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COVID-19 Community Journal Club

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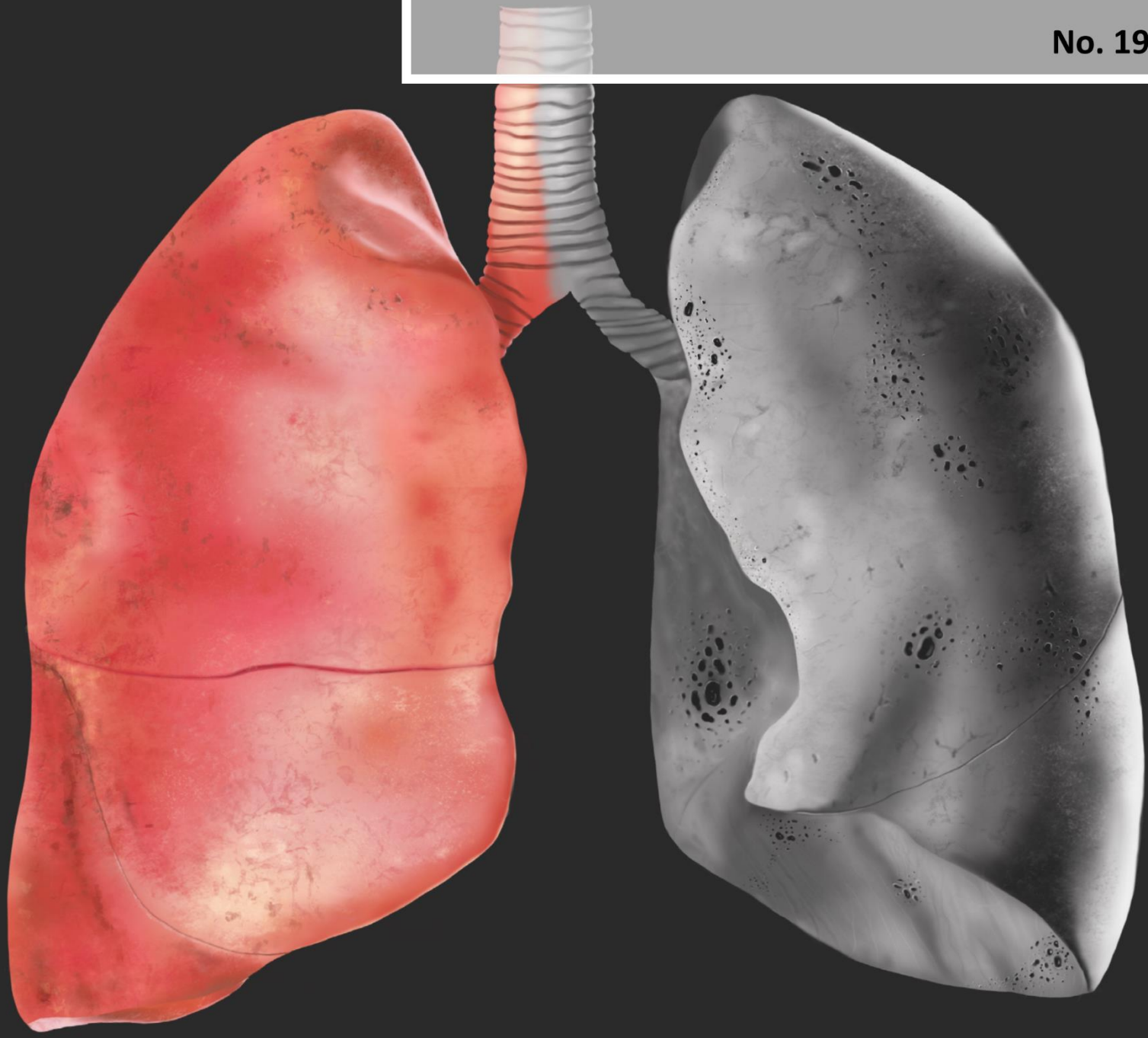

Artwork by *Lucy Chapman*

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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Coronavirus Disease 2019 Induced Inflammatory Response are Associated with Changes in Lipid Profiles **24**

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

Link: https://doi.org/10.21203/rs.3.rs-64766/v1

CAUTIONARY NOTE:

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

Antibodies

Antibody binding to SARS-CoV-2 S glycoprotein correlates with, but does not predict neutralization

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

Link: https://doi.org/10.1101/2020.09.08.287482

Summary:

In this study Shilei Ding *et al*. analyze how neutralization relates to binding of the Spike (S) in the context of viral or pseudoviral particles by a pseudovirus capture assay (VCA) previously developed by this same group and a neutralization assay. As it has been reported, although convalescent plasma therapy as some antibodies targeting S might potentially neutralize viral and pseudoviral particles, there is a proportion that won't. What they demonstrate is that the capacity of antibodies to bind the S glycoprotein at the surface of viral particles is required but not sufficient to mediate neutralization. Here they show the importance of the interaction between antibody and S in neutralization. Thus, it should be taken in consideration in the development of new vaccines.

