# COVID-19 Community Journal Club No. 5

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# School of Medicine, Cardiff University

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

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**It's not all about the spike protein……listen to this:**

https://m.facebook.com/probablytomfoolery/videos/925284527912453/

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**Corona virus activates a stem cell mediated defense mechanism that accelerates activation of dormant tuberculosis: implications for the COVID-19 pandemic.** **44**
Lekhika Pathak et al., BioRxiv, May 2020.
**Link:** https://doi.org/10.1101/2020.05.06.077883

# BAME COVID-19 DEATHS – What do we know?

## Sources:

Two related reports with overlapping datasets:

1. Coronavirus (COVID-19) related deaths by ethnic group, England and Wales: 2 March 2020 to 10 April 2020; *Office for National Statistics;* 7th May 2020; Chris White and Vahé Nafilyan
2. BAME COVID-19 DEATHS – What do we know? Rapid data and evidence review: 'Hidden in plain site;' *Centre for Evidence Based Medicine, University of Oxford;* 5th May 2020; Abdul Razaq (lead author) et al.

## Overall summary:

- The mortality rates and incidence of adverse outcomes following SARS-CoV-2 infections are higher amongst BAME (Black, Asian and Minority Ethnic) groups in England and Wales than predicted from the overall population makeup.
- Underlying causes need to be fully explored to inform decisions made by the NHS as both an employer and healthcare provider, as well as to address anxieties and suppress disease transmission and future mortality and morbidity caused by SARS-CoV-2 (Covid-19).

## Methodologies:

1. The ONS analyses include all hospital and community cases in England and Wales between March 2nd and April 10th 2020 where Covid-19 is mentioned on the death certificate. Over 68,000 of these deaths were uniquely linked to the 2011 Census data for stratification by ethnicity with adjustments for socioeconomic status, household composition, physical health, rural urban classification and deprivation.
2. The CEBM report is based on an ongoing meta-analysis of Covid-19 literature obtained via Google Scholar searches, supplemented with pre-publication material and information derived from government websites, multinational agencies and independent Covid-19 resource aggregators. Inclusion criteria are not stated. The report was allotted a 4-day time line for production.

## Key findings:

1. <u>Conclusions from the ONS report include:</u>
    - Mortality linked to SARS-CoV-2 infection in England and Wales affects ethnic groups disproportionately compared with the overall composition of the populations.
    - Adjusting for key socioeconomic factors including age, employment, health, disability, household composition, over-crowding, deprivation and urban location goes some way to redressing the discrepancies, but significant health inequalities remain:
        - The risk of a Covid-19-related death for males and females of Black ethnicity is 1.9 times higher than for those of White ethnicity.
        - Bangladeshi and Pakistani males were 1.8 times more likely to have a Covid-19-related death than White males; for females, the figure was 1.6 times more likely.

    <u>Important limitations include:</u>
    - Increased Covid-19 mortality risk may relate to factors not fully evaluated in these data, including the impact of public-facing occupations (security guards, bus drivers, health and care workers) for different ethnic groupings. Covid-19-related deaths have not been stratified by employment category for assessment of ethnicity as a truly independent variable.

- Comprehensive analysis of co-morbidities, including mental health issues, are also required for improved interpretation of the mortality rates. Importantly, these should include access to healthcare provision and the frequency of medical interventions that could increase viral exposure. Vaccination programs in countries of origin (where not the UK) might also be helpful.
- More detailed dissection of geographical influence would be valuable, rather than just the rural urban classification. Regional issues including population density, access to services, transport networks, international diversity, air pollution and cultural clustering may all differentially contribute to risk of exposure and risk of death.
- Finer stratification of cultural and ethnic groups might expose more subtle variables, given the enormous dataset available for subdivision.
- The authors emphasise the potential for inaccuracy from transposing 2011 Census data onto 2020 Covid-19 mortality rates, as well as the issues associated with evaluating emigration and the absence of data for low numbers of people born after 2011 and deaths with delayed registry.
- There is also some inconsistency in the data available from the different health boards in England and Wales, so additional resolution is necessary.
- Overall, the study has huge statistical power and provides essential insights into the particular contributions of diverse socioeconomic variables (based on unique linkage to Census data) for the Covid-19-associated mortality rates of distinct ethnic populations in the UK.

2. <u>Conclusions from the CEBM report include:</u>
   - BAME groups have higher but differential mortality risk and admission into intensive care units (ICU) from Covid-19 in England.
   - Contributing factors may include: overrepresentation of BAME populations in lower socio-economic groups, multi-family and multi-generational households leading to increased risk of transmission, disproportionate employment in lower band key worker roles who either work in high exposure care environments or are unable to implement safe social distancing due to their roles, co-morbidity exposure risks especially for cardiovascular disease, diabetes, renal conditions and complex multi-morbidities in ICU and increased exposure through employment in health and care settings.
   - The pattern of ICU outcomes by ethnic group broadly reflect the pattern of overall Covid-19 mortality by ethnic groups, suggesting the ICU deaths follows the overall death risk pattern overall for BAME communities.
   - BAME groups account for 21% NHS workers; 20% nursing and support staff and 44% medical staff. Notably, BAME workers account for 63% overall NHS Covid-19 deaths; 64% amongst the nursing and support staff and 95% of medical staff Covid-19 deaths. However, these figures are based on only 116 cases in total, with 34% from London and only 10% from the care sector, so may not be representative of the NHS across the UK.
   - The report provides useful background information concerning the aetiology and pathophysiology of SARS-CoV-2, as well as some interesting discussion of relevant health behaviours, comorbidities and health inequalities. Issues of racism and sources of anxieties for healthcare workers are also considered.

   <u>Important limitations include:</u>
   - Chaotic data collection and presentation - data sources are not always obvious and corresponding amongst sections of the report.

- Addition of international data that requires separate consideration of reporting methodology and contributing variables.
- Over-reliance on a very small dataset of NHS employees suffering Covid-19-related deaths to calculate enhanced risk – limited statistical power and capacity for extrapolation.
- Limited evaluation of adjustments made to initial data reports – accounting for geographical location alone has a very different impact on conclusions compared with dual accommodation of geography and demographics. Additional variables may have similar influence?
- Inclusion criteria for studies not discussed and contributing datasets and reports may not have been peer reviewed or independently-verified. There are key differences between cohorts and subdivisions (e.g. skews in age and sex) that may be important. It is very difficult to draw clear conclusions without proper assessment of data reliability.

For a summary of considerations published in the *Lancet*: see
https://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(20)30922-3.pdf

## Diagnostics, Therapeutics and Virus Engineering

### NIH clinical trial shows Remdesivir accelerates recovery from advanced COVID-19

https://www.nih.gov/news-events/news-releases/nih-clinical-trial-shows-remdesivir-accelerates-recovery-advanced-covid-19

The author reported another a randomized, controlled trial, regarding remdesivir in coronavirus, involving 1063 patients, carried out in US. They found hospitalized patients with advanced COVID-19 and lung involvement who received remdesivir recovered faster than similar patients who received placebo. This report is an initial report, more details will be revealed later.

**Main points:**

- When reviewed the data on 27$^{th}$ April, they noted that remdesivir was better than placebo from the perspective of the primary endpoint, time to recovery, a metric often used in influenza trials.
- Patients who received remdesivir had a 31% faster time to recovery than those who received placebo ($p<0.001$).
- The median time to recovery was 11 days for patients treated with remdesivir compared with 15 days for those who received placebo.
- Mortality rate is 8.0% for the group receiving remdesivir versus 11.6% for the placebo group ($p=0.059$).

**Clinical Impact:**

- Moderate to important.

**Towards the next phase: evaluation of serological assays for diagnostics and exposure assessment**
GeurtsvanKessel *et al*. (2020); medRxiv preprint
**Link**: https://www.medrxiv.org/content/10.1101/2020.04.23.20077156v2

GeurtsvanKessel et al (2020) et al. provide a comprehensive comparison of a selection of SARS-CoV-2 serological assays. Out of the 250+ immunoassays commercially available, they chose to evaluate assays that (1) target SARS-CoV-2 spike protein, particularly the receptor-binding domain (RBD), (2) had received European Conformity (CE) or equivalent marking, and (3) had necessary large-scale production capacity. PRNT50 was used as a reference. Important insights from this study support decisions of using RBD-based serological assays for diagnostics and assessing population exposure.

**Main findings:**
- Testing for cross-reactivity is vital for validating SARS-CoV-2 serological analysis (as presence of antibodies targeting other coronaviruses could impair specificity).
- Although *Wantai* assay (that uses RBD as coating antigen) is highly sensitive for detecting total immunoglobulins (Ig), it does not display good quantitative relationship, especially at high levels of neutralising antibodies.
- *DiaSorin Liaison* (IgG) and *Euroimmun* (IgG/A) assays are preferable for population screening as they measure long-lived IgG and/or memory IgA antibodies, providing good quantitative relationship between antibody measurement and protection.
- *Orient gene* rapid antibody test showed high specificity that could be useful for population screening in laboratory settings.
- Overall, IgM and IgG rapid diagnostic tests typically provide binary qualitative (yes/no) results, increasing risks of people interpreting positive test as protection.

**Highlights:**
- RBD-based serological assays are optimal for both patient diagnostics and population screening.
- Rapid antibody tests are not robust enough for personalised home-based testing.

**Clinical Impact:** Medium-High

**Important methodologies:** Evaluated specificity and performance of various commercial serological assays available for detecting SARS-CoV-2 antibodies.

**Limitations:**
- Excluded assays that did not target SARS-CoV-2 spike protein.
- Did not include samples from patients with subclinical disease during evaluation.
- Long-term follow-up of Covid-19 patients is required to improve understanding of relationship between antibody measurement and protection.
- Need to include evaluation of multiplexed serological immunoassays that can distinguish between different Ig isotypes present in a single serum sample.

