COVID-19 Community Journal Club

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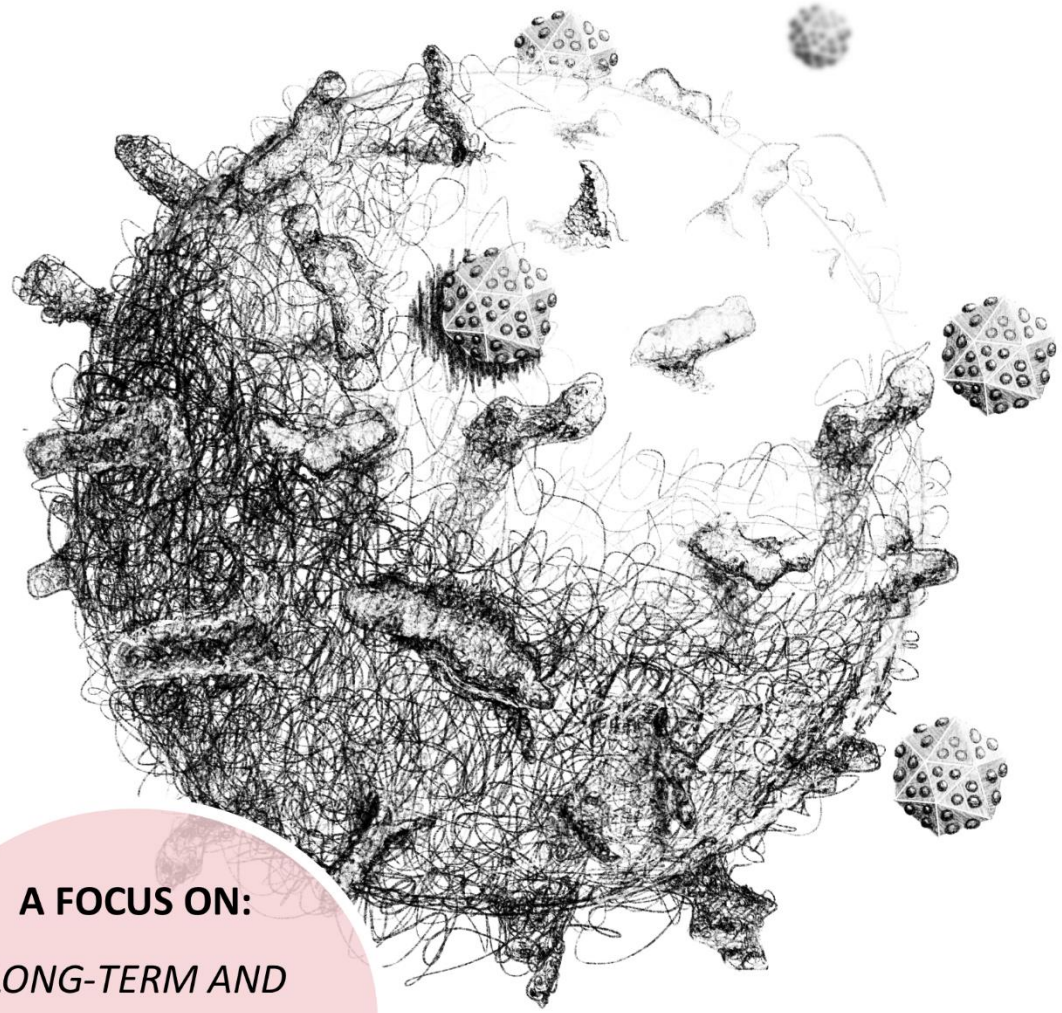

A FOCUS ON:

LONG-TERM AND HERD IMMUNITY

Pages 5-9

Artwork by
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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

Table of Contents

Page

Long-Term and Herd Immunity

News and Views

Journal Reviews

Vaccines

Immunopathology

Virology

Diagnostics and Therapeutics

CAUTIONARY NOTE:

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

Long-Term and Herd Immunity

News and Views

Herd Immunity and Implications for SARS-CoV-2 Control

Omer, S.B. *et al*. 2020. *JAMA*

Link: https://doi.org/10.1001/jama.2020.20892

Herd immunity is a historical term used to describe the protection of vulnerable individuals in a population from a pathogen when those around them have already developed immunity, either through infection or immunisation.

