlights:

1. Sensitivity and specificity of Serum Epitope Repertoire Analysis (SERA) is high >91%
2. Sex, age and race of healthy donors were evenly split
3. Proportion of seropositive samples increased steadily with time

Impact for COVID-19 research:

- A significant proportion of the population is likely asymptomatic for COVID-19 and authors raise the question of whether convalescent plasma donors could be identified among these "healthy" individuals.
- Authors demonstrate the utility of SERA for monitoring the antibody response to emerging infections.

Methodologies:

- Study Type: *in silico/cohort study*
- Key Techniques: *Serum Epitope Repertoire Analysis (SERA)*

Limitations:

- Was carried out at the beginning of the pandemic (March to July), as a lot of people were isolating during this period, it would be good to repeat this now to get a better representation.

Early induction of SARS-CoV-2 specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients

Tan, A.T. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.10.15.341958

Summary:

Tan *et al*. evaluate viral loads, antibody and T cell responses in 12 COVID-19 patients from acute symptomatic infection to convalescence or death. Patients with moderate symptoms eliminate the virus after 15 days of infection, while it persists longer in severe patients. Most patients develop NAbs, achieving the peak of neutralization activity 9-15 days post symptom onset. However, fast kinetics on NAbs correlate with severity. Antibody profiles show an early NP-biased antibody response in severe, while mild/moderate have a spike-dominant or balanced response. Early and specific ORF7/8 T cell responses correlate with mild disease but wane after resolution of acute infection. Overall, rapid and high induction of antibody responses correlated with disease severity, while early T cell responses associated with mild disease and increased viral clearance.

Research Highlights:

1. Persistent viral load correlated with disease severity and early T cell response correlated with viral clearance.
2. Most patients developed NAbs (peak of neutralizing activity 9-15 days post symptom onset), but those to early develop NAbs progressed to severe COVID-19.
3. Ab profile for severe COVID-19 showed an early NP-biased antibody response, while mild/moderate profile had a spike-dominant or balanced response
4. Waning of T cell responses after acute infection
5. Exclusive expansion of ORF7 and ORF8 specific CD4 T cells in the convalescents, with a robust IFN-y response in the early phases of infection and only in patients with mild disease.

Impact for COVID-19 research:

- Relating an early antibody response to disease severity might highlight a possible pathogenic role for rapidly induced antibodies. More data is needed to confirm this correlation. The different profile of the antibody response in mild vs severe also provides valuable information.
- The study highlights the protective role of T cell responses in the resolution of the infection

Methodologies:

- Study Type: *in vitro, cohort clinical study.*
- Key Techniques: *RT-PCR, surrogate virus neutralisation assay, Luminex, ELISpot, Flow cytometry.*

Limitations:

- Patient sample is small to make some conclusions.
- T cell responses are only evaluated only in convalescence
- Viral loads are detected by RT-PCR, but it would be informative to know the dynamics of active viral replication

Immunopathology and Clinical Interventions

Recovery of monocyte exhaustion is associated with resolution of lung injury in COVID-19 convalescence

Scott, N.A. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.10.10.20207449

Summary:

Peripheral blood samples from convalescent COVID-19 patients ~12 weeks after discharge, compared against acute inpatients and healthy controls. Monocytes are thought to infiltrate many organs in COVID-19, are associated with severity. Previously monocytes from acute hospitalised COVID-19 patients produced lower levels of inflammatory cytokines in response to a second microbial stimulus. This study showed that some patient's peripheral blood monocytes (PBMCs) from convalescent appear to have a more pro-inflammatory response to LPS than acute cases and a reduced expression of CCL2, a migration marker. Patients with "recovered" chest X-ray appear to differ most from healthy controls and are suspected to have slightly different monocyte profiles than "non-recovered" convalescents.

Research Highlights:

1. Took peripheral blood samples from 43 convalescent COVID-19 patients ~12 weeks after discharge, compared against 17 acute hospitalised inpatients (group) and healthy controls (between 11-23).
2. Some patients still have fewer peripheral monocytes produce IL-6 after LPS stimulation ~12 weeks post-discharge in patients that had abnormal chest X-rays.
3. Moderate/severe patients were more likely to have abnormal X-rays or dyspnoea.
4. Convalescent patients had a high proportion of $TNF\alpha^{+}$ PBMCs and a reduced percentage expressing CCL2 compared to acute cases post-LPS.
5. Migration makers (LFA-1, VLA-4, CD31/PECAM and CXCR6) were higher on "recovered" convalescent patients but did not correlate to clinical measures taken during hospitalisation.

