COVID-19 Community Journal Club

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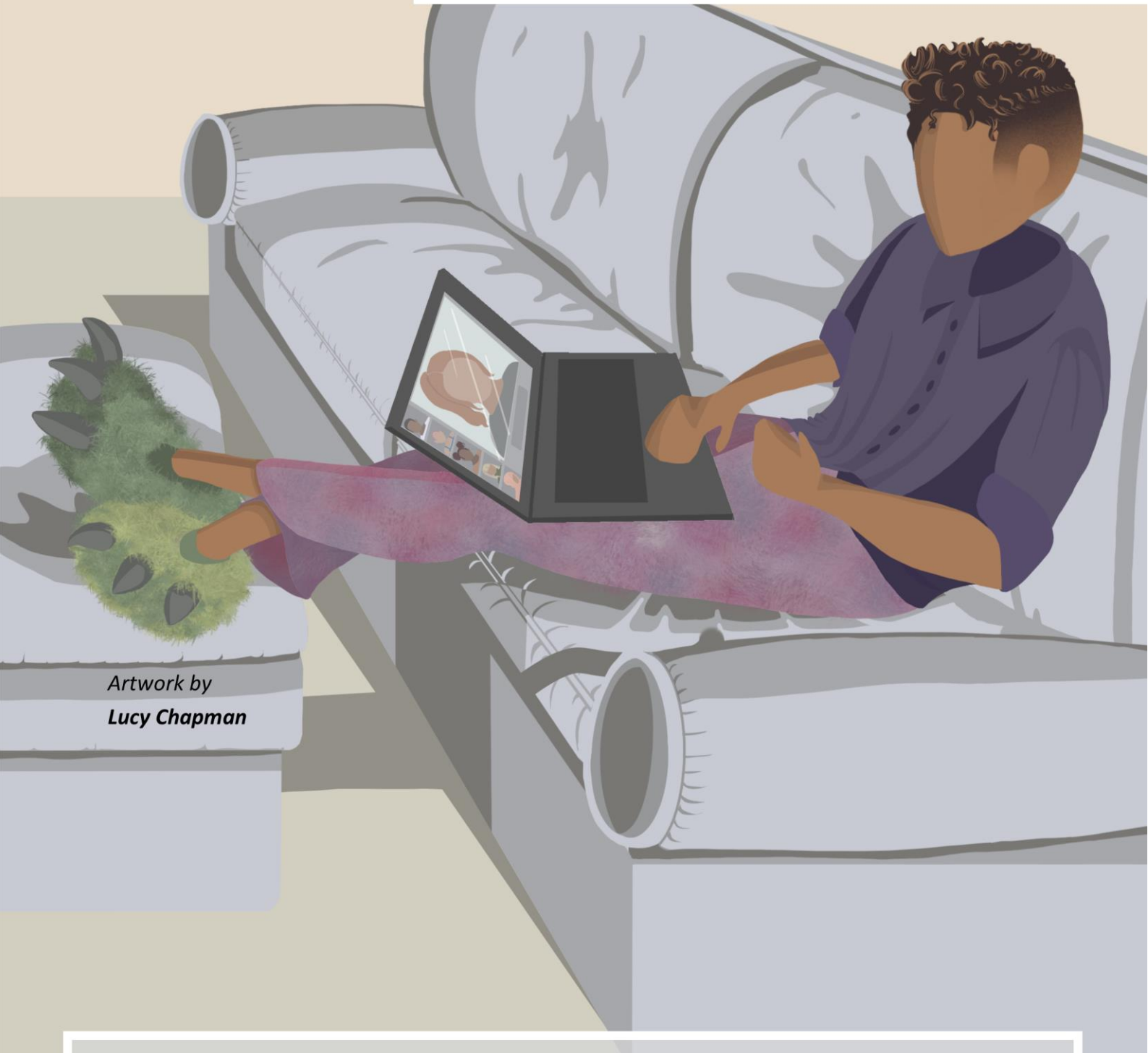

Artwork by
Lucy Chapman

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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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

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CAUTIONARY NOTE:

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

Learning From COVID-19 Autopsies

Multi-organ Proteomic Landscape of COVID-19 Autopsies

Nie, X. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.16.20176065

Summary:

Nie *et al*. aimed to characterise the changes SARS-CoV-2 induces in multiple organs using TMT shotgun proteomics. They sampled autopsies of lung, spleen, liver, heart, kidney, thyroid and testis from 19 COVID-19 patients as well as 56 non-COVID-19 patients. Interestingly, they identified CSTL rather than ACE2 to be significantly upregulated in the lung of COVID-19 patients. Furthermore, integrating a systematic pathway enrichment across multiple organs, glucose and fatty acid metabolism were affected in multiple organs, supporting virus replication by production of energy. Evidence suggests that SARS-CoV-2 uses the hyperinflammatory environment to induce tissue hypoxia which further stimulates inflammatory responses as well as induces abnormal angiogenesis and fibrosis across multiple organs.