Research Highlights:

1. Recognition of S glycoproteins at the surface of pseudoviral particles is required but no sufficient to neutralize
2. The decrease in neutralization over time might be due to the disappearance of antibodies able to recognize the S glycoprotein at the surface of viral particles.

Impact for COVID-19 research:

- Although this report do not alter our point of view of Covid-19, it does show how virus neutralization might vary depending on S binding which should be considered when using plasma from convalescent patients as a treatment

Methodologies:

- Study Type: *in vitro.*
- Important cell lines/viral models used: *Plasmids: Spike of SARS-CoV-2/1; pNL4.3 R-E-Luc: VSV-G-encoding plasmid (pSVCMV-IN-VSV-G). Cell lines: Cf2Th cells and 293T-ACE2 cell line*
- Key Techniques: *Virus capture assay to measure the capacity of plasma samples and monoclonal ab. Neutralization assay using sera.*

Limitations:

- Might have been worth it to do a time curse to analyze when ab/plasma lose the ability to bind the S glycoprotein.
- Compare neutralization ability plasma and ab.

SARS-CoV-2 infection severity is linked to superior humoral immunity against the spike

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

Link: https://doi.org/10.1101/2020.09.12.294066

Summary:

Guthmiller *et al.* evaluate plasma from hospitalised acutely SARS-CoV-2 infected and convalescent subjects. The antibody response against SARS-CoV-2 was present in the majority of patients and largely driven against the N (first) and S (later) proteins, with response to other antigens mainly found during acute infection. In both acute and convalescent, the authors correlated disease severity with increased antibody titers. In acute infection, those hospitalised longer mounted a larger antibody response against N protein and were more likely to mount a response against other SARS-CoV-2 antigens, like ORF8 and NSP. Among convalescent, high responders had high titers against N but also ORF8. Memory B cells from convalescent largely targeted the Spike and correlated with antibody titers, but for N. The antibodies against the spike appeared to target conserved epitopes, and to react against spike with the D614G mutation and cross-react to SARS-CoV-1 receptor binding domain. Overall, this preprint shows that disease severity directly correlates with the breadth and magnitude of the humoral immune response.

Research Highlights:

1. Subjects with a more severe SARS-CoV-2 infection exhibit a larger antibody response mainly directed against the spike and nucleocapsid protein, but also ORF8 and NSP.
2. Greater antibody response correlated with a larger memory B cell response against the spike, but not the nucleocapsid protein.
3. Antibodies against the spike can bind the D614G spike mutant and cross-react with the SARS-CoV-1 receptor binding domain

Impact for COVID-19 research:

- Helps understand the humoral immune response

Methodologies:

- Study Type: *clinical samples, ex vivo*
- Important cell lines/viral models used: *Clinical samples*
- Key Techniques: *Bioinformatics, ELISpot, Neutralisation assays, ELISA*

Limitations:

- The neutralizing capacity or the role of the antibodies in the pathogenesis is not addressed, but the authors comment on that.
- A link on the viral titers and the magnitude of the antibody response might have been a complementary and less biased measure than length of hospitalisation. But the authors mention in the discussion that length on hospitalisation may link to viral titers.

- There is no information on whether those hospitalised, all resolved the infection or some died, and in which case if the response was found different.
- It would have been informative to evaluate acute asymptomatic SARS-CoV-2 infected subjects.

Seroprevalence of SARS-CoV-2 in an Asymptomatic US Population

Rigatti, S. *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-80313/v1

Summary:

Rigatti S. *et al*. undertake serological testing of a large cohort of individuals to establish rates of seropositivity to SARS-CoV-2, extrapolating to generate an estimate of the overall prevalence of SARS-CoV-2 antibodies in the United States of America. The calculated seropositivity in the US was 3.8 times higher than the cumulative number of reported cases by the CDC, suggesting higher rates of exposure to SARS-CoV-2 than those indicated by diagnosed SARS-CoV-2 infections.