**Test performance evaluation of SARS-CoV-2 serological assays**
Whitman *et al*. (MedRiXV) 2020.
**Link:** https://doi.org/10.1101/2020.04.25.20074856

Whitman et al. describes test performance for 12 COVID-19 serology assays using a panel of 130 samples from 80 individuals with PCR-confirmed SARS-CoV-2 infection and 108 pre-COVID19 specimens. Each test quantified IgM and/or IgG antibodies by time period from onset of symptoms and assessed specificity and cross-reactivity. These data be useful to inform the medical community, public health, and government in planning for SARS-CoV-2 serological testing. The study will also provide feedback to manufacturers about necessary improvements to existing tests.

**Main findings:**
- The number of seropositive cases within the SARS-CoV-2 PCR positive samples increased with time - 81.8-100% samples were positive >20 days after the onset of symptoms.
- Higher positivity in LFA tests in patients admitted to ICU.
- Test specificity ranged form 84.3-100% in COVID negative samples – antibodies were detected in 5 specimens in >3 tests.
- IgM detection was more variable than IgG and detection was optimal when IgM and IgG results were combined.
- No consistent cross-reactivity was observed in tests.

**Highlights**
- Majority of tests evaluated were able to detect SARS-CoV-2 positive samples with high specificity and sensitivity.
- Data will inform the medical community, public health, and governmental institutions in planning for SARS-CoV-2 serological testing.
- Lastest COVID-19 testing updates on http://covidtestingproject.org

**Clinical Impact:**
- Highlight the importance of taking caution in ruling out COVID-19 infections solely based on a negative molecular test and that serological assays will provide a beneficial supplementary diagnostic tool when used at optimal time points.

**Important Methodologies:**
- Performed 10 Lateral flow and 2 ELISAs to detect SARS-CoV-2 antibodies using serum samples from confirmed COVID-19 positive individuals, pre-COVID-19 negative controls and samples from patients with other respiratory diagnoses to look at cross reactivity.

**Limitations:**
- No standardised samples available for comparisons/specific readings.
- Limited sample materials – some sample exhausted and therefore not run for all tests.

**A human monoclonal antibody blocking SARS-CoV-2 infection**
Wang *et al*. Nature Communications
**Link**: https://www.nature.com/articles/s41467-020-16256-y

Wang *et al*. have identified a human monoclonal antibody (47D11) that neutralises SARS-CoV-2 *in vitro*. The antibody can also neutralise the SARS-CoV virus. 47D11 engages the receptor binding domain of SARS-CoV-2 but does not block the interaction with ACE2 on the cell surface. The authors state that 47D11 could be used diagnostically in antigen detection tests or serological assays, or potentially to prevent and/or treat COVID-19.

**Highlights**
1. Identified a human monoclonal antibody, 47D11, that neutralises SARS-CoV-2 and SARS-CoV *in vitro*
2. Antibody targets a conserved communal epitope on the receptor binding domain of both SARS-CoV and SARS-CoV-2
3. Antibody does not neutralise the virus through blocking interaction with ACE2, but by an unknow mechanism

**Clinical Impact?**

**High** – Wang et al have identified a human monoclonal antibody that neutralises SARS-CoV-2. This could be used to develop antigen detection tests and serological assays. This antibody also could be used to prevent and/or treat COVID-19 potentially in tandem with other treatments. Moreover, this antibody neutralises the virus through binding to a conserved region, therefore, this antibody could provide protection from other viruses of the same family.

**Important Methodologies?**

- Antibody identified using ELISA-(cross) reactivity assays using supernatants from 51 SARS-S hybridomas from immunized H2L2 mice
- Murine antibody was reformatted to a fully human monoclonal antibody by cloning of human variable heavy and light chains into a human IgG1 isotype backbone – generating human mAb 47D11
- Binding kinetics of 47D11 assessed using biolayer interferometry
- Interaction of 47D11 and receptor binding domain assessed using a flow cytometry based receptor binding inhibition assay

**Limitations?**

- Authors did not find the mechanism by which 47D11 mAb neutralised SARS-CoV-2
- Cell culture data only, study would benefit from some *in vivo* data

**Structural Basis for Potent Neutralization of Betacoronaviruses by Single-domain Camelid Antibodies**
Wrapp *et al*. (Cell), 2020
**Link**: https://www.cell.com/pb-assets/products/coronavirus/CELL_CELL-D-20-00891.pdf

Wrapp et al. isolated single domain antibodies (VHHs) from a llama immunized with prefusion-stabilized coronavirus spikes which were able to neutralize MERS-CoV and SARS-CoV-1 Spike (S) pseudotyped viruses. Crystal structures of VHHs in complex with viral epitopes were resolved and found to occlude the receptor binding interface (RBD). SARS-CoV-1 RBD-directed VHH found to cross-react with the SARS-CoV-2 RBD and can block the receptor-binding interface. As a result, VHH engineered into a bivalent human IgG1 Fc-fusion and found that it can be produced at high yields in an industry-standard CHO cell system for potential use as a therapeutic.

**Main findings:**

- Immunized a llama subcutaneously with SARS-CoV-1 S protein and with MERS-CoV S protein
- Isolated clones had no cross-reactivity of MERS VHHs with SARS CoV-1 S and vice versa
- Performed in vitro neutralization assays using MERS-CoV England1 S and SARS-CoV-1 Urbani S pseudotyped lentiviruses
- High affinity MERS VHH-55, -12, -34 and -40 neutralized MERS-CoV S pseudotyped virus with $IC_{50}$ values ranging from 0.014 to 2.9 µg/mL (0.9 nM to 193.3 nM)
- SARS VHH-72 and -44 neutralized lentiviruses pseudotyped with SARS-CoV-1 S with $IC_{50}$ values of 0.14 (9 nM) and 5.5 µg/mL (355 nM)
- Tested binding to recombinant MERS-CoV S1, RBD, and N-terminal domain (NTD) and SARS-CoV-1 RBD and NTD by ELISA
- SARS VHH-72 and MERS VHH-55 127 target the RBD and not NTD
- Affinities of these VHHs determined by immobilizing recombinantly expressed VHH to an SPR sensorchip and determined the binding kinetics for their respective RBDs
- VHH-72 (1.2nM) and VHH-55 (79.2 pM)
- Resolved crystal structure of MERS VHH-55 bound to the MERS-CoV RBD (3.4Å)
- CDR3 of MERS VHH-55 is looped over the functional host receptor dipeptidyl peptidase 4 (DPP4) -binding interface, occluding DPP4 from productively engaging the MERS-CoV RBD
- RBD residue Arg542 has a critical role in MERS VHH-55 binding
- Crystal structure of binding between SARS VHH-72 and the SARS-CoV-1 RBD resolved (2.2 Å)
- ACE2 found to clash with the CDR-distal framework of SARS VHH-72, as opposed to classical receptor-blocking in which the CDRs would occupy the ACE2 binding interface
- ACE2 also carries an N-glycan modification at position Asn322 and when bound to the RBD, this N-glycan points into the space that is occupied by SARS VHH-72, forming even larger clash
- SARS VHH-72 binds to the SARS-CoV-1 RBD through a hydrogen bond network involving CDRs 2 and 3, in which backbone groups participate extensively (accounts for high affinity observed)

- Equilibrium dissociation constant of SARS VHH-72 for the SARS-CoV-2 RBD-SD1 was ~39 nM, substantially higher than for the SARS-CoV-1 RBD
- This is likely the result of variant residue Asn-439 in SARS-CoV-2 RBD which prevents formation of a salt bridge with Asp61 from SARS VHH-72
- Binding to RBDs for both VHH-72 and VHH-55 confirmed by Biolayer interferometry
- However high off-rate constant of the monovalent SARS VHH-72 for SARS CoV-2 RBD was found
- Engineered genetic fusion of SARS VHH-72 to the Fc domain of human IgG1 and purified from ExpiCHO cells
- Using SARS-CoV-2 S pseudotyped VSV with a luciferase reporter, VHH-72-FC led to neutralization with an $IC_{50}$ of approximately 0.2 ng/mL
- VHH-72-Fc construct reached expression levels of 300 mg/L in ExpiCHO cells

**Highlights**

- Crystal structures of these newly isolated VHHs in complex with their respective viral targets were resolved and binding mode examined
- Inherent thermostability and chemostability of VHHs may provide useful for nebulization and direct delivery via inhalation

**Clinical Impact:**

- Limited

**Important Methodologies:**

- Biolayer interferometry
- CoV pseudovirus neutralization
- Surface plasmon resonance
- ELISA

**Limitations:**

- No pre-clinical *in-vivo* models used to determine efficacy of VHH as a potential therapeutic
- SARS-CoV-2 S immunization not performed and may deliver more potent VHHs

**Alpha 1 antitrypsin is an inhibitor of the SARS-CoV-2 priming protease TMPRSS2**
Nurit P. Azouz et al. (bioRxiv), 2020
**Link**: https://doi.org/10.1101/2020.05.04.077826. #Sec3

Nurit P. Azouz et al. developed an experimental framework using HEK-293T cell line for quantifying TMPRSS2 proteolytic activity. Camostat mesylate, bromhexine hydrochloride (BHH), aminomethyl)benzenesulfonyl fluoride (AEBSF) and alpha 1 antitrypsin (A1AT) inhibit TMPRSS2 proteolytic function *in vitro*. The effects of BHH, AEBSF and A1AT are dose

dependent. It suggests these protease inhibitors may be potential antiviral agents for further COVID-19 treatment investigations.

**Main findings:**

- TMPRSS2 overexpression in HEK-293T – a human cell line results in overproduction of functional TMPRSS2. This framework allows to accurately measure TMPRSS2 proteolytic activity.
- Camostat mesylate inhibits TMPRSS2 proteolytic activity at the lowest concentration of 100 nM.
- A1AT and AEBSF inhibit TMPRSS2 proteolytic activity in a dose dependent manner (peak effects seen at 1 µM).
- BHH also inhibits TMPRSS2 proteolytic activity in a dose dependent manner but less potent than A1AT and AEBSF.
- Secretory leukocyte peptidase inhibitor doesn't inhibit TMPRSS2 proteolytic activity at any concentration.