Research Highlights:

1. Analysis of proteomic landscape in severe COVID-19 patients in multiple organs is presents a useful resource in understanding the molecular pathogenesis of SARS-CoV-2
2. SARS-CoV-2 triggers hyperinflammation which leads to tissue hypoxia, further enhancing the immune response and leading to abnormal angiogenesis and fibrosis
3. CSTL as opposed to ACE2 is enhanced in lung of severe COVID-19 patients, presenting an alternative drug target

Impact for COVID-19 research:

- Dissection of multi-organ pathology in COVID-19 patients; creation of proteomic landscape of severe COVID-19 patients which present a useful resource to investigate

Methodologies:

- Study Type: *patient case study*
- Key Techniques: *TMT shotgun proteomics*

Limitations:

- Sample size, healthy donors had other diseases which may perturbate proteomic landscape

SARS-CoV-2 infection of human iPSC-derived cardiac cells predicts novel cytopathic features in hearts of COVID-19 patients

Pérez-Bermejo, J.A. *et al*. 2020. *bioRxiv*
Link: https://doi.org/10.1101/2020.08.25.265561

Summary:
Cardiac damage, even after recovery, has been reported in a multitude of COVID19 patients with nearly half of mildly ill patients showing echo abnormalities. This preprint investigates the cellular alterations occurring during SARS-CoV-2 infection *in vitro* using human iPSCs. They confirm data that cardiomyocytes *in vitro* are most susceptible to SARS-CoV-2 infection via a cathepsin-L-dependent autolysosomal entry pathway. Infection of these cells results in strong transcriptional changes including downregulation of proteasomal degradation and contractile and structural integrity. The authors confirm the data through high-content imaging and find clear fragmentation of myofibrils, in parts which could be explained through proteasomal inhibition. This fragmentation seems to be independent of cell infection suggesting larger cytopathological effects of COVID19. Lastly, the verified in vitro data using post-mortem cardiac tissue from COVID19 patients observing similar cytoskeletal fragmentation and nuclei loss in the absence of SARS-CoV-2 protein or DNA marker. This study gives a potential explanation to the long-term cardiac effects observed in COVID19 patients and may allow educated approaches for intervention strategies.

Research Highlights:

1. The authors show that human iPSC-derived cardiomyocytes show highest infectibility in comparison to cardiac fibroblasts and endothelial cells, correlating with endogenous ACE2 expression.
2. Cytopathic effects derived from replicative virus exposure without infecting the cells.
3. SARS-CoV-2 enters through a cathepsin-L endolysosomal pathway into cardiomyocytes.
4. Interferon-beta pre-treatment can prevent viral entry into cardiomyocytes.
5. Transcriptomic analysis shows deregulation of contractile and structural integrity of CM which is further confirmed by high-content imaging identifying specific and periodic cleavage of myofibrils into sarcomeric fragments.
6. Post-mortem cardiac tissues show “extreme myofibrillar anomalies”, but no presence of virus could be identified.

Impact for COVID-19 research:

- COVID19 has been attributed a clear effect on the cardiac system through high levels of circulating cardiac damage markers (troponin-I and natriuretic peptides). The study dissects cardiac abnormalities that may lead to long term damage to cardiac tissue after SARS-CoV-2 infection. Thus, the molecular changes observed may give a possible explanation for the observed clinical cardiac alterations after COVID19 and give insights into new treatment options.

Methodologies:

- Study Type: *in vitro, post-mortem histology*
- Important cell lines/viral models used: *human iPSC-derived cardiomyocytes, cardiac fibroblasts, endothelial cells, post-mortem cardiac tissue, WA-1 strain (BEI resources) of SARS-CoV-2*
- Key Techniques: *iPSC cell culture, RNAseq, TEM/CLEM, histologies*.

Limitations:

- Some data shown by the authors would require more data points/repeats to ensure validity of the data (ANOVA over 2-3 replicates may result in some false positive results).
- To investigate the cytopathic effects on non-infected cells, it would have been beneficial to use condition medium and use blocking antibodies to identify the molecular component causing the effect.
- Read-depth of RNAseq dataset is at minimum especially considering that at later time points, viral RNA maps about 50% of all reads, it would have been more beneficial to increase read-depth.

Inflammation and Immune Dysregulation

Lipid droplets fuel SARS-CoV-2 replication and production of inflammatory mediators

da Silva Gomes Dias, S. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.22.262733

Summary:

Lipid droplets (LD) are lipid-rich organelles that regulate lipid and energy homeostasis. da Silva Gomes Dias *et al.* showed increased of LD accumulation in monocytes from COVID-19 patients compared with healthy volunteers. *In vitro* infection with SARS-CoV-2 modulated proteins involved in lipid metabolism including CD36, PPARγ and SREBP-1, and DGAT-1 enzyme, leading to accumulation of LD in monocytes and human lung cells. SARS-CoV-2 uses LDs as a replication site, demonstrated by co-localization of viral proteins and ds-RNA with LD in infected cells. Pharmacological targeting of LD formation by blocking DGAT-1 enzyme, inhibits SARS-CoV-2 replication and reduced the exaggerated inflammatory responses mediated by cytokine storm. Collectively, these data indicate that SARS-CoV-2 modulates cellular lipid metabolism and associates to LD potentially using them as replication platform, suggesting that blocking lipid metabolic enzymes could serve as a potential target for therapeutic intervention.

Research Highlights:

1. Monocytes from COVID-19 infected patients (an infected other cell line models) had an increased accumulation of lipid droplets.
2. SARS-CoV-2 infection promotes expression of key enzymes in lipid metabolism, increasing lipid droplets formation in human cells.
3. Using a DGAT-1 inhibitor limits cell death, viral spreading, release of pro-inflammatory cytokines and mediators.
4. Lipid droplets are potential sites for SARS-CoV-2 replication.