Research Highlights:

1. 50,255 adults with a median age of 42 years (IQR: 34-54) were tested, of which 56% were male
2. 3.13 % of tested individuals were positive for SARS-CoV-2 antibodies
3. No distinct differences in seropositivity were observed based on history of chronic illness, age or sex
4. Seropositivity from individuals in the state of New York was significantly higher (17.1%) compared to other areas
5. The total burden of SARS-CoV-2 infections in the US according to their estimates was 6.98 million, 3.8 times greater than reported cases by the CDC as of June 1 2020

Impact for COVID-19 research:

- Minimal, but highlights how the cumulative number of infections is likely significantly higher than the reported number of confirmed cases

Methodologies:

- Study Type: *Epidemiological*
- Key Techniques: *Antibody tests were performed using the Roche Elecsys 'Anti-SARS-CoV-2' kit (stated sensitivity of 100% and specificity of 99.8%)*

Limitations:

- Individuals in this study were all tested as part of the process of buying life insurance, skewing the population tested towards healthier and wealthier individuals than

average, introducing a potential bias in the data. The tested population was also skewed to towards young adults. Additionally, the study displayed an imbalanced representation of the US states. Finally, the calculations which they used to estimate seroprevalence across the US are not displayed.

T-cells

Identification and characterization of an immunodominant SARS-CoV-2-specific CD8 T cell response

Gangaev, A. *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-33197/v2

Summary:

Gangaev *et al.* aim to provide a CD8 T cell antigen landscape of the full SARS-CoV-2 genome for 10 prominent HLA-I types. By *in silico* analysis, 50 peptides are selected for each HLA (500 total) and tested against CD8 T cells from 22 severe and critical COVID patients. 9 specific peptides triggered SARS-CoV-2 CD8 T cell responses, 5 unique to SARS-CoV-2 and 4 shared with SARS-CoV-1, none within highly mutable regions. The majority of CD8 T cell responses (11/16) belonged to HLA-A*01:01 and to epitopes derived to ORF1ab, in which the responses were higher in magnitude. TTDPSFGLGRY (TTD) was the immunodominant epitope for HLA-A*01:01 patients. The TTD CD8-specific response was highly dysfunctional, unable to produce cytokines and based on scRNAseq data, with reduced ability to migrate.

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

1. TTDPSFGLGRY is an immunodominant epitope for the subgroup of patients positive for HLA-A*01:01
2. The majority of CD8 T cell responses (11/16) belonged to HLA-A*01:01 and to epitopes derived to ORF1ab, in which the responses were higher in magnitude

Impact for COVID-19 research:

- Description of a new immunodominant epitope for a specific HLA

Methodologies:

- Study Type: *in vitro, cohort study, in silico, ex vivo.*
- Important cell lines/viral models used: *Clinical samples*
- Key Techniques: *pHLA multimers conjugated to fluorescent dyes to assess CD8 reactivity, FACS, scRNAseq.*

Limitations:

- Small cohort with many participants being HLA-A*01:01
- Patients were in treatment or not, which may have influenced the activation state of the cells.
- HLA-A*01:01 type is highly associated with autoimmune diseases but the authors don't comment on that.
- The authors don't specify if they found any response with the other peptides incorporated in the study as previously described in the literature, which would be an informative control for the assay.

- The analysis of the scRNAseq is very limited and the differences between clusters C3-C6 are not well defined. One of the patients contributes substantially more than the others.
- The authors claim that there is no bias in having more peptides from ORF1ab as the contribution is similar to the proteome, but then state that ORF1ab is the least translated ORF of the SARS-CoV-2 genome; which is contradictory.

Drug Repurposing

Distinct mechanisms for TMPRSS2 expression explain organ-specific inhibition of SARS-CoV-2 infection by enzalutamide

Li, F. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.09.11.293035

Summary:

The authors aimed to characterize the antiviral efficacy of enzalutamide, an antiandrogen agent, on transmembrane serine protease 2 (TMPRSS2) expression. TMPRSS2 is a key component in mediating SARS-CoV-2 entry into host cells and has been shown to be reduced in prostate cancer cells following enzalutamide treatment. Enzalutamide efficiently blocked entry of SARS-CoV-2 in prostate cancer cells, however there was no antiviral activity in mouse and human lung epithelial cells duet to independence of TMPRSS2 expression of androgen receptor (AR) in the lung. Therefore, they conclude that enzalutamide is unlikely to exhibit any antiviral effect in COVID-19 treatment.

Research Highlights:

1. AR inhibition can prevent SARS-CoV-2 infection through reduction of TMPRSS2 expression in the prostate
2. Using lung cancer cells as well as organoids showed that TMPRSS2 expression was independent of AR expression in the lung
3. Application of Ad-ACE2 transduced mouse models confirmed in vitro results that enzalutamide did not inhibit SARS-CoV-2 entry into host lung cells
4. Distinct AR binding pattern between prostate and lung cells identified using ATAC-seq and ChIP-seq

Impact for COVID-19 research:

- The drug enzalutamide was proposed as a promising drug reducing the expression of TMPRSS2, however in this study, the authors found an organ-specific regulation of TMPRSS2. This renders expression of TMPRSS2 in the lung to be independent of AR, and therefore effect less for enzalutamide treatment.