When assuming no population immunity to SARS-CoV-2 and considering all individuals are both equally susceptible and infectious, the estimated ***herd immunity threshold*** for SARS-CoV-2 is between 50% and 67%, as the R_0 is between 2-3. However, these models assume random mixing within a population, which is not realistic. It is currently unclear what the effect of heterogenic social mixing has on herd immunity to SARS-CoV-2.

Infection-based herd immunity has been suggested for SARS-CoV-2 and relies on shielding the most susceptible while allowing the low-risk groups to continue life as normal and become infected. This method is rare, with the most recent success seen when Brazil overcame Zika. Sweden attempted to achieve infection-based herd immunity to SARS-CoV-2 but abandoned this approach by the end of March. A study found that seroprevalence in Stockholm was less than 8% by April, suggesting that infection-based herd immunity is unrealistic. This is especially the case when considering large populations such as USA: it is estimated that 198 million people would need to become immune to reach herd immunity threshold of 60%.

Vaccine-induced herd immunity will result from mass a reduction in the reproduction number from mass immunisations. This approach has successfully been seen with the eradication of smallpox. However, the durability of an immune response and the presence of memory is critical to understanding and achieving herd immunity. It is well known that natural immunity to seasonal coronaviruses is short-lived and so it is likely that a periodic vaccination program will be required to sustain herd immunity to SARS-CoV-2.

Journal Reviews

Robust SARS-CoV-2-specific T-cell immunity is maintained at 6 months following primary infection

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

Link: https://doi.org/10.1101/2020.11.01.362319

Summary:

A time-dependent decline in antibody responses to SARS-CoV-2 infection has been demonstrated in patients, leading to the question of whether there is protection against subsequent infection. However, the duration of cellular responses are yet to be characterised. Both $CD4^+$ and $CD8^+$ virus-specific T-cells were detected in all 100 patients 6 months post-infection, with T-cell responses being 50% higher in symptomatic individuals. $CD4^+$ T-cell responses appear to dominate over $CD8^+$ responses, with a marked increase in IL-2 production. A strong positive correlation was also found between T-cell responses and antibody titres. This suggests that the T-cell response 6-months after SARS-CoV-2 infection persists, which may have implications for long-term immunity and vaccine design.

Research Highlights:

1. Blood samples from 100 donors, both symptomatic and asymptomatic patients, were collected 6-months post SARS-CoV-2 infection. 95% of donors showed a SARS-CoV-2-specific T-cell response against 1 or more viral proteins using an IFNγ ELISPOT assay. 82% had T-cell responses against the spike (S) protein. ELISPOT T-cell responses were 50% higher in symptomatic donors, however, no correlation was seen with age.
2. Intracellular cytokine analysis of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T-cells revealed virus-specific cytokine responses in 96% of donors (including the 5 donors negative by ELISPOT). $CD4^+$ responses were double that of $CD8^+$ responses for both S and non-S antigen-specific T-cells.
3. $CD4^+$ T-cells had a marked increase in IL-2 responses compared to $CD8^+$ T-cells. However, cytokine responses differed depending on the T-cells antigen-specificity. Analysis of supernatant from *ex vivo* peptide-stimulated T-cells revealed varying levels of IL-2, TNFα and IFNγ.
4. Antibody (Ab) titres against S and nucleoprotein (NP) antigens peaked two months post-infection and declined by 50% the two months thereafter. S-specific Ab titres continued to decrease further, whereas NP-specific Abs persisted.
5. A strong positive correlation was found between S-specific T-cell responses and S-specific Ab titres, as well as between non-S-specific T-cell responses and NP-specific Ab titres. Donors with persistent NP-specific Abs had stronger NP- and membrane protein-specific T-cell responses. This was not seen for S-responses.
6. CXCR5 analysis of T-cells revealed expression on only 7% of virus-specific $CD4^+$ T-cells, with no correlation to antibody responses.

Impact for COVID-19 research:

- Investigating both cellular and humoral long-term immunity is vital for understanding protection against subsequent COVID-19 infection and may impact upon vaccine design.

Methodologies:

- Study Type: *Cohort study*
- Key Techniques: *IFNγ ELISPOT assay, intracellular cytokine staining (ICS), LEGENDplex supernatant cytokine analysis (cytokine panel 2).*

Limitations:

- Low sample numbers
- No comparison to blood samples taken during/immediately after infection for T-cells. Cannot investigate the decline/maintenance in T-cell responses like they did with Abs.