Impact for COVID-19 research:

- The research helps our understating of how cellular lipid metabolism is changed by SARS-CoV-2 infection and how SARS-CoV-2 may use of host lipid metabolism to support its replication.
- It also argues in favour of targeting lipid metabolism as therapeutic intervention.

Methodologies:

- Study Type: *in vitro*
- Important cell lines/viral models used:
    - *PBMCs from SARS-CoV-2 patients and healthy donors.*
    - *Human lung epithelial carcinoma cell line (A549)*
    - *African green monkey kidney (Vero subtype E6)*

  - *Human lung microvascular endothelial cell line.*

- Key Techniques:
  - *In vitro SARS-CoV-2 infection*
  - *fluorescence microscopy*
  - *SDS-PAGE and Western blot*
  - *Real-Time PCR*
  - *ELISA*
  - *Flow Cytometry analysis*

Limitations:

- DGAT-1 was only inhibited using one inhibitor, however, it would be useful to use a second inhibitor or another inhibitor in the same pathway to verify the initial data and to exclude any off-target effects of the inhibitor.
- The authors observe a reduced pro-inflammatory cytokine/lipid mediator production when the DGAT-1 inhibitor A922500 is used. However, it remains unclear whether this is due to reduced cell death or due to direct effects in lipid metabolism.
- The authors show regulation of SREBP-1, PPAR-g and CD36 on monocytes but it remains unclear whether these changes occur in all cell types.
- The authors performed A922500 treatment in inflammatory chemokine and cytokines reduction in *in vitro* infected cells, it would be interesting to investigate were similar effect will be observed from monocytes isolated from COVID-19 patients.
- Fatty acid uptake was measured by the FA transporter, CD36, however it would helpful to confirm the data by any fluorescence fatty acid uptake assays (BODIPY measurement) or lipid level measurement (LipidTOX)
- Further studies are needed to investigate indirect involvement of other host factors in accumulation in lipid droplets, given that glucose exposure and Reactive Oxygen Species (ROS) could play a role in Lipid droplets formation (LucaTirinato *et al* , 2019) and both have been demonstrated to be altered in monocytes and favours SARS-CoV-2 infection (Ana Campos Codo *et al* , 2020)

COVID-19 severity associates with pulmonary redistribution of CD1c+ DC and inflammatory transitional and nonclassical monocytes

Sánchez-Cerrillo, I. *et al*. 2020. *JCI*

Link: https://doi.org/10.1172/JCI140335

Summary:

The authors investigate myeloid cell populations in COVID-19, linking certain monocyte and DC subsets with disease progression. They suggest that increased levels of transitional monocytes in peripheral blood could be a marker of mild disease, while decreases in transitional and non classical monocytes as well as CD1c+ DCs could be associated with severe

disease. The authors further point out potential differences in myeloid cell activation status and a link between increased lung infiltration with inflammatory transitional and non classical monocytes/CD1c+ cDCs and decreased effector CXCR5+CD38+CD8+T cells in the lungs of critical patients.

Research Highlights:

1. COVID19 is associated with reductions in almost all myeloid subsets compared to healthy controls
2. Inflammatory transitional (CD14+CD16+) monocytes are increased in mild patients compared to healthy controls but deceased in severe and critical patients
3. Depletion of classical and transitional monocytes from the blood as observed in severe and critical patients correlates with high plasma levels of inflammatory markers (PCT, CRP, IL-6)
4. Expression of the maturation marker CD40 is decreased in all monocyte subsets in peripheral blood of critical patients
5. In critical patients, transitional and non classical monocytes as well as CD1c+cDCs are enriched in the lungs, expressing higher CD40 than those circulating in the blood
6. High ratios of inflammatory transitional and non classical monocytes /CD1c+ cDCs are negatively associated with proportions of CXCR5+CD38+CD8+T cells in the lungs of critical patients

Impact for COVID-19 research:

- At this point it is unclear whether myeloid dysregulation drives severity or is an effect of superinfection. If levels of myeloid cells are found to be dysregulated early in COVID-19 infection, this could be useful for early detection of severe disease.

Methodologies:

- Study Type*: cross-sectional comparison of healthy individuals, mild, severe and critical COVID-19 patients*
- Key Techniques: *analysis of peripheral blood samples from all participants, bronchoscopy samples from critical patients*

Limitations:

- Samples were not time matched, which is difficult since severe symptoms develop late when mild patients would already be recovering
- Between 80.95% and 100% of critical patients displayed microbial superinfection at the time of sample collection, it would be interesting to know whether changes in myeloid cell populations are due to superinfections or severe COVID-19 alone
- Most patients were on various kinds of treatments that could potentially affect myeloid populations differently
- Lung samples were only taken from critical patients, it would have been interesting to compare lung infiltrates between the different severity groups

Baricitinib restrains the immune dysregulation in severe COVID-19 patients

Bronte, V. *et al*. 2020. *JCI*

Link: https://doi.org/10.1172/JCI141772

Summary:

The authors report on an observational 14-day trial of the selective and reversible JAK1/JAK2 kinase off-label inhibitor Baricitinib (n= 20 patients). Baricitinib treated patients had a lower mortality (5%) than patients who received other treatments (25/56 died) and were weaned off oxygen therapy earlier. In addition, Baricitinib reduced pSTAT3 in circulating immune cells, inflammatory cytokines, and increased circulating IgG/IgA. Baricitinib appears to moderate inflammation in hospitalised COVID-19 patients, supporting another pilot study of 12 COVID-19 patients. This study improves understanding of how Baricitinib treatment alters COVID-19 progression and suggests markers of target engagement which could be used in larger Baricitinib trials, e.g. Eli Lilly's Phase III trial.