**Highlights**

- Camostat mesylate, BHH, AEBSF and A1AT, as TMPRSS2 inhibitors, may be useful strategy in COVID-19 treatment.

**Clinical Impact:**

- Minimal

**Important Methodologies:**

- To use PLX304 plasmid containing human TMPRSS2 open reading frame (PLX-TMPRSS2) and control PLX304 vector (PLX)
- To transfect HEK-293 cell line with PLX and PLX-TMPRSS2 via TransIT LT-1 transfection reagent.
- To quantify TMPRSS2 activity with fluorogenic substrate BOC-QAR-AMC and GloMax plate reader.
- To use gel electrophoresis western blotting

**Limitations:**

- To be entirely *in vitro* study.
- Didn't investigate in conditions with viral infection

**Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites**

Kang, S et al., *Acta Pharmaceutica Sinica B,* 2020

**Link**: https://www.sciencedirect.com/science/article/pii/S2211383520305505?via%3Dihub

Kang, S et al. present the crystal structure of the SARS-CoV-2 nucleocapsid N-terminal domain (SARS-CoV-2 N-NTD) at a 2.7 Å resolution. This study reveals specific structural changes in the SARS-CoV-2 N-NTD that are present compared with the mild coronavirus HCoV-OC43, highlighting that the introduction of a hydrogen-bond-forming moiety (a guanosine base like moiety) at the base recognition site and avoiding the steric clash at the

branching phosphate group binding site would benefit high-affinity ligand development during structure-based drug discovery.

**Main findings:**

1. Solving of SARS-CoV-2 N-NTD (47–173 residues of SARS-CoV-2 N protein) at 2.7 Å resolution
2. Superimposition of SARS-CoV-2 N-NTD with HCoV-OC43 N-NTD-AMP complex revealed:
   i. N-terminal tail movement of of SARS-CoV-2 N-NTD, contributing to easier access to nucleotide binding cavity
   ii. Larger sidechain residues in the phosphate group binding site, suggesting increased steric clashing with the ribonucleotide phosphate moiety
3. Accordingly, SPR analysis and Bio-Layer Interferometry interaction analysis for SARS-CoV-2 N-NTD with ribonucleotides (AMP/UMP/CMP/GMP), all except for GMP (KD value is 8 mmol/L), showed little binding signals in assay
4. Observations suggest that the introduction of a hydrogen-bond-forming moiety at the base recognition site and avoiding the steric clash at the branching phosphate group binding site would benefit ligand development during drug discovery

**Highlights**

- Solves SARS-CoV-2 N-NTD structure, highlighting factors that should be considered during structure-based drug discovery

**Clinical Impact:**

- Minimal

**Important Methodologies:**

- Crystallisation by hanging drop vapor diffusion method
- Imaging using Rigaku X-ray diffraction
- Surface plasmon resonance (SPR) analysis and Biolayer interferometry assays to assess binding to ribonucleotides

**Limitations:**

- Structure of SARS-CoV-2 N-NTD in complex with GMP not resolved despite study showing GMP binding

**Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform.**
Thao, T. T. N. *et al*. Nature (2020)
**Link**: https://www.nature.com/articles/s41586-020-2294-9

Study presents a yeast based synthetic genomics platform which can genetically reconstruct diverse RNA viruses such as *Coronaviridae* which previously were difficult to clone due to their size and occasional instability. The technique includes; viral sub genomic fragment

generation and reassembly in one step Saxxhaomyces cerevisae using transformation associated recombination cloning to maintain the genome as a yeast artificial chromosome. Using this platform the author has been able to rapidly engineer and resurrect chemically synthetized clones of the recent SARS-CoV-2.

**Main findings:**

- The results demonstrate the full functionality of a SARS-CoV-2 reverse genetics system.
- The fast, robust and versatile synthetic genomics platform will provide new insights into the molecular biology and pathogenesis of a number of emerging RNA viruses.
- Uses 'Transformation Associated Recombination' Cloning which has been generated for RNA viruses and can manage with the size of SARS-CoV-2. A main advantage of the TAR cloning system is that the viral genomes can be fragmented to at least 19 overlapping fragments and re-assembled efficiently. This allowed the cloning and rescue of rSARS-CoV-2 and rSARS-CoV-2-GFP to occur within one week. The author notes that they see considerable potential to reduce the time of DNA synthesis.
- The platform generated was suitable to genetically modify coronavirus genomes.
- Cloning of a range of coronaviruses in yeast was successful across all coronaviruses; irrespective of the virus source, the nucleic acid template or the number of DNA fragments.
- As the SARS-CoV-2 pandemic is ongoing, sequence variations and possibly phenotypic changes are possible. The platform generated makes it possible to rapidly introduce variations into the infectious clone and to functionally characterise SARS-CoV-2 evolution in real time.

**Highlights:**

- Generation of SARS-CoV-2 from chemically synthesized DNA could bypass the limited availability of virus isolates and allow genetic modifications and functional characterisations.
- Results demonstrate that the synthetic genomics platform provides the technical advance to rapidly generate molecular clones of diverse RNA viruses by using virus isolates, cloned DNA, synthetic DNA, or clinical samples as starting material.

**Clinical Impact:**

- Minimal

**Important Methodologies:**

- Generates a methodology which will be useful in genomics platforms for future RNA viruses.

## Neurosciences and Mental Health

**Do psychiatric patients experience more psychiatric symptoms during COVID-19 pandemic and lockdown? A case-control study with service and research implications for immunopsychiatry.**
Hao F, Tan W, Jiang L, Zhang L, Zhao Z, Zou Y, Hu Y, Luo X, Jiang X, McIntyre R, Tran B, Sun J, Zhang Z, Ho R, Ho C, Tam W (2020) Brain Behav Imm
**Link:** https://doi.org/10.1016/j.bbi.2020.04.069

**Summary**

This study is the first cross-sectional study that compared the prevalence of psychiatric symptoms between people with and without psychiatric illnesses during the COVID-19 pandemic within Chongqing, China during lockdown. 76 psychiatric patients and 109 healthy controls were recruited, with no COVID-19 diagnosis, to complete an online questionnaire. This study revealed that psychiatric patients displayed higher levels of depression, anxiety, PTSD symptoms, stress, insomnia, worries about physical health, anger and suicidal ideation compared to healthy controls. This reveals the importance of the continuation of psychiatric intervention to patients in some form during a pandemic of life-threatening infectious diseases.

**Highlights**

- PTSD-like symptoms, depression, anxiety, stress, suicidal ideation, and clinical insomnia were significantly higher in psychiatric patients compared to healthy controls
- No significant differences in auditory hallucination, paranoia, alcohol use or intention to harm others
- Respondents who reported recent physical symptoms similar to COVID-19 had increased depression, anxiety and PTSD scores, and those with psychiatric illnesses reported higher levels of these measures than controls.

**Clinical Impact?**

High, psychiatric patients may be at increased risk to develop worsening symptoms

**Important Methodologies**

- 3 questionnaires were used: IES-R to measure PTSD symptoms, DASS-21 to measure depression and anxiety and ISI to measure insomnia

**Limitations**

- The study involved an online questionnaire: patients who did not have access to the Internet were excluded, as well as more severely ill patients who might not be able to complete it
- The questionnaire was based on self-reported measures and was only performed in one hospital

**Does SARS-Cov-2 invade the brain? Translational lessons from animal models**
Natoli, S *et al.* (2020) European Journal of Neurology
**Link:** https://onlinelibrary.wiley.com/doi/abs/10.1111/ene.14277

This review considers animals models of previous coronavirus (CoV) infections, namely SARS and MERS and how they might inform our approach to understanding the current Covid-19 pandemic, caused by SARS-CoV-2. The common mechanism to all three viruses is the requirement for the presence of angiotensin-converting enzyme 2 (ACE2) as a cell entry receptor. Animal models have demonstrated the ability of coronaviruses to infiltrate the brain, but the mechanism of how this happens remains unresolved. This review highlights the need for SARS-CoV-2 research to focus on elucidating these mechanisms, particularly given the growing neurological impact of Covid-19.

**Main Findings**

- To produce a clinically relevant phenotype in an animal model of CoVs is challenging as it only occurs with manipulation of the animal or the virus.
- K18-hACE2 mice show neurons are susceptible to infection by SARS-CoV. The virus enters the brain via the olfactory bulb and spreads quickly transneuronally.
- In a MERS models model brain invasion occurs later than infection of the lungs and pathological changes were minimal and inconsistent.
- Analysis of WOM RNA seq data illustrated the lack of 2 key genes (ACE2 and TMPRSS2) in human olfactory sensory neurons. These are required for cell entry of SARS-CoV-2. However olfactory epithelial support cells do express these genes suggest a potential mechanism behind the anosmia some Covid-19 patients experience.

**Highlights**

- The pathogenicity of SARS-CoV-2 appears lower than SARS-CoV in mice demonstrating the need for the development of news tools to study SARS-CoV-2 infection that is translatable to humans.
- Challenging to produce a clinically relevant phenotype in animals

**Clinical Impact**

- Low

**Important Methodologies**

- Literature review of animal models of other coronaviruses.

**Limitations**

- Lack of neurological manifestations in SARS and MERS meant the corresponding research in animal models was not carried out.
- Does not highlight a common mechanism of how CoVs invade the nervous system, this may be via different routes and depend on disease stage

**Are we facing a crashing wave of neuropsychiatric sequelae of COVID-19? - Neuropsychiatric symptoms and potential immunologic mechanisms**
Emily A. Troyer, Jordan N. Kohn, Suzi Hong
**Link**: https://www.sciencedirect.com/science/article/pii/S088915912030489X?via%3Dihub

Neuropsychiatric sequelae have long been associated with viral infections. Past pandemics including "Spanish" influenza (1918), H1N1 (2009), SARS-CoV-1 (2003) and MERS-CoV (2019) have left in their wake a legacy of increased rates of depression, mania, psychosis, narcolepsy and seizures in patients. The article presents a summary of some early reports of COVID-19 patients presenting with neuropsychiatric symptoms and using more thorough studies from previous pandemic sets out a case for the huge magnitude of neurological complications we would see in the coming years post-COVID-19. Additionally, the mechanisms by which these symptoms may arise is also addressed again using data from previous viral pandemics.