Long-term persistence of neutralizing memory B cells in SARS-CoV-2

Bull, R. *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-92527/v1

Summary:

This study presents longitudinal analysis of SARS-CoV-2 humoral responses in 81 COVID-19 patients for up to six months after infection. While the antibody titres against SARS-CoV-2 receptor binding domain (RBD) maintained, neutralising activities (NAbs) were not detected in half of the participants. However, RBD-specific memory B cells persisted or even increased in number four to six months after infection in majority of the selected participants. These memory B cells are capable of generating antibodies with neutralizing responses. These data suggest that despite the declining of Nabs level in majority of seroconverted individuals, the persistence level of memory B cells with capacity of producing neutralising antibodies could provide hope for protection following natural infection.

Research Highlights:

1. The study performed longitudinal analysis of 81 participants with SARS-COV-2 infection for antigen binding and viral neutralization
2. Significant reduction of antibody binding to RBD and spike antigen after six months
3. The neutralising antibody level in half of the seroconverted individuals 35 of 81 (43%) had reverted to background neutralising levels.
4. While Nabs responses declined, robust memory B cell populations were observed in majority of the participants four to six months following infection.
5. Memory B cells express antibodies with neutralizing responses.

Impact for COVID-19 research:

- The study provides hope of protective immunity that could reduce reinfection severity upon exposure and promise for effective vaccine strategies based on NAb induction.

Methodologies:

- Study Type: *ex vivo longitudinal analysis of convalescent serum, plasma and PBMCs.*
- Key Techniques:
  - *flow cytometry live cell-based assay*
  - *SARS-CoV-2 neutralisation assay*
  - *Production of monoclonal antibodies from single-sorted RBD-specific B cells*

Limitations:

- The authors note that while the study sample incorporated a longitudinal monitoring, samples collected during early phase of infection (acute vs convalescent) would enable to evaluate if early immune responses (B or T cell responses) could influence memory B cell repertoire.
- Limitations noted by the authors include sample size of the study. Therefore future investigation in larger cohort will help in confirming the findings.
- While the study included participants with Asymptomatic or mild, moderate and severe infections, minimal attention was given to the level of memory B cell responses in individual with different the degree of the disease severity.
- Minimal evidence reported for clinical data and comorbidities of individuals. Therefore, it is not clear if any-existing conditions could contribute to the findings.

Declining prevalence of antibody positivity to SARS-CoV-2: a community study of 365,000 adults

Ward, H. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.10.26.20219725

Summary:

This study by Imperial College London has used random, non-overlapping populations of individuals over three months to investigate the antibody prevalence to SARS-CoV-2. Prevalence was determined through use of self-administered lateral flow immunoassays (LFIA) for anti-spike IgG. Overall, they found that antibody prevalence has declined from 6.0% to 4.4% between June and September, providing evidence of waning antibody positivity as we approach the second wave of the virus.

Research Highlights:

1. SARS-CoV-2 antibody prevalence in England delinked from 6.0% to 4.8% and 4.4% over three rounds between June and September, despite similar patterns of infection. Overall, this was a fall of 26.3%.
2. Antibody prevalence was highest in those ages 18-24 and lowers in those 75 and over. White ethnicity was associated with lower prevalence (3.6%) than people of black (13.85) and Asian (9.7%) ethnicity. Other socioeconomic factors associated with increased prevalence.
3. Individuals who had a PCR-confirmed case of COVID-19 demonstrated less antibody decline than those lacking a confirmed test (-22.3% vs. -64.0%).
4. Sera which tested positive in this study using the lateral flow assay had a significantly higher titre of neutralising antibodies than those that scored negative ($P<0.001$).
5. Sensitivity of assay for IgG 84.4% in PCR confirmed cases and specificity in pre-pandemic sera was 98.6%.

Impact for COVID-19 research:

- Investigating antibody prevalence is important for epidemiological studies and the impacts of national lockdowns. It also helps understand the kinetics of the humoral response to SARS-CoV-2.
- It is important to note that whist this study highlights that antibody prevalence has decreased it does not necessarily mean that any long-term immunity to SARS-CoV-2 has been affected, which many media outlets have suggested. Studies looking at memory B-cell populations and reinfection studies would help in this regard.