Research Highlights:

1. 20 COVID-19 patients received 2x 4mg for 2 days then 1x 4mg for 7 day, 56 COVID-19 controls received other treatments and 6 healthy donors.
2. Baricitinib treatment reduced inflammatory cytokines (IL-6, IL-1β and TNF-α) and C-reactive protein, increased circulating B/T cells, especially CD4+ T Cells, and SARS-CoV-2 spike protein antibodies.
3. Phosphotylated-STAT3 (p-Tyr705) was assessed in 6 Baricitinib treated patients in T cells, NK cells, monocytes and neutrophils leukocyte subsets, and was found to be reduced after 4 days of treatment. P-STAT1 (p-Tyr701) was unchanged in these patients.
4. Monocyte expansion was seen at day 7 irrespective of treatment received.
5. CD14+ monocytes assessed in one Baricitinib treated patient (n=1) were shown to suppress activated T-cells at a higher level in the ICU compared to when they were released from the ICU.

Impact for COVID-19 research:

- This study suggests Baricitinib could be used to treat severe COVID-19 but needs to be validated in a larger cohort.

Methodologies:

- Study Type: Clinical Trial ID (NCT04438629).
- Key Techniques: *ELISAs and Flow Cytometry used to measure various parameters in patient blood/serum.*

Limitations:

- Small number of patients (n=20), and some missing data from patient follow-up, monocyte function only studied in one patient.
- Control group received other treatments (n=56).
- Trial was held over a short timeline (14 days), long term impact of Baricitinib unknown.

Longitudinal analyses reveal immunological misfiring in severe COVID-19

Lucas, C. *et al*. 2020. *Nature*

Link: https://doi.org/10.1038/s41586-020-2588-y

Summary:

Lucas *et al*., present novel insight into features of the dysregulated immune response to SARS-CoV-2 infection. It is newly shown that features of immune responses to pathogens other than viruses occur in COVID-19 disease.

Research Highlights:

1. $CD4^+$ and $CD8^+$ T cells were reduced in association with disease severity, but remaining T cells were activated and T cells in moderate and severe disease produced IFNγ. Increased monocytes, eosinophils and low-density neutrophils correlated with disease severity, although neutrophils did not increase over time. Circulating monocytes had reduced HLA-DR expression.
2. COVID-19 core immune signalling signature: moderate and severe disease had high circulating IL-1a, IL-1b, IL-12p70, IL-17a and IFNα. Further markers specifically associated with severe disease included thrombopoietin (TPO), IL-33, IL-16, IL-21, IL-23, IFNλ, eotaxin, eotaxin 3 and cytokines associated with cytokine release syndrome.
3. Longitudinal correlates: persistence of the 'core signature,' particularly IFNα, IFNλ, and IL-1Ra, 10 or more days from onset of symptoms, occurred in severe patients, whereas they decreased in moderate patients.
4. Although viral titre did not correlate with disease severity, it did correlate with levels of certain signalling molecules: IFNα IFNγ and TRAIL.
5. Patients clustered into 3 groups which had distinct acute immune features, disease severity and outcomes. The clusters were characterised by 4 immune signatures. Signature A included growth factors and wound healing factors. Signature B consisted of a mixture of Types 2 and 3 immunity signalling mediators. Signature C involved a mixed response including type 1, type 2 type 3 immunity. Signature D mainly featured chemokines. Cluster 1 patients experienced moderate disease and limited mortality, which were associated with Signature A. Clusters 2 and 3 patients both experienced more severe disease and increased mortality. Cluster 2 had higher levels of Signature C and D markers (i.e. mixed immune responses and chemokines, respectively). Cluster 3 patients had high levels of type 2 and 3 responses (Signature B) in addition to features of Signatures C and D.

Impact for COVID-19 research:

- This paper presents a novel angle on the variable immunopathology in COVID-19 disease. It suggests that poor control of SARS-CoV-2 infection may occur not only due to dysfunction of the 'appropriate' antiviral immune response but also due to the activation of 'inappropriate' immune responses to other forms of pathogen, or a mix of these and the antiviral response, reducing the efficacy of the latter.
- The longitudinal clustering analysis provides important insight into immune signatures of COVID-19 disease severity, clinical course and outcome. A biomarker window was

described which could inform clinical practice: increased IFNα IFNλ, IL-16, IL-2 and certain chemokines within 12 days of symptom onset predicted longer hospital stays and higher mortality.

Methodologies:

- Study Type: *Longitudinal, Case- control study: COVID-19 patients (n=113; 253 data points over 7 longitudinal collection points) and healthcare workers (n=108 serving as controls.*
- Key Techniques: *SARS-oV-2 infection was detected using the US CDC RT–qPCR primer/probe sets for 2019-nCoV_N1, 2019-nCoV_N2, and the human RNase P (RP) as an extraction control. Cytokines and chemokines were measured using the Human Cytokine Array/Chemokine Array 71-403 Plex Panel (HD71). Flow cytometry cell surface and intracellular analysis antibody information is provided: manufacturer, clone and dilution used. Groups comparisons were made by ANOVA (parametric) and Kruskal-Wallis (non-parametric) statistical tests with Dunn's and Tukey's post hoc tests, respectively. Clustering was performed using the k-means algorithm, correlations analysis by Spearman's coefficient and risk ratio by Poisson regression*.