Methodologies:

- Study Type: *in vitro, in vivo mouse models*
- Important cell lines/viral models used: *Pseudovirus, full SARS-CoV-2 virus (isolated from clinical case), LNCaP & VCaP (prostate cancer cell lines), A549, H1437 & H2126 (lung cancer cell lines), lung organoids, Ad-ACE2-transduced mouse model*
- Key Techniques: *ATAC-seq, CHiP-seq, immunofluorescence, luciferase assay, qRT-PCR*

Limitations:

- Although AR and TMPRSS2 staining's were implemented in the lung, no colocalization has been applied
- No consideration of human lung microenvironment with stromal cells potentially displaying antiviral activity following enzalutamide treatment

Baricitinib treatment resolves lower airway inflammation and neutrophil recruitment in SARS-CoV-2-infected rhesus macaques

Hoang, T.N. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.09.16.300277

Summary:

Baricitinib is a clinically approved JAK1/2 inhibitor and currently being evaluated in COVID-19 clinical trials. *Hoang et al* investigated the efficiency of Baricitinib in rhesus macaque (RM) model of SARS-CoV-2 infection. Baricitinib was detected in plasma and tissues after treatment but didn't limit viral replication. Similar SARS-CoV-2 RNA levels detected in nasal, throat, or BAL between the baricitinib treated and the untreated group. However, the lung pathology, systemic inflammatory markers and neutrophil infiltration reduced in treated compared with untreated animals. Additionally, RNA-Seq profiling of cells isolated from BAL showed that baricitinib treatment suppressed inflammatory pathways while keeping innate antiviral signaling largely intact. In Blood, lower neutrophils extracellular trap (NET) activity and migration to lung was detected in treated animals, suggesting lower inflammation level. Lastly, baricitinib treatment reduced level of T cell activation (measured by CD38 and HLA-DR expression) without affecting their antiviral responses.

Research Highlights:

1. Baricitinib treatment was well-tolerated without direct evidence of treatment-induced clinical pathology and detectable in plasma and tissues, but did not limit viral replication in SARS-CoV-2 infected RMs
2. Baricitinib reduced lung pathology and inflammation in SARS-CoV-2 infected rhesus
3. Gene signatures of inflammation and neutrophil degranulation reduced in the BAL of treated SARS-CoV-2 infected rhesus macaques compared with untreated group.
4. Baricitinib treatment abolishes inflammatory cytokine and neutrophil chemoattractant expression in bronchoalveolar macrophages of SAR-CoV-2 infected rhesus macaques.
5. Lower frequency of BAL neutrophils and reduced neutrophil extracellular trap (NET) activity in blood were observed in Baricitinib treated RMs.
6. The level of T cell immune activation was reduced in Baricitinib treated SARS-CoV-2 infected rhesus macaques.

Impact for COVID-19 research:

- Understanding virological and immunological parameters associated with clinical outcome of baricitinib treatment.

Methodologies:

- Study Type: *animal study*
- Important cell lines/viral models used: *SARS-CoV-2-infected rhesus macaque*
- Key Techniques: *Quantitative PCR (qPCR), RT-PCR for SARS-CoV-2 quantification from necropsy samples, LC-MS/MS analysis for baricitinib measurement in plasma, tissues, and CFS samples, Histopathologic examination, Immunohistochemical (IHC) staining, Quantitative image analysis, RNAscope in situ hybridization, RNA-sequencing (RNA-seq) analysis, 23-parameter flow cytometric analysis, Bioinformatic analysis for ScRNA-Sep data.*

Limitations:

- As authors pointed, the group size is relatively small (untreated n=4 and treated n=4). Therefore, future studies are needed to confirm the findings in larger group size.
- The model used in the study is moderate SARS-CoV-2 infection with animals aged 11-17 years. While this model allowed longitudinal collection of various samples as pointed by authors, it limits the ability of studying the impact of Baricitinib treatment in more severe infection.
- Although the same dose and route infection was applied between the two groups, limited discussion in the clinical outcomes of RMs following infection between the two groups.
- While the authors showed reduction in neutrophils chemoattractant expression 4 days post infection and 2 days post treatment in BAL samples from treated animals, the flow cytometry data showed an increase in the frequency of neutrophils in BAL 4 and 7 days and the decline was more prominent 10 days after infection in treated animals.
- In this study the treatment was inoculated 2 days following SARS-CoV-2 infection, however it's not clear if the same timing could be applied in human studies as the infection might not be detected until later stage in patients.