Methodologies:

- Study Type: *Cohort Study using a home-based lateral flow immunoassay with random, non-overlapping populations covering most of the population of England.*
- Important cell lines/viral models used: *Vero E6 Cells to assess neutralising capacity of sera against SARS-CoV-2.*
- Key Techniques: *Sensitivity analysis of LFIA using live neutralisation tests from PCR-positive health care workers.*

Limitations:

- Authors comments that the lateral flow assay only detects spike protein, and the threshold of detection has not been stated by the manufacturer. Given this study included a large cohort this was probably the easiest way to measure levels but better assays, with a quantitative value, would give a better insight.
- It is also noted that antibody waning may be attributed to inclusion of random non-overlapping populations and those exposed to COVID-19 would less likely take part at the later rounds. I think inclusion of a few individuals whereby the antibody levels were sequentially taken would give a good insight.

Vaccines

Soluble Spike DNA vaccine provides long-term protective immunity against SAR-CoV-2 in mice and nonhuman primates

Seo, Y.B. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.10.09.334136

Summary:

GX-19 is a synthetic soluble SARS-CoV-2 spike (S) DNA based vaccine candidate. In this study Yong Bok Seo *et al*. demonstrate the GX-19 efficiency against S in comparison to pGX27-S. GX-19 can induce antibody responses in mice and in non human primates (NHP) and also protect against SARS-CoV-2 in in NHP. The results obtained in this study prove the efficacy of DNA vaccine platform as a good alternative in vaccine development for emerging infections. In particular, the data shown explains the promising immunogenicity of the GX-19 showing protective effect against Covid-19. Although the ab titers in the pGX27-SATM group were higher than in the pGX27-S group at post-prime and post-boost which differs from what has already been reported in vaccinated macaques.

Research Highlights:

1. GX-19 induce strong humoral and cellular immune responses in mice and NHP
2. GX-19 protect NHPs from wild-type SARS-CoV-2 infection (after 10 weeks following the last vaccination)

Impact for COVID-19 research:

- Despite this study do not alter the point of view of Covid-19 it does demonstrate the efficiency of GX-19 as a possible candidate to treat Covid-19. Suggesting this synthetic soluble SARS-CoV-2 spike (S) DNA as a possible vaccine.

Methodologies:

- Study Type: *in vivo.*
- Important cell lines/viral models used: BALB/c mice strain and macaques as animal models.
- Key Techniques: *Antigen binding ELISA from serum and BAL of macaques to investigate the induced response. Live virus neutralization assay. IFN-γ ELISPOT and intracellular cytokine staining. Cytokine rofile analysis by cytometric bead array (CBA) to analyse cytokine patterns. To calculate the values of TCID50/ml they used the Reed and Muench method*

Limitations:

- Number of animals insufficient. Although there is a difference in the results pointing a tendency there is no statistical significance.
- The mice strain used is BALB/c which tend to express more Th2.

Immunopathology

Distinct cellular immune profiles in the airways and blood of critically ill patients with COVID-19

Saris, A. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.10.29.360586

Summary:

Saris *et al.* analyse Broncho Alveolar Lavage Fluid (BALF) and blood samples from 17 severe COVID-19 patients and compare their immune landscape. In the lungs, the most abundant cells were found to be macrophages and T cells. Blood and BALF monocytes revealed a very different phenotype. T cell phenotypes in blood showed central memory CD4 and terminally effector CD8, while in the lung CD4 T cells also showed a terminally differentiated effector memory phenotype. Exhaustion PD-1 marker was highly upregulated in both plasma and BALF as well as markers for T proliferation and differentiation. Prolonged ICU stay correlated with lower T cell activation status and higher frequencies of alveolar and monocyte-like macrophages. Non-surviving COVID-19 patients showed an increased T cell subset activation in blood but decreased in BALF. However, inflammatory mediators were higher in BALF, suggesting very different immune profiles in peripheral blood and in the lungs.

Research Highlights (*Around 5 bullet points is sufficient)*

1. For severe COVID-19, in blood CD4 T cells showed a central memory phenotype while and CD8 were RA+ effector memory. In the lung CD4 T cells also showed a terminally differentiated effector memory phenotype.
2. Exhaustion PD-1 marker was highly upregulated in both plasma and BALF
3. Cytokines stimulating T cell proliferation and differentiation were upregulated in severe COVID-19.
4. Prolonged ICU stay correlated with lower T cell activation status and higher frequencies of alveolar and monocyte-like macrophages
5. BALF levels of many cytokines are higher than their plasma levels

Impact for COVID-19 research:

- The study shows a comparison on the immune landscape between BALF and blood samples from severe COVID-19 patients.