Limitations:

- Recruitment of healthcare workers as controls which relied on viral testing may not have exclude those who were negative at the time of testing but who previously contracted SARS-CoV-2, which could also be more likely due to their occupation.

Clinical Biomarkers

Laboratory biomarkers associated with COVID-19 severity and management.

Keddie, S. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.18.20168807

Summary:

S Keddie *et al*. study which englobes 100 patients of COVID-19 investigates the routine laboratory test and cytokines implicated in the disease for their potential application as biomarkers of disease severity, respiratory failure and need of higher-level care. Demonstrating the importance of IL-6, CRP, IL-10, LDH and TNF-α as biomarkers in different aspects of COVID-19, supporting other studies previously done.

Research Highlights:

1. IL-6 and CRP were most significantly indicative of the level of respiratory support
2. IL-1β is the only biomarker which significantly differentiated by gender, with higher levels in males
3. IL-1β did not correlate with severity measures presence of ARDS or level of respiratory support

Impact for COVID-19 research:

- This study consists in brief cohort of different biomarkers that could help to classify patient according to the severity of illness. Although it supports other previous studies.

Methodologies:

- Study Type: *cohort study.*
- Key Techniques: *four-PLEX cytokine ELISA-based electrochemoluminiscent immunoassay to measure IL-6, IL-1β, IL-10 and TNF-α.*

Limitations:

- The number of patient is 100 of which only 86% was confirmed by PCR. The rest were considered based on internationally recognized diagnostic criteria.
- The only technique used in the study to check the levels of biomarkers is an ELISA. Although it is efficient might have been worth it to also corroborate by using other techniques

T-cells

Impaired cytotoxic CD8+ T cell response in elderly COVID-19 patients

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

Link: https://doi.org/10.1101/2020.08.21.262329

Summary:

Many previous studies have showed age-related disease severity and mortality outcomes in COVID-19 disease, which appear to be associated with dysfunctional T cell responses. This study shows that the cytotoxic T cell response in COVID-19 is mediated by $CD8^+$ T cells, with negligible contribution from $CD4^+$ T cells. However, the cytotoxic response is impaired in older patients, characterised by depletion of $CD8^+$ T cells, lack of expansion of effector phenotypes and no increase in the production of cytotoxic effector molecules. Since these data were obtained a mild cohort, further data on cytotoxic responses in severe COVID-19 is warranted.

Research Highlights:

1. $CD4^+$ T cells were reduced compared to healthy controls but did not show an age-related decrease in COVID-19 patients. Contrastingly, $CD8^+$ T cells were reduced compared to healthy controls in both age groups, but also in patients aged >80 years compared to younger COVID-19 patients.
2. Unsurprisingly, controls aged 80-96 had higher frequencies of terminally differentiated effector (TEM) and lower frequencies of naïve $CD8^+$ T cells than controls aged <80 years. However, proportions of these phenotypes were not altered in COVID-19 patients aged 80-96, nor did the production of cytotoxic effector molecules increase.
3. Patients aged <80 demonstrated an ongoing $CD8^+$ T cell response, characterised by increased TEM and effector memory (EM) and slightly reduced naïve $CD8^+$ T cell frequencies compared to age-matched controls. $CD8^+$ T cells in this cohort produced significantly more cytotoxic effector molecules (granzymes A and B and perforin) compared to age matched controls.
4. PD-1 expressing $CD8^+$ T cells in patients of all ages produced granzyme A, although only patients aged 29-79 produced significantly more granzyme B and perforin than age-matched controls.
5. The majority of TEM and EM $CD8^+$ T cells in patients of all ages produced at least two of granzymes A, B or perforin; it is unknown whether polyfunctional cytotoxic effector molecule production is protective or contributes to immunopathology.

Impact for COVID-19 research:

- The impairment of cytotoxic T cell responses in elderly COVID-19 patients is likely to contribute to poorer infection control and greater disease severity in association with age. However, more in-depth characterisation of the T cells in association with differing disease severities/ trajectories is required to support these conclusions.

Methodologies:

- Study Type: Age matched case-control study of acute mild COVID-19 disease.
- Key Techniques: *PBMC directly isolated from patient blood and analysed by flow cytometry without ex-vivo stimulation. Clone and manufacturer information for the antibodies used are provided.*

Limitations:

- Relatively small study (n=30). Cohort numbers could be more clearly described in figures and supplementary data is missing.
- Since the study included only mild cases, it is not possible to correlate the reduced cytotoxic response in the elderly patients to disease severity.
- Phenotyping of the T cells was relatively limited; by comparison other studies have highlighted increased frequencies of $CD4^+$ or $CD8^+$ T cells expressing activation markers HLA-DR and CD38 (Mathew *et al*., 2020, Kuri-Cervantes *et al*., 2020) and cytokines e.g. IFNγ (Chen *et al*., 2020).