Virology

SARS-CoV-2 manipulates the SR-B1-mediated HDL uptake pathway for its entry

Wei, C. *et al*. 2020. *bioRxiv*

Link: https://doi.org/10.1101/2020.08.13.248872

Summary:

Viral entry of SARS-CoV-2 is mediated through receptor recognition of angiotensin-converting enzyme 2 (ACE2). However, eventual co-receptors which could promote viral uptake into cells remain largely unknown. In this preprint, Wei et al. demonstrate that in the presence of high-density lipoprotein (HDL) particles, SARS-CoV-2 shows increased uptake into cells which is dependent on the S1 subunit of the viral spike protein. This uptake is mediated by the HDL receptor SR-B1. Although S1 does not directly bind to SR-B1, the authors find evidence that S1 interacts with the cholesterol and thereby increasing the chances to find ACE2 for viral uptake. Overexpression of SR-B1 in the presence of HDL and ACE2 increases viral replication, conversely transitory inhibition of SR-B1 inhibits viral replication. Inhibition of SR-B1 pharmacologically inhibits viral production and viral entry. It remains to be seen if this pathway is exploited by the virus *in vivo*, especially since cholesterol is significantly lowered in more severe COVID-19.

Research Highlights:

1. SARS-CoV-2 spike protein associates to HDL and cholesterol using cholesterol recognition motifs in the S1 subunit.
2. SR-B1 (HDL uptake receptor) overexpression increases uptake of SARS-CoV-2, while SR-B1 siRNA-mediated downregulation decreases viral infection.
3. SR-B1-enhanced viral uptake requires ACE2 expression since SR-B1 overexpression in the absence of ACE2 does not lead to viral infection.
4. SARS-CoV-2 spike protein does not bind to SR-B1 directly, but rather to cholesterols.
5. Pharmacological inhibition of SR-B1 using ITX5061 reduces SARS-CoV-2 viral entry and replication.

Impact for COVID-19 research:

- This research outlines a potential mechanism through which specific patient groups may have increased risk of severe COVID-19 pathology, such as atherosclerotic patients who have higher SR-B1 expression in arteries than non-atherosclerotic patients.
- These results provide an interesting new candidate for inhibiting viral entry in vivo (but further in vivo validation is required).

Methodologies:

- Study Type: *in vitro*

- Important cell lines/viral models used: *various cell lines (293T, Vero E6, MDCK, Hepa1-6, Huh-7), SARS-CoV-2-pseudovirus (based on HIV backbone) and SARS-CoV-2 (clinical isolate)*
- Key Techniques: *Standard molecular, cellular and virological tools*

Limitations:

- Distinction of viral uptake and viral replication sometimes difficult. Since many viral uptake data is based on expression of luciferase vector which could also arise from differences in viral replication speed due to changed cellular cholesterol uptake. Non-replicating vector may be preferred to answer the question of sole effect on viral uptake.
- Relevance of this finding in the context of in vivo decrease of circulating cholesterol levels would be of interest.
- In vivo data of SR-B1 expression from post-mortem lung tissue as well from animal models are required for determination of biological relevance in disease pathology.
- Since the structure of S1 is known, it would help the manuscript to identify the structure position of the cholesterol binding sites and whether they are also crucial in the context of lipid raft recognition

Inflammation and Immunopathology

Monocytes and macrophages, targets of SARS-CoV-2: the clue for Covid-19 immunoparalysis

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

Link: https://doi.org/10.1101/2020.09.17.300996

Summary:

Clinical outcomes of SARS-CoV-2 infection associated with aberrant activation of immune system. Boumaza *et al.* investigated the impact of SARS-CoV-2 infection on myeloid compartment. *In vitro,* SARS-CoV-2 can infect monocytes and macrophages and stimulate production of pro- and anti-inflammatory cytokines (IL-6, IL-10, and IL-1β and TGF-β). Transient changes in the transcription profile of monocytes was observed following incubation with SARS-CoV-2. SARS-CoV-2 infection induced transient transcriptional changes detected 24h after infection in macrophages with increase in genes associated with M1 and M2 profile. However, macrophages polarization had no significant impact in the viral load. In COVID-19 patients, a shift from activation to immunoregulatory phenotype (measured by HLA-DR and CD163) was observed in patients and was partly recapitulated by incubating control monocytes with SARS-CoV-2, suggesting that SARS-CoV-2 infection induces such an alteration. However, changes in monocytes phenotype was not associated with disease severity.

Research Highlights:

1. SARS-CoV-2 infects monocytes and macrophages and stimulates cytokine release by immune subsets.
2. SARS-CoV-2 induces a specific transient transcriptional program in monocytes and macrophages
3. Early transcriptional program of macrophages showed that SARS-CoV-2 does not induce clear polarization but rather a delayed shift toward a M2-type.
4. The macrophages polarization didn't impact SARS-CoV-2 infection
5. Monocytes phenotype is altered in COVID-19 patients and in monocytes from control after incubation with SARS-CoV-2 with a shift from activation to immuneregulatory phenotype.

Impact for COVID-19 research:

- Understanding impact of SARS-CoV-2 infection on myeloid compartment.
- Monocytes and macrophages manipulation may open the way for therapeutic perspectives.