**Main findings:**
SARS-CoV-2 infection is associated with acute neurological symptoms:

1. $1/5^{th}$ COVID-19 patients in a Wuhan hospital displayed altered consciousness lasting for more than 24hrs
2. Loss or changes in taste and smell have been widely reported in COVID-19 cases

Previous viral epidemics highlight several sub-acute, psychotic and degenerative disorders that may emerge SARS-CoV-2 infection:

1. Reports from the 2003 SARS epidemic show 39% of survivors being later diagnosed with depression.
2. PTSD – 54.5% of survivors of the 2003 SARS outbreak were later clinically diagnosed with PTSD and other psychiatric disorders: panic disorder (34.5%), OCD (15.6%)
3. Viral infection in utero drastically increases risk of developing schizophrenia
4. Increased rates of MS have been previously been associated with infections of specific strains of human coronaviruses
5. Parkinsonism has been reported after influenza infections however the same association has not been reported with any coronavirus infection.

Six mechanisms by which neurological symptoms arise have been proposed

1. Viral infiltration into CNS
2. Cytokine network dysregulation

3. Peripheral immune cell infiltration into CNS
4. Autoimmunity
5. Dysregulation host immune system due to immunomodulatory drug treatment
6. Gut microbiome alteration

**Highlights**

The incidence of a large array of neurological disorders could drastically increase following the COVID-19 pandemic.

**Clinical Impact**

Medium/high

**Important methodologies**

N/A

**Limitations**

Most of the data presented in based on previous coronavirus strains

The mechanisms proposed are purely speculative

**The differential psychological distress of populations affected by the COVID-19 pandemic**

Jie Zhang, Huipeng Lu, Haiping Zeng, Shining Zhang, Qifeng Du, Tingyun Jiang, Baoguo Du

Brain, Behavior, and Immunity

**Link**: https://doi.org/10.1016/j.bbi.2020.04.031

**Summary**

This study used a cross-sectional design to compare psychological distress in three different populations: those suffering from Covod-19 (n=57, those under quarantine (n=50), and the general population (n=98) in the city of Zhongshan. Participants used app-based versions of standard depression and anxiety questionnaires (PHQ-9 and GAD-7) to self-report on their mental wellbeing. Significant differences were found between those under quarantine and the other groups investigated.

**Main findings**

- Both infected patients and the general population showed increased levels of depressive symptoms compared to those under quarantine, who showed the lowers levels of adverse mental heath measures.
- Infected patients and the general population showed increased levels of severe depressive symptoms compared to those under quarantine.
- Anxiety was not significantly different between groups, although there was a strong trend towards difference in comorbid depression and anxiety.

**Clinical Impact**

- The study suggests that psychological impact for those in quarantine may be lower than might be expected.

**Important Methodologies**

- App-based mental health questionnaires.

**Limitations**

- The study, described as a pilot by the authors themselves, was limited in size.
- There were significant demographic differences between groups (eg marital status and hometown) which may affect results.
- The study lacks a proper control group. Ideally, the comparison group would a general population sample that wasn't affected by Covid-19, but this is obviously unavailable at this time.

## Clinical

**The values of coagulation function in COVID-19 patients**
Jin *et al*. (MedRxiv), 2020
**Link**: https://www.medrxiv.org/content/10.1101/2020.04.25.20077842v1.full.pdf+html

Jin and colleagues set out to elucidate the state of blood coagulation function in patients diagnosed with COVID-19. The analysis of blood from 147 patients revealed increased levels of several coagulation factors in COVID-19 patients when compared with healthy controls, being the levels of these factors positively correlated with disease severity. They have also observed that the same factors were increased in patients with thrombotic disease, who also presented higher mortality rate. Finally, they propose that t-PA/PAI-1 Complex and D-Dimer can be used as biomarkers to predict mortality in COVID-19 patients.

**Main findings:**

- COVID-19 patients had significantly higher values of thrombin-antithrombin complex (TAT), α2-plasmininhibitor-plasmin Complex (PIC), thrombomodulin (TM), t-PA/PAI-1 Complex (t-PAIC), prothrombin time (PT), international normalized ratio (INR), fibrinogen (FIB), and D-Dimer (DD) than healthy controls.
- COVID-19 patients with thrombotic disease had significantly higher levels of TAT, PIC, TM, t-PAIC, PT, INR, FIB, and DD than those without thrombotic disease.
- Levels of TM, t-PAIC, PT, INR and DD were positively correlated with disease severity.
- ROC curves analysis demonstrated that TM, t-PAIC, PT, DD were good predictors of mild vs severe/critical disease.
- COVID-19 patients who succumbed to the disease had significantly higher levels of TAT, TM, t-PAIC, PT, INR, activated partial thromboplastin time (APTT) and DD, but lower platelet levels than the ones who survived.
- t-PAIC and DD can independently predict the mortality risk for COVID-19.

**Highlights**

- Coagulation factors are increased in COVID-19 patients.

**Clinical Impact:**

- Moderate

**Important Methodologies:**

- Criteria for patient stratification

**Limitations:**

- Preprint has yet to be peer-reviewed
- To better evaluate the influence of thrombotic disease in COVID-19 patients the analysis should have been made between thrombotic patients with and without COVID-19.

**Pulmonary Embolism and Increased Levels of d-Dimer in Patients with Coronavirus Disease**

Daniel O. Griffin et al. EID Journal. 2020

**Link**: https://wwwnc.cdc.gov/eid/article/26/8/20-1477_article

Coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2, has been extensively reported since the outbreak in Wuhan, China, and can progress to involve major respiratory complications. During the second week of illness, decompensation occurs in some patients, possibly driven by the cytokine storm associated with increased levels of Interleukin 6. This study report 3 case-patients with COVID-19 who were improving after successful treatment during the critical period but showed development of pulmonary emboli (PEs) despite deep vein thrombosis (DVT) prophylaxis. Computed tomography angiograms performed on symptom day 18 showed bilateral PEs. Monitoring disseminated intravascular coagulation and measurement of platelet counts, d-dimer and fibrinogen levels, and trending International Society of Thrombosis and Haemostasis scores might be beneficial for early diagnosis of PE in patients with COVID-19.

**Main findings:**

- Case-patient 1, a 52-year-old male former smoker with a history of asthma, hospitalised on day 12 of symptoms, showed bilateral PEs in CTA on day 18. The patient was given enoxaparin, transitioned to rivaroxaban, and discharged receiving supplemental oxygen.
- Case-patient 2, a 60-year-old female non-smoker with a history of chronic bronchitis, ovarian cancer postoophorectomy, and provoked deep vein thrombosis 18 years earlier, was admitted on day 8 of symptoms. On day 18 of symptoms, she was persistently hypotoxic and had tachycardia and hypotension. CTA showed multiple bilateral segmental and subsegmental PEs with suggestion of cardiac strain. The patient was given rivaroxaban and discharged receiving supplemental oxygen.
- Case-patient 3, a 68-year-old male non-smoker with a history of hypertension, and type 2 diabetes mellitus, was admitted on day 14 of symptoms. On day 22 of symptoms, he showed development of hypotension, and his oxygen saturation was <90% with a 100% nonrebreather mask. The patient was given enoxaparin and showed improvement.

**Highlights**

- PEs can occur after the cytokine storm in COVID-19 patients, despite DVT prophylaxis.
- Patients might continue to have high or increasing oxygen requirements because of development of thromboembolic disease.
- Low levels of platelets, increased levels of d-dimer, and increasing levels of prothrombin in COVID-19 were associated with poor outcome, which might be explained by thromboembolic complications in patients with severe disease
- Severe endothelial dysfunction, driven by the cytokine storm and associated hypoxemia, leads to disseminated intravascular coagulation, causing thromboembolic complications.

**Clinical Impact:**

- Moderate

**Important Methodologies:**

- Computed tomography angiograms

**Limitations: None**

**Neutrophil extracellular traps and thrombosis in COVID-19**
Zuo *et al.* medRxiv (2020)
**Link**: https://www.medrxiv.org/content/10.1101/2020.04.30.20086736v1.full.pdf

Neutrophil extracellular traps (NETs) are extracellular webs of chromatin and microbicidal proteins that may be influencing inflammation and thrombosis. NETs carry enzymes such as myeloperoxidase (MPO), NADPH oxidase and nitric oxide synthase. They also carry significant cytotoxic potential and when formed, NETs can block the blood vessels. Curiously, early studies of COVID-19 reported a high risk of morbid arterial events. In this study, four COVID-19 patients were reported to have elevated neutrophil activation and NET markers, despite slightly varying neutrophil counts. The authors suggest combatting NETs in COVID-19 patients to alleviate possible co-morbidities.