The analysis of the long-term impact of SARS-CoV-2 on the cellular immune system in individuals recovering from COVID-19 reveals a profound NKT cell impairment

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

Link: https://doi.org/10.1101/2020.08.21.20179358

Summary:

Liu *et al.* performed a phenotypic study of peripheral blood mononuclear cells in convalescent patients with mild/moderate COVID-19. They utilized samples from two cohorts, a Chinese cohort (n=30 convalescent individuals (CI) and n=26 matched uninfected individuals (UI)) and a German cohort for invariant NKT analysis (n=16 CI and n=6 UI) at 3.5 months and 1.5 months respectively after initial diagnosis. They report reduced frequencies of invariant NKT and NKT-like cells in CI relative to UI, likely due to increased cell death in the recovery phase, and an expansion of DCs in CI. Analysis of the T cell compartment showed an increase in the frequency of regulatory T cells, higher expression of TIM-3 on CD4 and CD8 T cells, lower GzmB expression with a slightly enhanced proliferation status in CI and intact effector function to TCR stimulation. DCs and B cells showed increased expression of costimulatory molecules, suggesting a slightly enhanced activation status. Together these data suggest that SARS-Cov-2 infection elicits a sustained impact on immune cell composition in the extended convalescent phase and an ongoing restoration of immune homeostasis.

Research Highlights:

1. A decline in invariant NKT and NKT-like cells in convalescent COVID compared to controls.
2. Increased proportion NKT-like cells of convalescent COVID that are undergoing apoptosis and/or necroptosis even after recovery from COVID-19
3. Long-term suppression of cytotoxic capacity, as based on GzmB production, of T and NKT-like cells after resolving from SARS-CoV-2 infection.
4. No differences in PD-1 expression but 20-30% increase of other exhaustion markers as Tim-3 and TOX in both CD4 and CD8 T cell populations.
5. General effector functions maintained, with enhanced activation and proliferation of T cells, suggesting ongoing restoration of the immune homeostasis.

Impact for COVID-19 research:

- Understand long-term of SARS-CoV-2 infection in cellular immune cells

Methodologies:

- Study Type: *in vitro and ex vivo*
- Important cell lines/viral models used: *PBMCs from SARS-CoV-2 patients and healthy donors. Viral model was natural infection of SARS-CoV-2.*
- Key Techniques: *flow cytometry analysis.*

Limitations:

- Phenotypic analysis is limited and it is not integrated with antibody levels, clinical phenotypes (presence of co-morbidities), length of recovery and/or persistent symptoms in the extended convalescent phase. As such only limited conclusions can be reached from this study.
- iNKT cells were studied from a different demographically distinct cohort (German cohort) with more recent COVID-19 recovery and what looks like milder disease on the basis of the limited information provided. Further studies are needed to corroborate these findings and investigate whether a potential redistribution of iNKT cells could contribute to the reduced frequencies observed in the peripheral blood.
- The authors performed *ex-vivo* analysis based on GzmB, as a marker of cytotoxicity. A wider evaluation of functional responses/cytotoxicity of NKT-cells in response to innate cytokine stimulation and iNKT cells in responding to α-GalCer stimulation would strengthen these observations.
- The study analysed T cell responses and their proliferating capacity to TCR stimulation. However, this is limited to n=5 CI and n=5 UI and shows a wide range of responses particularly for IL-2 and TNFα production in the CI group. It is unclear how the donors were selected for the functional study i.e. disease severity and how the functional data relate to phenotype and transcription profile.
- Although not a limitation per se, it would be of great interest to examine responses to SARS-CoV-2 antigens and analyse virus-specific populations.

Antibodies and Convalescent Plasma

Decline of SARS-CoV-2 specific IgG, IgM and IgA in convalescent COVID-19 patients within 100 days after hospital discharge

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

Link: https://doi.org/10.1101/2020.08.17.20175950

Summary:

Ma *et al*. measured the levels of IgG, IgM and IgA against SARS-CoV-2 RBD for 27 COVID-19 convalescent patients (1 critically ill, 4 severe, 20 moderate and 2 mild patients) on the day of discharge from hospital and on a voluntary revisit 28-99 days (median 91 days) later. A significant reduction in RBD-specific antibody levels was observed overall, and patients with low levels of antibodies at hospital discharge remained low at revisit. Finally, an exponential decay model of antibody kinetics following infection was used to predict the number of days post hospital discharge when RBD-specific antibodies would reach undetectable levels.

Research Highlights:

1. Observed that after 90 days post discharge from hospital, symptomatic patients had a significant decrease in RBD-specific IgG, IgM and IgA.
2. Predicted RBD-specific IgG to become seronegative 273 days, IgM 150 days and IgA 108 days post hospital discharge.
3. Observed that symptomatic patients being discharged from hospital with low RBD-specific antibody levels, remained low at revisit.

Impact for COVID-19 research:

- Observed that symptomatic patients 90 days after discharge from hospital had a significant decrease in RBD-specific antibody levels which may have an impact on long-term immunity for infected individuals

Methodologies:

- Study Type: *Cohort study*
- Important cell lines/viral models used: *SARS-CoV-2*
- Key Techniques: *PCR, previously established chemiluminescence kit detecting RBD-specific antibodies*

Limitations:

- Small number of patients.
- Number of repeats for each sample is not mentioned or was not carried out.
- Would have been interesting to have investigated antibody responses to the whole spike protein of SARS-CoV-2 rather than just the RBD.

- Would have been interesting to have measured the antibody responses at additional time points (the majority were at >85 days post hospital discharge with a few at 29-38 days), for example at 50-70 days or a later time point than 100 days – this might also increase the accuracy of their antibody seronegative predictions.
- Would have been informative to have also examined cellular immune responses in the patients.

Convalescent plasma for patients with severe COVID-19: a matched cohort study

Rogers, R. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.08.18.20177402

Summary:

In this paper Rogers *et al*. investigate the efficacy of convalescent plasma (CP) therapy for COVID-19 patients. 64 patients who received CP were compared to 177 patients that previously received standard of care in the same centre. The control patients were chosen for similar age, sex, pre-existing conditions and COVID diagnosis. The study did not show any differences in the primary and secondary outcomes, there was no impact of CP on all cause in-hospital mortality or in the length of the hospitalization. 3 patients (2.8%) experienced adverse events. CP treatment showed improvement in time to hospital discharge in a subgroup of patients over 65.