Methodologies:

- Study Type: *in-vitro study*

- Important cell lines/viral models used: *SARS-CoV-2 strain IHU-MI3, Vero E6 cells and THP cell lines.*
- Key Techniques: *Quantitative PCR (qPCR), In-vitro virus infection, SARS-CoV-2 Viral RNA extraction and q-RTPCR, Immunoassay kits to measure soluble markers, Flow cytometry analysis, Immunofluorescence*

Limitations:

- The decline of monocytes activation measured by HLA-DR has been reported to be associated with disease severity (Brandt D. Pence 2020). The study didn't detect such an affect. The variation in the findings could be explained by study cohort used in both studies.
- The activation status of monocytes was measured by HLADR and CD163 MFI. Comparison in frequency of positive cells will be helpful.

Functional myeloid-derived suppressor cells expand in blood but not airways of COVID-19 patients and predict disease severity

Falck-Jones, S. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.08.20190272

Summary:

The authors compare blood and respiratory samples from patients with different severities of COVID-19 to influenza A and healthy controls to investigate changes in M-MDSC. They report alterations in COVID-19 patients similar to those suffering from influenza including increased plasma frequencies of M-MDSC, decreased T cells and alterations in cytokine levels. Importantly, the authors suggest that an expansion of M-MDSC early in SARS-CoV-2 infection is indicative of increased disease severity and could be used to identify patients with high risk of developing severe disease.

Research Highlights:

1. M-MDSC are increased in blood of influenza and moderate and severe COVID-19 patients compared to healthy controls, with numbers increasing with disease severity
2. M-MDSC are increased in NPA of influenza patients but not NPA or ETA of COVID-19 patients
3. Purified M-MDSC from COVID-19 patients suppress CD4 and CD8 T cell proliferation and IFNγ secretion in an Arg-1 dependent manner *in vitro*
4. Plasma but not NPA levels of IL-6 are increased in influenza and COVID-19, increasing with disease severity in COVID-19
5. Numbers of CD4 and CD8 T cells are decreased in COVID-19 patients, with lower expression of the CD3ζ chain, but no correlation between blood M-MDSC frequency and T cell count is found

6. M-MDSC numbers are associated with age and male gender

Impact for COVID-19 research:

- M-MDSC frequency in the first two weeks from onset of symptoms could potentially predict more severe outcomes of COVID-19

Methodologies:

- Study type: *In vitro*
- Key Techniques: *flow cytometry of PBMCs and cells from nasopharyngeal aspirates (NPA) and endothracheal aspirates (ETA), T cell suppression assay, ELISA*

Limitations:

- The MDSC terminology is controversial, according to flow cytometry gating these cells could also be monocytes
- NPA and ETA do not necessarily reflect what's happening in the lower airways and it would be interesting to see whether these cells are increased deeper in the lungs

The Monocyte Chemotactic Protein 1 as a Potential Indicator for SARS-CoV-2 Infected Mild COVID-19 Patients

Xi, X. *et al*. 2020. *Research Square*

Link: https://doi.org/10.21203/rs.3.rs-71401/v1

Summary:

The authors compare chemokines and their receptors in mild and severe cases of COVID-19 to healthy controls, suggesting that mild (and severe) disease is associated with increased serum levels of MCP-1, but only severe disease is characterized by increased IL-8 and IP-10. They show that mild cases do have elevated PBMC expression of the MCP-1 receptor CCR2 and that MCP-1 upregulation is associated with reduced levels of IRF3 and a reduced IFN-β response.

Research Highlights:

1. Mild (and severe) cases of COVID-19 show higher serum levels of MCP-1 than healthy controls
2. Levels of IP-10 and IL-8 are high in severe but low in mild cases
3. Levels of CCR2 but not CXCR3 and CXCR2 are elevated in PBMC in mild disease compared to healthy controls
4. Levels of IRF3 and IFN-β are reduced in mild disease compared to healthy controls
5. IRF3 downregulation is negatively correlated with MCP-1

Impact for COVID-19 research:

- Low

Methodologies:

- Study type: *In vitro*
- Key Techniques: *ELISA, real-time PCR*

Limitations:

- The authors focus on the difference in serum chemokines between mild COVID-19 and healthy controls. It would be more useful to investigate differences between mild and severe cases.
- Chemokines were only measured in serum, not in lung fluid.
- The authors conclude that MCP-1 may be an effective indicator in mild patients, which is wrong as MCP-1 levels are elevated in severe patients to the same extend.
- The authors conclude that early use of interferon has a good antiviral therapeutic effect which they did not test. A downregulation of IFN-β in mild cases compared to healthy controls does not necessarily mean increasing IFN-β would be beneficial. The difference of IFN-β in mild compared to severe cases was not investigated.