Impact for COVID-19 research:

- Highlights importance of taking samples from patients' post-hospitalisation to better understand if/how/when COVID-19 immunopathology is resolved.

Methodologies:

- Study Type: *Cohort Study*
- Key Techniques: *PBMCs, IL-6 and TNFα measurement*

Limitations:

- Currently low n number in some groups mean that differences between "recovered" and "non-recovered" patients may be lost.
- Showed that convalescent male monocytes produced more TNFα than females, but do not mention if this is controlled for in other analysis.
- Methods appear to be missing from the PDF, do not know if patients were discharged by the same criteria.

Effects of Tocilizumab in COVID-19 patients: a cohort study

Vu, C. *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-49532/v2

Summary:

In this retrospective observational study they report the results obtained after the early administration of tocilizumab. Despite it is not the first study done about the effects of tocilizumab it does support other studies although it might differ with others. Unlike other studies, they didn't observe sustained reductions in CRP but did observed an overall increase in PaO2/FiO2. However, their results suggest a possible mortality benefit with tocilizumab as has been already reported in many studies. The findings of this study show the benefits of treating patients with tocilizumab and its effects when patients are treated with other concomitant therapies.

Research Highlights:

6. Tocilizumab might help patients achieve clinical improvement
7. A rebound effect with C-reactive protein was observed
8. Tocilizumab may increase the risk of infections

Impact for COVID-19 research:

- Although it doesn't alter our view of the disease it might help clinicians if they consider to use tocilizumab as a therapy for Covid-19 as this study reports the effects of using tocilizumab as a therapy and what to expect if they use it with other reported therapies such as hydroxychloroquine.

Methodologies:

- Study Type: *cohort study*
- Key Techniques: *Tocilizumab was administered intravenously and data was collected on day -1, 0, 1, 2, 3, 4, 5, 7, 10, 14 and 30 relative to tocilizumab administration. Oxygenation was assessed by calculating* PaO_2/FiO_2.

Limitations:

- The sample size of the study was small (n=63)
- The dose approved for tocilizumab by the FDA is 8mg/kg whereas in this study the average dose they use is 4.75mg/kg
- Many patients received other therapies such as hydroxychloroquine that might impact clinical outcomes
- All the infections diagnosed were based on tracheal aspirates which might affect to the quality of the culture making it difficult the diagnosis of pneumonia.
- This is a descriptive study so is not possible to determine tocilizumab efficacy.

A placebo-controlled double blind trial of hydroxychloroquine in mild-to-moderate COVID-19

Dubée, V. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.10.19.20214940

Summary:

Since the start of the COVID-19 pandemic, there have been conflicting reports surrounding the efficacy of hydroxychloroquine. Therefore, this multi-centre randomised double-blind placebo-controlled clinical trial (NCT04325893) evaluated this drug in elderly patients with mild-to-moderate COVID-19. Overall, treatment with 800 mg on Day 0 followed by 400 mg for 8 days did not benefit patients compared to control.

Research Highlights:

1. No significant differences were seen with hydroxychloroquine treatment regarding primary endpoint (composite of death and the need for invasive mechanical ventilation within 14 days after randomisation). 6.5% of placebo patients vs. 7.3% of treated individuals died within 14 days and the relative risk for death or ventilation was 1.23.
2. Using the WHO Ordinal Scale for Clinical Improvement for COVID-19, no difference between treatment groups were seen by day 14 and day 28. Similar proportions of individuals had returned home or demonstrated clinical improvement.
3. Viral shedding at days 5 and 10 was not significantly affected by hydroxychloroquine.

Impact for COVID-19 research:

- Controlled trials demonstrates the limited efficacy of hydroxychloroquine in vulnerable populations, allowing better clinical practice going forward.

Methodologies:

- Study Type: *Clinical Trial – patient eligibility: ≥75 years of age, 60-74 years old with the presence of at least one comorbidity* (obesity, arterial hypertension or diabetes mellitus requiring treatment), Need for supplemental oxygen to reach a peripheral capillary oxygen saturation of more than 94% (SpO2 >94%)
- Key Techniques: *RT-PCR*

Limitations:

- Study was prematurely stopped due to low inclusion numbers as pandemic slowed in France.