Research Highlights:

1. CP does not improve all cause in-hospital mortality
2. CP does not improve length of the hospitalization
3. Patients experienced an unusually high number of adverse events

Impact for COVID-19 research:

- This study shows that CP treatment (just approved in the USA for emergency use) does not improve COVID-19 outcomes for hospitalized patients
- Bigger studies are needed to see if the trend towards benefit seen especially in older patients can be repeated or if it is a result of the limited number of patients in the study

Methodologies:

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

Limitations: The study addresses its own limitation in an extensive way. These limitations are:

- Limited number of patients.

- The control cohort was not collected at the same time as the treated cohort. Standard of care for COVID-19 treatment has shifted between the two cohorts.
- The antibody title of the CP given to the patients was not measured in advance. Some of the patients received suboptimal amounts of antibody, this could have impacted the efficacy of the treatment
- The patients experienced a greater than expected number of adverse effects. It is difficult in the case of severe COVID-19 patients to determine the cause of the adverse effect and some could have been attributed to the treatment in error.
- The study highlights how the treatment could be effective in older patients, but the number of patients in this subgroup is too small to draw definitive conclusions

Limited window for donation of convalescent plasma with high live-virus neutralizing antibodies for COVID-19 immunotherapy

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

Link: https://doi.org/10.1101/2020.08.21.261909

Summary:

Convalescent plasma is used as immunotherapy for COVID-19 patients, however, the optimal timeframe for donation is currently unknown. 175 COVID-19 plasma donors were found to have robust IgM and IgG, and potent neutralisation responses to SARS-CoV-2 100 days post-symptom onset (DPO). Virus neutralisation capacity began to decline 60 DPO. Higher antibody titres were found in sera of those who were >30 years of age and had more severe disease, indicating this population as optimal donors for convalescent plasma. These data aid in understanding the persistence of serological responses to SARS-CoV-2, for immunotherapy in COVID-19 patients.

Research Highlights

1. 2.3% of 175 convalescent plasma donors had undetectable levels of IgM/IgG to the RBD or ectodomain of the S protein. Whilst, 25.4% had undetectable virus neutralisation titres.
2. IgG and IgM titres were detectable until 140 DPO and peaked at 30 DPO. IgG levels were consistently higher, with IgM declining 60 DPO.
3. 80% of individuals had a virus neutralisation titre >160 (the FDA-recommended value for use in COVID-19 convalescent plasma therapy), until 60 DPO.
4. RBD antibodies titres >1350 in early infection (1-30 DPO), were a promising marker for virus neutralisation titres >160. This may help in identifying good convalescent plasma donors.
5. Only 8.3% of individuals had an increase in virus neutralisation capacity after the first blood donation, highlighting the importance of fast screening for donors.

6. Those with more severe disease had higher antibody titres, as did those over 30 years of age. IgM titres also persisted longer in these groups.

Impact for COVID-19 research:

- Understanding serological responses to COVID-19 and optimal donor characteristics enables clinicians to improve convalescent plasma therapy.

Methodologies:

- Study Type: *Cohort study.*
- Important cell lines/viral models used: *Vero E6 cells for virus neutralisation assay.*
- Key Techniques: *Virus neutralisation assay, recombinant S ectodomain and RBD ELISAs for IgG and IgM.*

Limitations:

- No serum IgA analysis.

The immunodominant and neutralization linear epitopes for SARS-CoV-2

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

Link: https://doi.org/10.1101/2020.08.27.267716

Summary:

Through stimulation of three-dimensional structures of SARS-CoV-2 Spike (S), Membrane (M), Envelope (E) and Nucleocapsid (N) proteins, Lu *et al*. were able to predict 33 surface-accessible epitopes; the majority of which induced antibody production in immunised mice. Immunodominant epitopes that induced neutralising antibodies mostly differed in sera of domestic (China) and European sera, which is likely the result of S/N mutations (e.g. D614G). However, as 24/33 share >80% sequence homology to SARS-CoV and bat RaTG13 strains, there is potential that such epitopes may be useful to guide pan-coronavirus vaccine development.

Research Highlights:

1. 33 exposed B-cell epitopes identified from stimulation of 3D structures of SARS-CoV proteins induced antibody responses against its own epitope peptide in immunised/boosted mice.
2. Of all epitopes, only N152-170 was immunodominant in both domestic and European samples. Gene sequencing of SARS-CoV-2 genes in both cohorts highlighted mutations between the two: D614G in S and K203R204/G189R203G204/R203G204/R203G204S344 in N.

3. 8 epitopes induced antibody responses in mice that could inhibit both SARS-CoV-2 D614 and G614 pseudovirus infection. A further 3 could prevent D614 infection only while 9 could inhibit G614 entry.
4. Most neutralising epitopes were spatially clustered near the N-terminal domain, receptor-binding domain or S2' cleavage site.

Impact for COVID-19 research:

- Majority of their findings with particular epitopes are supportive of other studies.
- Epitopes which can induce neutralising antibody responses to SARS-CoV-2 irrespective of mutations will be of great importance for global vaccine design, particularly as there are mutational differences between populations.