Bacterial pulmonary superinfections are associated with unfavourable outcomes in critically ill COVID-19 patients

Buehler, P.K. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.10.20191882

Summary:

Isolation of relevant respiratory bacteria associated with more severe disease course, prolonged ICU stay and use of ventilators in critically ill COVID-19 patients. Mainly gram-negative pathogens were isolated, in line with previous studies and the authors list the most frequently isolated bacteria and fungi detected. Authors conclude that, based on the superinfections identified, broad-spectrum antimicrobial therapy could be stopped and patients only treated if bacteria are detected, which may reduce the prevalence of drug-resistant bacteria.

Research Highlights:

1. Clinically relevant bacterium or fungus was detected in 42.2% of patients *(higher than previously recorded, although this could be due to strict sample collection schedule and the severely ill patients in the cohort)*
2. 124 relevant microorganisms were detected in tracheobronchial secretions (TBS), but only seven different bacterial species in 12 positive blood culture pairs
3. On average clinically relevant pathogens were detected on day 10 after ICU admission
4. Multidrug resistant bacteria was detected in 22.2% of patients

5. Patients with pulmonary superinfections had substantially lower 28-day ventilator-free survival than those without and they were ventilated for significantly longer, had significantly longer stays in the ICU and increased hospitalization time.

Impact for COVID-19 research:

- Identifying the bacterial strain means it can be treated specifically rather than with broad-spectrum antibiotics, resulting in a reduction of morbidity and mortality in COVID-19 patients

Methodologies:

- Study Type: *Prospective Cohort Study*

Limitations:

- Small number of patients - Total of 45 patients, skewed towards male (77.8%)
- Majority of patients treated with empirical broad-spectrum antibiotic therapy at admission (>90%)
- Investigated only critically ill COVID-19 patients
- Single centre design

Broncho-alveolar inflammation in COVID-19 patients: a correlation with clinical outcome

Pandolfi, L. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.07.17.20155978

Summary:

Pandolfi *et al.* assess and compare the broncho-alveolar inflammatory environment between COVID-19 patients admitted to Intensive Care Unit (ICU) and Intermediate Medicine Ward (IMW) at two main COVID-19 centers of Italy (located in Pavia/Milan). Inflammatory cell and cytokine analysis of bronchoalveolar lavage (BAL) samples suggest that hyperactivation of innate immune effectors, particularly neutrophil infiltration, contribute to COVID-19 associated alveolitis in severe cases. Furthermore, elevated levels of pro-inflammatory cytokines (IL-6 and 8) correlate with alveolar inflammatory status and patient clinical outcome.

Research Highlights:

1. ICU patients showed a significant increase in neutrophils, and relatively lower lymphocyte and macrophage levels, compared to IMW patients. Neutrophil infiltration at alveolar level did not correlate with clinical outcome.
2. BAL collected from COVID-19 ICU patients revealed extensive cytopathic damage in all cell types, including loss of nuclear & plasma membrane integrity, pseudopodia

formation and cytoplasm vacuolization. Presence of viral particles were also observed in cytopathic epithelial and mononuclear cells.

3. Pro-inflammatory IL-6 and IL-8 cytokines were significantly higher in BAL samples derived from ICU compared to IMW COVID-19 patients. Additionally, non-survivors (N=12) had higher IL-6/IL-8 BAL levels compared to survivors (N=16).
4. Patients treated with steroids had lower BAL IL-6 compared to patients on tocilizumab or antivirals (remdesivir, lopinavir/ritonavir or hydroxychloroquine).
5. Direct correlation between BAL IL-6/IL-8 and neutrophil count. In contrast, IL-6/IL-8 levels inversely correlated with percentage of macrophages. BAL IL-8 also inversely correlated with lymphocyte count.

Impact for COVID-19 research:

- Increased IL-6 and IL-8 levels in broncho-alveolar environment of COVID-19 patients correlates with neutrophil infiltration and is associated with clinical outcome. Steroid treatment could reduce IL-6 levels in severe COVID-19 cases, potentially leading to better outcome.

Methodologies:

- Study Type: *Patient cohort*
- Key Techniques: *Assessment of BAL cell differentials, ultrastructural cell analysis and cytokine profiling*

Limitations:

- Small patient cohort (N=33; 28 ICU and 5 IMW). Majority of patients were male.
- Small sample size limited the possibility of additional analysis (e.g. flow cytometric evaluation cell surface activation markers).
- The study is specific to a particular time & place and cannot be generalised.
- Paired assessment of inflammatory cytokines in peripheral blood was not possible due to lack of stored serum samples from acute COVID-19 patients/healthy donors.
- Pre-existing comorbidities may have influenced cellular infiltration and cytokine levels.