Vaccines and Long-Term Immunity

The effect of influenza vaccination on trained immunity: impact on COVID-19

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

Link: https://doi.org/10.1101/2020.10.14.20212498

Summary:

Long term boosting of the innate immune system or 'trained immunity' is suggested to induce a cross-protection between influenza vaccination and COVID-19. *In vitro* investigation of the 2019-2020 influenza vaccine in the Netherlands found a trained immunity response that resulted in protection against subsequent SARS-CoV-2 infection. SARS-CoV-2 infection was also found to be less common in Dutch hospital workers who had received the seasonal influenza vaccine compared to those who hadn't. This suggests a benefit to receiving the season influenza vaccine to protect against the current COVID-19 pandemic.

Research Highlights:

1. PMBCs isolated from healthy blood were stimulated with a quadrivalent inactivated influenza vaccine (Vaxigrip Tetra), which is the 2019-2020 seasonal influenza vaccine in the Netherlands. An increase in cytokines was not seen with Vaxigrip alone, but coupled with the BCG TB vaccine resulted in increased cytokine production (TNFα, IL-6, IL-1β), dose-dependently, compared to BCG alone.
2. 6 days post vaccine treatment, PBMCs were restimulated with SARS-CoV-2 or LPS. High levels of IL-1RA were subsequently detected, however this was significantly lower in the Vaxigrip/BCG treated cells. IL-6 was upregulated higher in the Vaxigrip/BCG treated groups as was IFNγ, but not TNFα in the SARS-CoV-2 stimulated cells.
3. Influenza vaccination statuses of 184 Dutch healthcare workers with a documented PCR positive SARS-CoV-2 test were collected. Incidence of COVID-19 in those vaccinated with the influenza 2019/2020 vaccine was 1.33% while it was 2.23% in non-vaccinated individuals. A statistically significant negative association between influenza vaccination and COVID-19 incidence was found (p= 0.0008). No association with disease duration was found.

Impact for COVID-19 research:

- The seasonal influenza vaccine is readily available to many of the population, whereas there is no vaccine against SARS-CoV-2 currently. Further research into the protective effects of the influenza vaccine against SARS-CoV-2 could lead to a prophylactic method of protecting vulnerable members of the population against SARS-CoV-2 infection.

Methodologies:

- Study Type: *In vitro* & *cohort study*.

- Key Techniques: *In vitro 'influenza training model' using PBMCs, ELISA (IL-1RA, TNFα, IL-6, IL-1β, IFNγ).*

Limitations:

- Influenza vaccination status in SARS-CoV-2 negative healthcare workers was not known.
- SARS-CoV-2 negative employees were documented to have less direct contact with SARS-CoV-2 patients which may be a confounding factor.

Rabies virus-based COVID-19 vaccine CORAVAX™ induces high levels neutralizing antibodies against SARS-CoV-2

Kurup, D. *et al*. 2020. *npj vaccines*

Link: https://doi.org/10.1038/s41541-020-00248-6

Summary:

The authors introduced SARS-CoV-2 S1 protein into an attenuated rabies virus vector (CORAVAX) and immunised mice with live or inactivated CORAVAX with and without adjuvant. They report titres of virus neutralising antibodies at higher levels than in the sera of convalescent patients, suggesting the vaccine as a potential candidate for clinical trials.

Research Highlights:

1. Live CORAVAX included a balanced Th1/Th2 response, while inactivated vaccine was biased toward Th1 responses
2. Levels of virus neutralising antibodies were present in all groups, highest in inactivated CORAVAX and higher in all vaccinated mice than convalescent patients

Impact for COVID-19 research:

- Moderate: while several vaccines are in clinical trials, the authors question their ability to induce virus neutralising antibodies. This vaccine could be a good candidate to enter clinical trials.

Methodologies:

- Study Type: *In vivo/in vitro*
  Key Techniques: *virus construction, analysis of mouse serum after vaccination*
  SARS-CoV-2 neutralisation assays with infected vero CCL81 cells exposed to mouse serum

Limitations:

- The authors focused on IgG antibodies. Other studies have suggested that IgM antibodies are more protective than IgG so it would be interesting to see whether those isotypes are also induced by the vaccine.

- The authors did not investigate how long neutralising antibodies persisted after vaccination (serum was analysed 56 days after immunisation but not later)
- In SARS-CoV/macaque models anti-spike IgG caused fatal acute lung injury, it will need to be investigated whether that is a risk with this vaccine