Methodologies:

- Study Type: *in silico, in vitro, in vivo*
- Important cell lines/viral models used: *ACE2-293T cells, D614 and G614 SARS-CoV-2 pseudoviruses*
- Key Techniques: *HBC-S-peptide VLP vaccine design, ELISA, Pseudovirus neutralisation assay, prime-boost regimen for BALB/c mice.*

Limitations:

- Few samples used for the domestic and imported (European) samples (n=20 total). In particular, results determining the immunodominant epitopes binding antibodies in sera have large range which may skew averages.
- Refer to a Table 1 in text which was not in report.

Genetic Variants and Severity

Presence of Genetic Variants Among Young Men With Severe COVID-19

van der Made, C.I. *et al*. 2020. *JAMA*

Link: https://doi.org/10.1001/jama.2020.13719

Summary:

The authors investigate genomic variations in 2 pairs of young (20-35) brothers suffering from severe COVID-19. They identify a loss of function mutation affecting X-chromosomal TLR7 in one set of brothers that is heterozygous in the carrier mother, and a missense variant in the same gene predicted as deleterious in the second set of brothers. Upon PBMC imiquimod stimulation TLR7 expression is upregulated in healthy controls but not in the patients. TLR7 downstream signalling is impaired, as is IFN-γ production in response to imiquimod but not *Candida albicans*. The authors suggest a vital role for TLR7 in the defence against SARS-CoV-2 and a potential explanation for higher risk of severe disease in males.

Research Highlights:

1. 4 men from 2 different families with severe COVID-19 show loss of function mutations in TLR7
2. Both rare TLR7 variants (loss of function and missense mutation) result in defective upregulation of type I IFN–related genes in the TLR7 pathway in response to imiquimod and lack of IFN-γ production in response to imiquimod but not *Candida albicans*

Impact for COVID-19 research:

- TLR7 variability could help in the identification of patients at high risk for severe COVID-19 although loss of function TLR7 varients are rare across the general population
- X chromosome linked expression of TLR7 could explain higher levels of basal TLR7 expression in females and higher risk of severe COVID-19 in males

Methodologies:

- *In silico*, case series, *in vitro*
- Key Techniques: *Rapid whole exome genome sequencing, peripheral mononuclear blood cell isolation and exposure to imiquimod and heat killed Candida albicans yeast*

Limitations:

- Very small number of subjects studied, index cases were only compared to parents of family 1 which seemed to have lower TLR7, IRF7, ISG15 and IFNB1 expression than healthy controls. Would have been interesting to also see comparisons to parents from family 2.
- In the stimulation of PBMCs with *Candida albicans* only 1 female and 1 male control were used

Virology

The SARS-CoV-2 Envelope and Membrane proteins modulate maturation and retention of the Spike protein, allowing optimal formation of VLPs in presence of Nucleoprotein

Boson, B. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.24.260901

Summary:

Boson *et al.* characterised how the intracellular trafficking and maturation of the SARS-CoV-2 S protein are regulated by the other structural proteins E, M and N. Co-expression of E or M were found to decrease S cleavage, alter the N-glycosylation patterns in the S2 subunit and to retain S protein in the endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC) or cis-Golgi, known CoV assembly sites. The S protein expressed alone was found widely distributed within the cell and to promote syncytia, which was prevented with SARS-CoV-2 infection or co-expression with E or M. In contrast, N protein does not appear to have any effect on S maturation or localisation. While E protein was found to influence the level of S expression by inducing a non-specific retention of glycoproteins in the ER and to slow down the cell secretory pathway; M influenced S localisation through direct interaction with S C-terminal moiety. Finally, all structural proteins proved essential for VLP formation.

Research Highlights:

1. SARS-CoV-2 E and M proteins alter S cleavage, maturation and intracellular trafficking.
2. SARS-CoV-2 E and M proteins promote S retention at ERGIC or cis-Golgi compartments by different mechanisms and induce furin-mediated processing independently of its mechanisms of intracellular retention.
3. SARS-CoV-2 E protein induces an ER non-specific retention of glycoproteins and slows down the cell secretory pathway
4. M-mediated retention at the ERGIC requires the C-terminal motif in the S cytoplasmic tail.
5. SARS-CoV-2 M, E and N are required for VLP formation.

Impact for COVID-19 research:

- Understand the virology and cell biology of SARS-CoV-2/COVID19

Methodologies:

- Study Type: *in vitro*
  Important cell lines/viral models used: *Homo sapiens codon optimized SARS-CoV-2 S (Wuhan-Hu-1, GenBank: QHD43419.1) was cloned into pVAX1 vector. The delta 19 truncation of S form was generated by site directed mutagenesis introducing a stop*

codon after Cys1254. SARS-CoV-2 E, M and N genes 405 (Wuhan-Hu-1, GenBank: QHD43419.1) were synthesized and cloned into pCDNA3.1(+) vectors; VeroE6, 293T, temperature-dependent folding mutant of VSV-G, Huh-7.5 cells, hepatitis C virus (HCV) p7.

- Key Techniques*: Transfection, Endo-H assessment for intracellular trafficking, western blot, flow cytometry, Immunofluorescence and confocal microscopy, cell-to-cell fusion assay.*

Limitations:

- There is no housekeeping protein in the western blots (GAPDH, beta-tubulin and beta-actin) in Figure 1 and 2.
- In Figure 1, 3B there is no indication of how many times the experiment was performed.
- No other marker for any other cell localisation (as for negative control) is shown in the immunofluorescent studies.