Gastrointestinal involvement attenuates COVID-19 severity and mortality

Livanos, A.E. *et al*. 2020. *medRxiv*

Link: https://doi.org/10.1101/2020.09.07.20187666

Summary:

Livanos *et al*. investigated the involvement of gastrointestinal (GI) in SARS-CoV-2 pathogenesis in three large cohorts from United States and Italy. GI symptoms (nausea, vomiting or diarrhoea) were inversely correlated with disease severity and mortality. Patients

who presented with GI symptoms had reduction of developing severe infection with an accompanying decrease in circulating inflammatory cytokines levels (IL-6, IL-8, IL-17 and CCL28). A robust expression of ACE2 receptor was observed in small intestinal enterocytes in both COVID-19 patients and controls, and SARS-CoV-2 viral particles and proteins were detected in small intestinal tissue of patients, suggesting that these cells can harbor SARS-CoV-2 antigens. However, these virions failed to cause cytopathology when co-culture with Vero E6 cells, suggesting a low number of SARS-CoV-2 present in the GI tract. Analyzing immune cell signature of intestinal tissues from COVID-19 patients showed a reduction in pro-inflammatory DCs and pDCs but increase in effector T cells, compared with controls. In addition, the RNA-Seq analysis of lamina propria (LP) and epithelial compartment (EC) showed a transcriptional remodeling of GI tissues by SARS-CoV-2 characterized by downregulation of pathways associated with inflammation and antigen presentation, yet activation of viral response signaling genes in the EC.

Research Highlights:

1. COVID-19 severity was reduced in patients with GI symptoms when compared to those without GI symptoms.
2. Presence of GI symptoms can be used to predict reduced disease severity and mortality 225 in patients with COVID-19
3. COVID-19 patients with GI symptoms have reduced levels of circulating cytokines associated with inflammation and tissue damage.
4. The GI mucosa was endoscopically uninflamed in COVID-19 patients regardless of the severity.
5. Small bowel enterocytes have robust expression of Angiotensin converting enzyme-(ACE2) and harbour SARS-CoV-2 antigens
6. SARS-CoV-2 viral particles and protein are detectable in intestinal tissue of COVID-19 patients, but infectious virions could not be isolated from the GI tissues.
7. Pro-inflammatory dendritic cells are depleted, and pro-inflammatory pathway are downregulated in the GI lamina propria in COVID-19 patients

Impact for COVID-19 research:

- Understand involvement and immunological landscape of GI tissues in attenuation of SARS-CoV-2 pathogenicity.
- For clinical setting, the study suggest that GI symptoms are inversely correlated with severity of SARS-CoV-2 illness and argues in favor that GI symptoms could be used to predict disease severity.

Methodologies:

- Study Type: e.g. in vitro analysis
- Important cell lines/viral models used: PBMCs from SARS-CoV-2 patients and healthy donors. African green monkey kidney epithelial cells (Vero E6) cells.
- Key Techniques: *Drop ELLA Cytokine measurement, Multiplexed proteomic assay, Mass cytometry (CyTOF), Virus isolation and viral infection assays, RNA isolation and RT-qPCR for Viral Determination, RNA-sequencing (RNA-seq) analysis, Integrating and clustering techniques for data analysis*

Limitations:

- As authors pointed, some analysis was performed in later stage of acute infection
- Large variations if the sample collection dates for the GI biopsies with some patients, samples collected during early infection and others during later time-point.
- COVID severity was defined based on internal scoring system of the department.

Coronavirus Disease 2019 Induced Inflammatory Response are Associated with Changes in Lipid Profiles

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

Link: https://doi.org/10.21203/rs.3.rs-64766/v1

Summary:

In this study Chuanwei Li *et al*. evaluate the effects of Covid-19 on the lipoprotein profiles as it has been demonstrated previously that there is an association between changes in lipids and lipoprotein and viral pneumonia. This is the first preliminary study descriptively evaluating the effects of Covid-19 on 242 different patients between 53-69 in Wuhan. This study shows that patients with covid-19 exhibit atherogenic lipid changes which might correlate with exuberant inflammatory responses.

Research Highlights:

1. Marked decrease in serum levels of HDL-c and apoA-I in Covid-19 patients
2. Consistent with the decrease in apoA-I and increase in apoB, the apoB/apoA-1 ratio, which worked as indices for risk of acute myocardial infarction, were notable increased

Impact for COVID-19 research:

- As is it shown in this study, patients with covid-19 show a lipid profile different to healthy patients which could be correlated with exuberant inflammatory responses

Methodologies:

- Study Type: *cohort study.*
- Key Techniques: *Biochemical measurements and lipid profiles including total cholesterol, high density lipoprotein, low density lipoprotein cholesterol, non-high density lipoprotein cholesterol, apoA-1 and apoB.*

Limitations:

- No data recorded after the recovery of patients
- No data regarding the viral load to check whether it exists a correlation between the viral load, cytokine storm and lipid levels.