**Main findings:**

- 3 patients had markedly elevated D-dimers and neutrophil-to-lymphocyte ratios early on
    - 2 of which had elevated levels of NET markers such as cell-free DNA, MPO-DNA complexes and citrullinated histone H3. Also, elevated levels of neutrophil activation marker S100A8/A9
- 4$^{th}$ patient had elevated neutrophil activation and NET markers 8 days after admission, however D-dimer and neutrophil count were only mild

- **4 patients developed COVID-19-associated venous thromboembolism (VTE)**
- **All 3 markers for NETs, as well as neutrophil activation marker S100A8/A9 were elevated in patients diagnosed with VTE**

**Highlights:**

- Patients with COVID-19 associated thrombosis and hyperactive neutrophils may benefit from more aggressive anticoagulation while hospitalized
    - Dismantling NETS with deoxyribonucleases, as well as prevent NET release with neutrophil elastase inhibitors and peptidylarginine deiminase 4 inhibitors
        - NETs can activate extrinsic and intrinsic coagulation pathways

**Clinical Impact:**

- Moderate-High

**Important Methodologies:**

- Cell-free DNA was quantified in sera using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, P114946)
- Citrullinated-histone H3 was quantified in sera using the citrullinated Histone H3 (Clone 11D3) ELISA Kit (Cayman, 501620)
- MPO-DNA complexes were quantified as described by author

**Limitations:**

- Study only accounted for 4 patients with no data on previous health conditions or life style
- A fair amount of data was not available

**Incidence of thrombotic complications in critically ill ICU patients with COVID-19**

F.A. Klok (Thrombosis Research), 2020

**Link**: https://www.thrombosisresearch.com/article/S0049-3848(20)30120-1/pdf

Due to excessive inflammation, hypoxia, immobilisation and diffuse intravascular coagulation, COVID-19 patients may be predisposed to venous and arterial thromboembolism. Authors observe 184 patients with proven COVID-19 pneumonia – all patients received at least basic standard dose of thromboprophylaxis. Cumulative incidence was 31% - 27%-venous thromboembolism and 3.7% arterial thrombotic events, pulmonary embolism was the most frequent complication. 31% incidence is remarkably high; therefore suggest to strictly apply pharmacological thromboprophylaxis in all COVID-19 patients admitted to the ICU, possibly increasing levels to high doses. Physicians should be vigilant for signs of thrombotic complications and order diagnostic tests at a low threshold.

**Main findings:**

- The cumulative incidence of the composite outcome was 31% - 27%-venous thromboembolism and 3.7% arterial thrombotic events.
- Out of 31 cases –
    - 25 were pulmonary embolisms (PE, 81%) – 18 cases in segmental arteries, 7 limited to sub segmental arteries.
    - 3 were other venous thromboembolic events – 1 proximal deep-vein thrombosis of the leg and 2 catheter related upper extremity thrombosis
    - 3 arterial thrombotic events – all ischemic strokes

**Highlights**

- Indicates that a high percentage of COVID-19 patients develop thrombotic complications and these must be taken into consideration during treatments.

**Clinical Impact:**

- Moderate

**Important Methodologies:**

- Observational Study

**Limitations:**

- Authors state that this is a conservative estimation and the real number is likely to be higher due to several limitations including:
    - The majority of patients (76%) were still on ICU at the end of the study, therefore could develop thrombosis after the observation period
    - Venous thromboembolism is more difficult to recognise in intubated patients and diagnostic imaging tests were difficult due to the strict isolation
    - The three hospitals had different thromboprophylaxis regimens and the dose increased over time, the findings were not adjusted for the actual administered doses and the effect of the changes in the protocols were not studied

**A clinical and biological framework on the role of visceral fat tissue and leptin in SARS-CoV-2 infection related respiratory failure**

van der Voort *et al*., (medRxiv), 2020

**Link:** https://www.medrxiv.org/content/10.1101/2020.04.30.20086108v1.full.pdf

Reports are emerging that SARS-CoV-2 infected patients admitted to the intensive care unit (ICU) with respiratory failure are overweight. Van der Voort *et al*., report the association of increased leptin with body mass index (BMI) in the serum of 31 SARS-CoV-2 patients admitted to ICU requiring mechanical ventilation (BMI range 24.8 - 48.4). This finding was not observed in the control group comprising 8 ICU patients with no signs of SARS-CoV-2 infection (BMI range 22.4 - 33.5). The clinical characteristics associated with SARS-CoV-2 patients admitted to the ICU match with hyperleptinemia and implicate a central role of adipose tissue on the pathophysiology of respiratory failure.

**Main findings:**

- SARS-CoV-2 ventilated patients have significantly increased serum levels of leptin compared to control patients

- Leptin levels correlate with BMI (r=0.555, *P=0.0012*)

- SARS-CoV-2 patients with a similar BMI to control patients have significantly higher serum levels of leptin (mean = 21.2 (6.0 - 85.2)) compared with the control group (mean = 5.6 (2.4 - 8.2)) µg/L (*P=0.0007*).

**Highlights:**

- The unmet clinical need for studies investigating the impact of obesity and the pathogenesis of COVID-19 and other infectious diseases

- Design of a clinical and biological framework to explain the clinical characteristics of COVID-19 patients. In it, they hypothesise that binding of ACE2 receptors by SARS-CoV-2 in the lung epithelia promotes a loss of ACE2 receptor at the cell surface, thereby enhancing leptin levels that drives pulmonary inflammation. This is compounded by SARS-CoV-2 infection of visceral fat causing further increases in leptin in an already obesogenic host characterised by chronic inflammation.

**Clinical impact:**

- Minimal

**Important methodologies:**

- Detection of leptin in serum collected from routine blood sampling of SARS-CoV-2 and other ICU patients

**Limitations:**

- Unclear whether control patients were tested for SARS-CoV-2
- Requirement for BMI-matched control patients in order extrapolate any meaningful conclusions from the data. Small sample size (n=8 controls).
- Were all patients tested for other inflammatory markers (e.g. CRP?)
- Very little data (one figure)
- Association study - No mechanistic data to support the proposed model
- Proposed model is hypothetical

**Classification of the cutaneous manifestations of COVID-19: a rapid prospective nationwide consensus study in Spain with 375 cases**
Galván Casas, C. *et al*. (2020). British Journal of Dermatology.
**Link**: https://onlinelibrary.wiley.com/doi/10.1111/bjd.19163

Cutaneous symptoms of COVID-19 have previously been poorly characterised. In this study, 5 cutaneous manifestations associated with COVID-19 have been identified: Pseudo-chilblain (19%), other vesicular eruptions (9%), urticarial lesions (19%), other maculopapular eruptions (47%), and livedo or necrosis (6%). Disease severity and temporal relationship to classical COVID-19 symptoms were related to the type of cutaneous manifestation presented. Cutaneous manifestations may aid COVID-19 diagnosis, particularly paucisymptomatic cases, as well as provide prognostic information. The recognition of paucisymptomatic patients could also be helpful for epidemiological control.

## Main Findings

- Data was collected and analysed from 375 patients from Spain. Patients had either suspected or confirmed SARS-CoV-2 infection and displayed an unexplained recent eruption on their skin.
- 5 major groups of cutaneous manifestation were identified, of which nearly all patients could be classified.
- **Acral areas of erythema-oedema with vesicles or pustules (pseudo-chilblain)** were present in 19% of patients. These lesions appeared later in disease, with 59% presenting after COVID-19 symptoms. Pseudo-chilblain affected younger patients and presented with less severe COVID-19.
- **Other vesicular eruptions** (9%) appeared earlier in disease, with 15% presenting before other symptoms. They affected middle aged patients and associated with moderate COVID-19.
- **Urticarial** (19%) and **other maculopapular lesions** (47%) appeared around the same time as other disease symptoms and associated with more severe disease. Itching was highly reported with these lesions (97% and 88%, respectively).
- **Livedo or necrosis** (6%) occurred in older patients with more severe disease (10% mortality). Degree of manifestation varied, suggesting occlusive vascular disease, although transient livedo was also seen.
- Similar trends were seen when only analysing patients with a laboratory confirmed SARS-CoV-2 infection – inclusion of suspected cases didn't bias results.
- A familiar cluster of vesicular eruptions was seen in siblings suspected of SARS-CoV-2 infection.
- An increased number of herpes zoster (shingles) were perceived in COVID-19 patients

**Highlights**

- The first study to characterise the cutaneous manifestations of COVID-19 based on morphological and clinical data

**Clinical Impact?**

- Moderate. Impacts for prognostic tools and clinical COVID-19 diagnosis.

**Important Methodologies**

- Dermatologist nationwide case collection survey over 2 weeks. Use of a standardised questionnaire and pictures.

**Limitations?**

- Cross-sectional study limited to 2 week period in Spain. Severity of disease may have changed.
- Patients with the most severe disease were not included – difficulties in obtaining consent.
- Manifestations may have other causes other than COVID-19

**COVID-19 in Newborns and Infants-Low risk of severe disease: silver lining or dark cloud?**
Munmun Rawat et al. (Am J Perinatol 2020-05-07).
**Link**: https://www.ncbi.nlm.nih.gov/pubmed/32380565

Review which goes through all the different theories about the low prevalence of neonates, infants and children that have been exposed to SARS-CoV-2 not suffering from severe disease.

**Main findings:**

- Low severity of SARS-CoV-2 infection in this population is associated with a high incidence of asymptomatic or mildly symptomatic infection making them efficient carriers

**Highlights**

- There is a low prevalence of novel coronavirus disease in neonates, infants and children
- The fetal haemoglobin may play a protective role against coronavirus in neonates
- Immature angiotensin converting enzyme (ACE2) interferes with coronavirus entry into cells

**Limitations:**

- Short review which only mentions the different hypothesis that might explain the decreased impact of Covid-19 in neonates. A deeper understanding is needed.

## Infection and Transmission

**SARS-COV-2 was already spreading in France in late December 2019**
Deslandes et al., 2020, International Journal of Antimicrobial Agents.
**Link:** https://doi.org/10.1016/j.ijantimicag.2020.106006

The authors perform a retrospective study on ICU patient nasopharyngeal samples if admitted with flu-like illness. If patient samples were negative for common flu-like illness causing viruses and the patients presented with symptoms now thought typical of COVID19, the samples were chosen for further analysis. This consisted of RT-PCR for SARS-COV-1 and -2 envelope protein and confirmation by RT-PCR of three SARS-COV-2 specific genes. 1/124 ICU patients who presented with flu-like illness retrospectively tested positive for SARS-COV-2. The admission was 1-month prior to the first confirmed case of COVID-19 in France and this patient represents the new first reported case.