Methodologies:

- Study Type: *Immune profile of clinical samples*
- Important cell lines/viral models used: *samples from patients*
- Key Techniques: Spectral *Flow cytometry, Luminex*

Limitations:

- The authors don't discuss possible recruitment from blood to site of infection
- The sample is quite small (17) and only of severe ICU cases, it would be informative to know if the same differences are found in non-ICU but hospitalized patients and mild patients.
- The authors discuss that they don't observe a dramatic lymphopaenia and that might be due to late sampling, which may also bias the rest of the results as more of those entering convalescence rather than acute infection.
- Although an incredible amount of data has been generated, the discussion could be more extensive.

COVID-19 cytokines and the hyperactive immune response: Synergism of TNF-α and IFN-γ in triggering inflammation, tissue damage, and death

Karki, R. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.10.29.361048

Summary:

In this paper Karki *et al*. use published data to identify cytokines systemically upregulated during SARS-CoV-2 infection. They go on to test the effect of these cytokines on BMDM and identify the combination of TNFα and IFNγ as the only one capable of cause cell deaths. In vivo treatment of mice with TNFα and IFNγ causes cytokine shock that partially mirror COVID19 symptoms. By using KO mouse models, they show that the cell death is a combination of pyroptosis, apoptosis and necroptosis and can be abrogated by blocking the STAT1 and Casp8 pathways. Treatment with antibodies against TNFα and IFNγ is also protective in murine models of SARS-CoV-2 infection

Research Highlights:

1. TNFα and IFNγ are upregulated in SARS-CoV-2 infection and together can trigger cell death
2. TNFα and IFNγ administration in mice triggers cytokine storm that mirrors some symptoms of COVID19
3. TNFα and IFNγ induce pyroptosis, apoptosis and necroptosis in BMDM
4. TNFα and IFNγ induced cell deaths is dependent on STAT1, Casp8 and iNOS
5. Treatment with antibodies against TNFα and IFNγ is protective in murine models of SARS-CoV-2 infection

Impact for COVID-19 research:

- The link between the disease model used (TNFα + IFNγ treatment) and COVID19 is somewhat tenuous but the final experiment on SARS-Cov2 infected mice make the

case that blocking IFNγ and TNFα could help reduce COVID19 symptoms. The study does not investigate the optimal time for the treatment

Methodologies:

- Study Type: *online database analysis, in vitro, in vivo*
- Important cell lines/viral models used: *SARS-CoV-2 isolate USA-WA1/2020*
- Key Techniques: *cell death imaging, immunoblot, survival studies, flow cytometry, gene expression analysis*

Limitations:

- The study fails to demonstrate a clear connection between the model used (TNFα + IFNγ) and COVID19. While the results of the study seems valid the possible transability to the disease is remote.
- The study suggest anti-TNF and IFNγ Ab as possible therapy, while this is a reasonable strategy (considering the established use of steroids in the clinic) there are no indications on the best timing for administration

Virology

Transferrin receptor is another receptor for SARS-CoV-2 entry

Tang, X. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.10.23.350348

Summary:

Angiotensin-converting enzyme 2 (ACE2) has been repeatedly suggested as the receptor for SARS-CoV-2 entry into cells, this paper proposes an alternative entry with the transferrin receptor (TfR). By using knockout mice, the authors demonstrate that a lack of ACE2 does not completely block virus entry, and SARS-CoV-2 can infected mice with overexpressed humanized TfR and without humanized ACE2. TfR is shown to co-localise with the virus at the membrane and in the cytoplasm and treatment with anti-TfR antibody indicated promising anti-viral effects in mice.

Research Highlights:

1. TfR expression was increased in the respiratory tract and lung tissue of SARS-CoV-2 infected monkeys and mice.
2. Interaction between TfR and spike:
    a. Association rate constant (Ka) = 2.69×10^{5} $M^{-1}s^{-1}$
    b. Dissociation rate constant (Kd) = 7.92×10^{-4} s^{-1}
    c. Equilibrium dissociation constant (KD) = 2.95 nM
3. Two peptides were designed to interfere with the binding of TfR and spike according to the docking model of the interaction and these were shown to be successful. Soluble TfR, Tf, anti-TfR antibody and the designed peptides all blocked virus infections to Vero E6 cells
4. ACE2 KO reduced but did not eradicate SARS-CoV-2 infection. In these cells, addition of an anti-TfR antibody inhibited infection

Impact for COVID-19 research:

- If confirmed, an alternative entry point for SARS-CoV-2 would provide an alternative target for prevention and treatment of COVID-19.