**Main findings:**

- In late December 2019, one patient presented with flu-like illness including bilateral ground glass opacity in the inferior lobes and retrospectively tested positive for SARS-COV-2. The patient admitted to the ICU with lymphopenia, elevated C reactive protein and fibrinogen. The patient had not travelled outside of France since August 2019 indicating domestic transmission of SARS-COV-2 one month prior to the first reported case in France.

**Highlights**

- Domestic transmission of SARS-COV-2 began 1 month prior to what was previously thought.
- The authors do not overstate their findings and confirm the results using two independent RT-PCR tests and teams so despite the finding of only case, the finding appears robust.

**Clinical Impact:**

- None, this paper is of epidemiological interest.

**Important Methodologies:**

- Definition of 124 ICU patients with flu-like illness: Fever greater than 38.5 $^{o}$C, cough, rhinitis, sore throat, myalgia, ground glass opacity.
- FilmArray PCR for other common virus to exclude 44 patients.
- Non COVID-19 symptoms exclude 66 patients.
- 14 samples tested by RT-PCR according to the Charite protocol for the envelope protein of SARS-COV-1 and -2 prior to confirmation using the gene finder COVID19 plus real-amp kit.

**Limitations:**

- Only 1/ 124 patients admitted in December was found to be positive although this is acknowledged by the authors and they note: they exclude patients who were PCR positive for other common viruses whilst co-infection has been reported in the literature, and they only tested ICU cases whilst 18 – 23 % are asymptomatic and most cases are mild, not requiring ICU admission.
- The results here are robust but the PCR results would have been well supported by antibody tests as an alternate method of confirming the result.

**Cryptic transmission of SARS-CoV-2 in Washington State**
Bedford *et al*. (medRxiv Preprint), 2020.
**Link:** https://www.medrxiv.org/content/10.1101/2020.04.02.20051417v2

326 SARS-CoV-2 genomes were sampled between 20 February and 15th March 2020 from infected patients in Washington State, USA. The majority of cases are believed to have originated from 1 introduction event into the state in late January/early February 2020. Exponential doubling is estimated between 2.4-5.1 days. In communities where large scale transmission is not yet recognised, early identification of the virus, extensive testing and immediate self-isolation of infected persons is recommended.

**Main findings:**

- The first confirmed case of COVID-19 in the US was travel-associated, detected in Snohomish County, Washington State, on 19th January 2020. This infection was recorded as strain USA/WA1/2020 (WA1) and appears closely related to viruses from infections in China.
- On 28th February 2020, a community case was identified close to the location of the original Snohomish County case.
- 346 SARS-CoV-2 viruses from Washington State were analysed for their sequence between 20th February-15th March 2020.
- 293 (85%) of these viruses are closely related to WA1. This is consistent with the WA1 strain transmitting locally after arrival into the US.
- By analysing Washington State virus sequences within the WA1 'clade', the median estimated date for a common ancestor was 1st February 2020, with a doubling time between 2.4-5.1 days.
- 7 virus samples from the Grand Princess cruise ship group into the same outbreak clade as WA1.
- 53 SARS-CoV-2 genomes from Washington State fall outside the primary outbreak clade and into multiple separate clusters. 38 (11%) of this clade of viruses are closely related to viruses from the European outbreak, and likely represents a second introduction occurring in February 2020.
- SARS-CoV-2 was circulating undetected in Washington State since January 2020.

**Highlights:**

- The use of pathogen genomics to inform epidemiological understanding.

**Clinical Impact:**

- Moderate.

**Important Methodologies:**

- Metagenomics & Phylogenetics.

**Limitations:**

- Limited to 1 geographical area.
- Only a small proportion of infections in China have been sequenced.

**Shedding of infectious SARS-CoV-2 in symptomatic neonates, children and adolescents**
Isabella Eckerle et al. (medRxiv 2020-05-01)
**Link:** https://www.medrxiv.org/content/10.1101/2020.04.27.20076778v1

In this study they focus on whether children across all age groups and despite the high proportion of mild or asymptomatic infections should be considered as transmitters. To prove it they will use cell culture to assess the presence of cultivable SARS-CoV2 in the upper respiratory tract in a cohort of our instituition's first 23 symptomatic neonates, children and teenagers with covid-19 diagnosed by RT-PCR

**Main findings:**

- SARS-CoV-2 shedding patterns of culture competent virus in symptomatic children resemble those observed in adults

**Highlights**

- Initial VLs at diagnosis in symptomatic children is comparable to those in adults
- Symptomatic children of all ages shed infectious virus in early acute illness

**Clinical Impact:**

- medium

**Important Methodologies:**

- Sample collection flocked swab for testing using eMAG extraction and Charite RT-PCR, BD SARS-CoV-2 reagent kit for BD Max system and Cobas 6800 SARS CoV2 RT-PCR
-

**Limitations:**

- Small number of children assessed
- They use left-over material which means that some samples might be treated differently affecting to the virus infectivity and the initial number of viable particles
- No consecutive sampling over the course of the disease

**A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells**

Hoffmann *et al*./ Molecular Cell/2020

**Link:** https://www.sciencedirect.com/science/article/pii/S1097276520302641

The authors report that the cleavage of the SARS-CoV-2 spike protein by ubiquitously expressed cellular proprotein convertases is essential for entry of the virus into human lung cells. This cleavage depends on the presence of a multibasic cleavage site which is not a feature of animal-restricted coronaviruses and is likely a major virulence factor allowing systemic spread of viral particles.

**Main findings:**

- For coronavirus infection the surface unit S1 of the spike protein binds to a cellular target, while the transmembrane S2 enables fusion of viral and cellular membrane
- The S1/S2 site in SARS-CoV-2 forms an exposed loop with multiple arginine residues (=multibasic cleavage site) in contrast to monobasic cleavage sites in related coronaviruses in bats
- The presence of this multibasic site is essential for cleavage of the spike protein by the ubiquitously expressed host cell proprotein convertase furin
- Cleavage at the S1/S2 site by furin is essential for efficient viral entry into human lung cells as well as for cell-cell fusion

**Highlights**

- SARS-CoV-2 like MERS-CoV depends of furin-mediated cleavage of spike protein at the S1/S2 site for entry into lung cells. Furin inhibitors could be considered as a treatment option.

**Clinical Impact:**

- limited

**Important Methodologies:**

- generation of vesicular stomatitis virus particles bearing SARS-CoV-2 spike glycoprotein with multiple mutated versions (alterations at the S1/S2 cleavage site)
- western blot analysis of S protein processing
- transfection of Vero cells with S protein expression plasmids, analysis with confocal laser scanning microscope

**Limitations:**

- The group did not use authentic SARS-CoV-2 viral particles
- The authors infected Vero cells but not primary human lung cells
- Furin is essential for normal cell function and prolonged blockade will have adverse effects

## Innate and Adaptive Immunity

**A Dynamic Immune Response Shapes COVID-19 Progression**
Ong *et al.* (Cell Host & Microbe), 2020
**Link**: https://doi.org/10.1016/j.chom.2020.03.021

The authors followed a severe COVID 19 case and 2 non severe cases via transcriptomic profiling of a panel of immune genes in peripheral blood and found that the early immune response in COVID-19 patients is highly dynamic. In the severe case, most inflammatory gene expression peaked only after the lowest point of respiratory function, except for expression in the IL1 pathway. Data also shows an important role for T cells which could exacerbate disease or prolong infection. They suggest that IL1 (and its pathway) may be an indicator for severe disease as well as a therapeutic target for COVID 19.

**Highlights**

Aim of study: Analyse if the immune- and inflammatory response to COVID 19 is fluctuating (dynamic) early in infection to guide therapeutic interventions and address pathogenesis.

1. Highly dynamic expression of pro-inflammatory genes in the severe case. However, the expression of most of these genes (such as IL2, IL6, TNF and INFA1/13) peaked 1 day (day 6 of illness) after the worst point of respiratory function (day 5 of illness). Only IL1A and IL1B were expressed before the nadir of respiratory function. This could indicate that global cytokine expression is not the cause of declining respiratory function. Instead, COVID 19 pathogenesis may be driven by the IL1 pathway and IL1 receptor antagonists could potentially be used in the treatment or prevention of severe cases of COVID-19.

2. Compared to the 2 non-severe cases as well as the healthy controls, the severe case showed reduced transcription of the MHC class II and T cell activation pathway. mRNA levels of CD4, CD8B and CD8B were also the lowest in the severe case. According to the authors this can mean two alternative things:

    a. A reduced activation of adaptive immune genes in the severe case, which unleashed the TLR-mediated pro-inflammatory response.

    b. mRNA of CD4+ and CD8+ T cells could not be found in the peripheral blood, because they were recruited to sites of infection and the draining lymph nodes by the increased expression of pro-inflammatory cytokines in the severe case. (This may explain the mostly negative RT-PCR findings in throat swabs of this patient).

    The lower pro-inflammatory responses in the mild cases could have not only lead to milder symptoms but also to a more attenuated T-cell activation and prolonged viral shedding (RT-PCR positive for up to 4 weeks).

Understanding the role of T cells in COVID 19 pathogenesis and prolonged infection should be a priority according to the authors.

**Clinical Impact?**

Mild/Moderate – only 1 case. The findings need to be confirmed in a larger cohort. But I think this would be important to do.

**Important Methodologies?**

Transcriptome analysis of mRNA in peripheral blood. This uses a small blood sample volume which is indicated for severe patients as they reach the limit of blood collection volume quickly. Blood was collected directly into a denaturing guanidine-based lysis buffer which inactivates any viremia. And samples don't have to be handled in a level 3 facility.

**Limitations?**

There is very small n-number of 1 severe and 2 mild cases.
Only one method was used in this study, but this was due to the limited amount of blood available.

**Early phases of COVID-19 are characterized by a reduction of lymphocyte populations and the presence of atypical monocytes**
Lombardi, A. *et al.* MedRxiv preprint server (2020)
**Link:** https://www.medrxiv.org/content/10.1101/2020.05.01.20087080v1

Lombardi *et al.* examined the peripheral blood mononuclear cells profile of 63 patients with COVID-19 at diagnosis and the association with inflammatory biomarkers and 28-days mortality. At diagnosis, patients had lymphocytopenia with reduced CD8+ lymphocytes, NK and NKT cells. Monocytes had reduced expression of CD14 and HLA-DR and a subpopulation had increased scatter properties. The Th1/Th2 ratio skewed to Th2. Serum IL-6 and Th2 frequency were correlated. Patients who died in the 28 days from admission, when compared to those who did not, displayed lower numbers of CD3+ and CD4+ cells and higher numbers of CD8+/CD38+/HLA-DR+ lymphocytes.
**Main findings:**

- At diagnosis white blood cells (WBC) count, monocytes and neutrophils numbers were normal
- Lymphocyte counts were below the lower reference limit, in line with the findings of other groups
- Median CD3+, NK, NKT and CD8+ cytotoxic T cells (CTLs), were decreased, (but theCD38+HLA-DR+ subset of CTLs was increased).
- CD4+ T cells and CD19+ lymphocytes (B cells) were not significantly decreased.

- Th1/Th2 ratio was altered in favour of increased Th2 (in agreement with some, but not all, of the published literature)
- HLA-DR+ monocytes decreased, mirroring the findings of other groups
- Patients had a subpopulation of monocytes with high FSC and SSC. Blood smears showed atypical monocytes with vacuoles in their cytoplasm
- Median expression of CD14 and HLA-DR on high-scatter monocytes was lower than on monocytes of healthy donors
- The serum levels of IL-6 showed a moderate correlation with the median frequency of Th2 lymphocytes but were not correlated to monocyte counts
- Patients who died within 28 days from admission had higher age, LDH values, CD4/CD8 ratio and percentages of activated CD38+/HLA-DR+ CTL compared to those alive. The deceased also had lower P/F ratio (measure of oxygen level in blood), CD3+ and CD4+ T cell counts.

**Highlights:**

- A detailed and well conducted description of the immunological changes occurring in the early phases of severe disease
- Lymphocyte profile confirms the findings of other groups

**Clinical Impact:**

- May suggest prognosis upon admission to hospital, based on PBMC profile

**Important Methodologies:**

- Modified flow cytometer parameters with reduced photomultiplier tube values in order to analyse monocytes with high FSC and SSC

**Limitations:**

- Enrolled patients upon admission to hospital (i.e. with severe disease), on average 5 days after onset of symptoms. It would be great to repeat this study with patients recruited at the onset of symptoms.
- Not clear if reference values are corrected for age and sex of patients

**Functional alteration of innate T cells in critically ill Covid-19 patients**
Jouan *et al.* 2020
**Link:** https://www.medrxiv.org/content/10.1101/2020.05.03.20089300v1

Aim to investigate how inflammatory status of SARS-CoV-2 (COVID-19) patients differ from healthy controls, and how patient-matched Endotracheal Aspirates (ETA) and plasma samples differ. Focused on profiling cytokines and 3 innate T-cell populations: Muscosal-Associated Invariant (MAIT), γδT and invariant Natural Killer (iNKT) cells.
COVID-19 patients had higher cytokine production in ETA samples than Plasma and proportions of some innate T-cells differ. Profiling suggested the reduced number and CD69 expression of iNKT and MAIT cells were the best indicators of disease severity and could be used to identify patients in need of intubation.

**Main findings:**

- COVID-19 ETAs had higher IL-1β, IL-6, IL-1RA and IFN-α2 than matched plasma samples (though patient plasma still had higher levels than controls).
- ETA samples had higher IFN-γ and IL-17A production than matched plasma samples.
- Patient blood had reduced MAIT and iNKT cells compared to controls, γδT population did not differ.
- PMA stimulated blood innate T cells, produced less IFN-γ but more IL-17A compared to healthy donors.
- Got T-cells from 12 patients ETAs, matched samples had higher numbers of MAIT cells compared to blood, and ETA MAIT cells also had higher CD69 and PD-1 expression.
- Temporally tracked blood samples had CD69 expression was significantly reduced in all 3 innate T-cell subsets between 1 and 7 or 14 days, but CD69 levels appeared to stabilise at a lower level after 7 days.
- PD-1 showed a similar temporal pattern in MAIT cells.
- Patients with greater hypoxia at day 7 had significantly reduced CD69 expression on MAIT and iNKT cells.

**Highlights:**
Profiled innate T-cells in COVID-19 patients, in lungs and plasma. Found MAIT and iNKT cells numbers were reduced in plasma samples as disease progressed and CD69 expression on these cells was reduced.

**Clinical Impact:**
Mid, preliminary data on how the blood reflects changes in the lungs suggests CD69 expression on innate T-cells could predict level if patient hypoxia.

**Important Methodologies:**
Flow cytometric profiling of T-cell populations.
Utilising Luminex bead assay to screen for multiple cytokines.

**Limitations:**
Not yet peer reviewed
Small patient numbers (30), even lower number of patients with T-cells in ETA (12).

**Baseline pulmonary levels of CD8+ T cells and NK cells inversely correlate with expression of the SARS-CoV-2 entry receptor ACE2**
Author Pascal H.G. Duijf 2020
**Link**: https://www.biorxiv.org/content/10.1101/2020.05.04.075291v1

SARS-CoV-2 infects cells in the lung with high efficiency via the ACE2 receptor. However, clinical studies have shown an impaired immune response, including low interferon production and depletion of T cells. This study hypothesized that the pre-existing immune cell composition in lung tissue could contribute to these aspects of SARS-CoV-2 infection. *In silico* analysis carried out in normal lung tissue found high ACE2 expression correlated with low levels of T cells and NK cells. Therefore, it could be worth measuring ACE2 expression in COVID19 cases and analysing $CD8^+$ T cells and NK cells in ACE2 high and low cohorts to determine if this impacts the clinical course.

**Main findings**

- Analysis of n=578 normal lung samples from the GTEx database showed ACE2 expression negatively correlated with $CD8^+$ T cell, resting and activated NK cell and M1 macrophage frequencies.
- There was no statistically significant correlation between ACE2 expression and $CD4^+$ T cells, dendritic cells, or neutrophils.
- None of the phenotypic characteristics of the dataset: age, sex, BMI, ethnicity, or smoking status impacted ACE2 expression. There is a lack of consensus as to the impact of these characteristics, in particular sex and smoking status, on ACE2 expression and immune correlates.
- In an independent normal lung tissue dataset, n=1349, the correlations relating to ACE2 expression were upheld in relation to $CD8^+$ T cells and activated and resting NK cells (but not M1 macrophages). However, it was not determined if any of these correlations were associated with phenotypes within the dataset.

**Highlights**

- The number of $CD8^+$ T cells, resting and activated NK cells in normal lung tissue inversely correlate with ACE2 expression. This is suggested to underpin increased susceptibility to SARS-CoV-2 infection and contribute to a more severe clinical course of COVID19.

**Clinical Impact**

Minimal unless validated in clinical samples.

**Important Methodologies**

- *In silico* flow cytometry: a published method (Newman et al., 2015), demonstrating how the composition of different cell types in fresh, frozen or fixed tissue can be estimated. This involves measuring RNA expression in the tissue against a reference gene set which comprehensively describes the gene expression profiles of the cell types of interest. Used in this study to estimate the frequencies of 6 immune cell subsets involved in an antiviral response: NK cells, CD8 T cells, CD4 T cells, DC, M1 Macrophages and neutrophils.
- Gene expression levels in the two datasets were reported in the source datasets in transcripts per million (log transformed) and highest median absolute deviation, respectively.
- The correlations were derived by univariate or multivariate regression analysis.

**Limitations**

- The analysis was carried using two healthy tissue datasets; there are no direct links to SARS-CoV-2 infection outcomes.
- The phenotypic information of the dataset was only compared to the immune cell subset frequencies in one of the datasets (n=578.)
- The study was carried out *in silico*: these findings need to be further validated by experimental and clinical observations e.g. to determine any mechanism linking ACE2 signalling to immune cell chemotaxis to the lung.

- While the *in silico* mRNA analysis method has previously been reported and validated using 'real-world' samples, there may be some discrepancies between mRNA expression and protein levels, which could impact the estimation of immune cell subsets.

**Cross-talk between the airway epithelium and activated immune cells defines severity in COVID-19**
Chua *et al*. (medRxiv), 2020
**Link**: https://www.medrxiv.org/content/10.1101/2020.04.29.20084327v1

Chua et al. presents a comprehensive study of mucosal immune responses in COVID-19 patients that aims at understanding the cellular and molecular basis of different severities of disease. The lung pathology of moderate, critical and severe COVID-19 patients was assessed using single cell RNA sequencing data from respiratory samples. Increased expression of CCL2, CCL3, CCL5, CXCL9, CXCL10, IL8, IL1B and TNF in macrophages identified as likely cause of lung pathology. Therefore, immunomodulatory therapy along the CCL2, CCL3/CCR1 axis may be a promising option to prevent and treat the critical course of COVID-19 disease.