Methodologies:

- Study Type: *in vitro, in vivo*
- Important cell lines/viral models used: *Vero E6 cells*

Limitations:

- The significance and plotted means of the work in mice is questionable.
- Some of the figures raise questions that are not addressed in the text and more experiments would have been beneficial.
- Additional groups would have been beneficial in both in vivo and in vitro experiments for comparison, e.g. hACEs to compare to hTfR and untreated controls in the KO experiments.

Diagnostics and Therapeutics

An ultra-sensitive, ultra-fast whole blood monocyte CD169 assay for COVID-19 screening

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

Link: https://doi.org/10.1101/2020.10.22.20215749

Summary:

RT-PCR testing for SARS-CoV2 is the gold-standard but sensitivity depends on sampling quality leading to false negatives and can take 1+ hours. Exploring if a flow cytometric whole-blood CD169 expression test on monocytes as it has been proposed as a COVID-19 biomarker. Test was thought to be more sensitive, took 15-30 minutes and required a few microliters of blood. Monocytic CD169 expression could identify early and asymptomatic acute viral infections but would need SARS-CoV2 RT-PCR to confirm COVID-19 as the cause. Needs to be verified in larger cohorts with patients with varying disease severity.

Research Highlights:

1. 80 early stage COVID-19 patients (≤14 days after symptom onset), 71 late-stage (≥15 days) and 26 asymptomatic patients.
2. CD169 expression was higher in early stage and asymptomatic cases compared to late stage.
3. Specificity vs Sensitivity plots confirmed AUC of 0.93 in early COVID-19 cases, 0.95 in asymptomatic cases but only 0.58 in late-stage patients.
4. There was a weak correlation between SARS-CoV2 RT-PCR and CD169 blood test.
5. Neutrophil CD64 expression was unchanged between patient groups, and monocyte HLA-DR only differed between early and late stage cases.

Impact for COVID-19 research:

- Propose that monocyte CD169 expression could reduce demand on SARS-CoV2 RT-PCR testing by quickly identifying individuals with acute viral infection.

Methodologies:

- Study Type: *Cohort study*
- Key Techniques: *Flow cytometric analysis of monocytes from whole blood using a CD169-PE/HLA-DR-APC/CD64-PacBlue Antibody Cocktail (Beckman Coulter Life Sciences).*

Limitations:

- Increased monocyte CD169 expression marks acute viral infection, would still need SARS-CoV2 RT-PCR to confirm COVID-19 as the cause.
- Currently only 177 patients investigated, needs a larger cohort.

- Have not looked at how disease severity may impact the accuracy of this test.
- Five authors are employed by Beckman coulter, who developed this assay.

Tocilizumab in nonventilated patients hospitalized with Covid-19 pneumonia

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

Link: https://doi.org/10.1101/2020.10.21.20210203

Summary:

This article summarises the results of the EMPACTA clinical trial (ClinicalTrials.gov NCT04372186), evaluating safety and efficacy of tocilizumab in patients hospitalised with Covid-19 pneumonia. The trial included high-risk and minority populations, suggesting that tocilizumab demonstrates efficacy over placebo in reducing the likelihood of requiring mechanical ventilation or death.

Research Highlights:

1. The proportion of patients who required mechanical ventilation or had died by day 28 was 12% for tocilizumab and 19.3% in the placebo group
2. Median time to hospital discharge was 6 days for tocilizumab, 7.5 days for placebo
3. Adverse effects were reported in 50.8% of the tocilizumab and 52.8% of the placebo arm, with deaths occurring in 11.6% for tocilizumab and 11.8% for placebo

Impact for COVID-19 research:

- Moderate: there was a significant difference in patients' progression to mechanical ventilation suggesting tocilizumab could be beneficial to prevent severe outcomes.

Methodologies:

- Study Type: *Clinical trial*
  Key Techniques: *patients were randomised (2:1) to intravenous tocilizumab or placebo, if clinical signs did not improve a second dose could be administered 8-24 hours after the first. Efficacy was evaluated on day 28 and patients were followed for 60 days*

Limitations:

- Patients received different combinations of treatments including antiviral treatment and systemic corticosteroids (standard of care) that could have influenced outcomes but are stated to have been balanced between groups