**Main findings:**

- COVID-19 nasopharynx samples showed 21 different cell states spread between epithelial cells and immune cells. Subpopulation of basal cells with strong IFNγ response signature. Not able to identify NK cells which were only present in lower respiratory tract samples.
- Moderate vs critical COVID-19 patients: depletion of basal cells, strong enrichment for neutrophils but should be carefully interpreted.
- ACE2 co-expression with either or both priming proteases exceeded those detected in non-infected controls by 2- to 3-fold. Most ciliated, secretory and FOXN4+ cells predominately expressed ACE2 with at least one of the two priming proteases. Secretory and ciliated cells also highest fraction of virally infected cells.
- Virus shedding decreased around 10 days after onset of symptoms
- Different dynamics of epithelial differentiation upon SARS-CoV-2 infection: predicted an alternative differentiation path leading from basal cells directly into ciliated cells dependent on IFNγ mediated basal cells mostly driven by ISGs like *ISG15*, *IFIT1*,*IFIT3*, and *IFITM3.*
- Significant correlation between the percentage of both $ACE2^{+}$ ciliated and secretory cells and the intercellular-signalling strength of those cells with CTLs
- In moderate COVID-19 patients, secretory cells strongly expressed chemokine ligand encoding genes *CXCL1, CXCL3, CXCL5*, and *CXCL16*, which promote the recruitment of neutrophils and CTLs, respectively
- Ciliated cells expressed *CCL15* which may contribute to an inflow of monocytes/macrophages and neutrophils via CCR1.

- Increased numbers of epithelium-immune cell interactions in critical COVID-19 cases are consistent with a higher activation status of nrMa, moMa, and CTL
- The immune characteristics of individual patients are also described in the paper

**Highlights:**

- Anti-C3 agents for reducing the activation of innate immunity in the lung and controlling the maladaptive inflammatory response

**Clinical Impact:**

- Targeting chemokine receptors for treating exaggerated immune responses could offer a promising therapeutic option. Specifically, CCR1 inhibition, possibly combined with CCR5 blockage may be the most promising strategy for Sars-CoV-2 infection.

**Important Methodologies:**

- 3' single cell RNA sequencing (scRNAseq) on nasopharyngeal or pooled nasopharyngeal/pharyngeal swabs (NS), bronchiolar protected specimen brushes (PSB), and bronchoalveolar lavages (BAL)

**Limitations:**

- Small sample size: 14 patients, 10 males, 4 females, aged 21-75 years, 5 moderate, 9 critical including 2 deceased in the clinical course
- Only 4 patients sampled longitudinally
- Small control group: one influenza B patient which was SARS-CoV-2 negative and 2 healthy controls
- Different timepoints of sampling

**Beyond the Spike: identification of viral targets of the antibody response to SARS-CoV-2 in COVID-19 patients**
Hachim *et al*, MedRxiv, 2020
**Link:** https://www.medrxiv.org/content/10.1101/2020.04.30.20085670v1.full.pdf

In this preprint, Hachim *et al* report the evaluation of anti-SARS-CoV-2 antibody profiles to 15 different SARS-CoV-2 antigens by cloning and expressing them in mammalian cells (Cos1). Antibody responses in COVID-19 patients to antigens were then screened by the luciferase immunoprecipitation system (LIPS). Responses were detected to 11 out of the 15 SARS-CoV-2 antigens in COVID-19 patients, with open reading frames (ORFs) ORF3b and ORF8 in particular allowing antibody detection early in infection. These ORFs are also previously reported to be the most unique genes to SARS-CoV-2, sharing low homology with other human CoVs. In combination, ORF3b, ORF8 and nucleocapsid (N) antigens were sufficient to diagnose all COVID-19 patients early in infection, while the spike protein alone failed to do so.

**Main findings:**

- Among the 15 proteins assessed, 11 antigens showed higher responses in COVID-19 patients compared to healthy controls
- Antibodies to spike proteins S1 and cleaved S2 were higher in COVID-19 patients but not to uncleaved S2
- 6 out of 8 clones ORF1ab, ORF3a, ORF3b, ORF7a, ORF7b and ORF8 induced a humoral response in COVID-19 patients
- By calculating a cut-off value, they report that only ORF3b, ORF8 and N antigens were useful in diagnostic testing with high performance
- N, ORF3b and ORF8 antigens in combination identify all COVID-19 patients at early stages of infection while spike protein alone does not

**Highlights**

- LIPS allowed for detection of antibody responses in COVID-19 patients to 11 out of 15 SARS-CoV-2 antigens, identifying novel immunogenic targets
- ORF3b and ORF8 antigens allow early detection of antibody in a SARS-CoV-2 specific manner and demonstrate immune-dominance of the N antigen in COVID-19 patients
- Most extensive assessment of antibody responses of COVID-19 patients reported to date

**Clinical Impact:**

- Limited – reports novel data of a wide variety of SARS-CoV-2 antigens but limited by sample size, time points and antibody profile investigation

**Important Methodologies:**

- SARS-CoV-2 gene cloning by RT-PCR, gel extraction, restriction digest, T4 DNA ligase into pREN2 plasmid, transformation and plasmid purification
- Constructs with pREN2-Renilla luciferase plasmid containing SARS-CoV-2 antigen were recovered by transfection in Cos1 cells by Fugene 6 and Ruc-antigen levels measured using luminometer plate reader
- Antibody response measured by Luciferase Immunoprecipitation System (LIPS)
- Enzyme-linked (HRP) immunosorbent assay
- Microneutralisation assay

**Limitations:**

- Small sample size (26 patients) and small number of sera collected
- Lack of paired samples from multiple time points across all patients
- Need to investigate antibody profiles with disease severity

**Seroprevalence of antibodies against SARS-CoV-2 among health care workers in a large Spanish reference hospital**
Garcia-Basteiro *et al*. bioRxiv preprint 2020
**Link**: https://doi.org/10.1101/2020.04.27.20082289

Garcia-Basteiro et al obtained over a 12-day period nasopharyngeal swabs (rRT-PCR for SARS-CoV-2) and plasma samples (SARS-CoV-2 antibody titres) from 578 healthcare workers (HCW) from a major hospital in Barcelona*. The prevalence of SARS-CoV-2 infection in this population was 11.2%. 38.9% of seropositive individuals had not previously been diagnosed with Covid-19, and over half had displayed no symptoms of disease. However, the odds of being seropositive was greater in patients who reported at least one Covid-19 symptom. In this study, working in a Covid-19 unit was not associated with seropositivity.
(* see limitation below)

**Main findings:**

- The prevalence of SARS-Cov-2 infection in HCW tested was 11.2%
- 9.3% of HCW were seropositive for IgM and/or IgG and/or IgA or IgG. Antibody titres peaked between 18 – 25 days after onset of symptoms. Antibodies were only detectable after day 6 of symptom onset.
- 15% of HCW previously diagnosed with Covid-19 by rRT-PCR did not exhibit seropositivity.
- The odds of being seropositive were higher in HCW who reported Covid-19 symptoms (OR: 8.84). Symptoms most strongly associated with seropositivity were anosmia (loss of smell (OR: 83)), ageusia (loss of taste (OR 71.4), fever (OR: 11.4) and fatigue (OR: 11.2).
- There was no statistical association with seropositivity in HCW workers who worked in Covid-19 units, had close contact with Covid-19 cases or those who had direct daily contact with hospital patients.

**Highlights**

- Most HCW positive for Covid-19 did mount an antibody response. The rate of infection in this population was lower than expected.

**Clinical Impact:**

- Minimal

**Important Methodologies:**

- Quantification of IgM, IgA and IgG antibodies directed against the RBD of the spike protein of SARS-CoV-2 by Luminex.
- Detection of SARS-CoV-2 by rRT-PCR.

**Limitations:**

- Only a 12-day period was studied, and by the authors own admission they were unable to recruit 26% of those approached. These potential participants are possibly HCW with the most contact with Covid-19 cases, who were too busy to take part.

Furthermore, the term HCW includes around 25% of individuals who have no direct involvement with patients (i.e. laboratory technicians). Therefore, the reported rate of infection in HCW may not take into account those most at risk from transmission from infected patients.

- 7.4% of the rRT-PCR's performed in this study yielded an invalid result, this suggests that the method used is neither robust or reliable and therefore casts doubt on the remaining results reported.
- 78% of the recruited HCW are female.
- *The HCW were all selected from one hospital that, by the authors admission, has the greatest provision of PPE and contact-tracing systems in place in Spain. Therefore, these HCW may have much better protection from patient transmission than HCW in other hospitals. Hence, this study may at best provide an insight into the necessity for PPE, rRT-PCR screening and contact tracing to reduce infection rates, although greater details within the paper of the PPE, screening etc. would have been useful to facilitate accurate comparisons.
- Participants are enrolled into a longitudinal study over a 12month period. Examining the antibody titres over time may yield the most important data from this study.

**The production and clinical implications of SARS-CoV-2 antibodies**
Hu *et al*. BioRxiv
**Link**: https://www.medrxiv.org/content/10.1101/2020.04.20.20065953v1.full.pdf

SARS-CoV-2 infection induces production of IgG and IgM antibodies; IgM indicates current/recent infection and IgG indicates recovery or past infection. Therefore, testing of IgG and IgM antibodies can aid diagnosis and infection status. Hu et al., analysed 211 confirmed COVID-19 patients for detection of IgG and IgM antibodies during the course of infection and 14 days following discharge. They reported peak antibody concentration on day 19-21. Discharged patients with detectable SARS-CoV-2 RNA displayed reduced IgG and IgM after 7 days.

**Main findings:**

- Concentration of SARS-CoV-2 IgM and IgG antibodies peaked 19-21 days post symptom onset (Median 17.38 IgM and 5.59 IgG where IgM decreased gradually and IgG remained high)
- IgM antibodies were detected in 73.58% of cases on day 13-15 and IgG in 97.87% on day 16-18 (2 patients did not develop IgG or IgM antibodies during course of disease)
- Significantly higher concentration IgG in critically ill patients than those with mild-moderate disease but no difference in IgM.
- PII (pulmonary inflammation index) only factor higher in negative IgM than positive IgM group
- Concentration of IgG or IgM antibodies on day 16-21 is not correlated with disease course or outcome
- Positive SARS-CoV-2 mRNA in up to 52.7% recovered patients at 14 days after discharge. Lower IgG concentration within 7 days but no change in IgM

**Highlights:**

- Antibody concentrations peaked at days 19-21; IgM then decreased gradually while IgG remained high
- IgG level was significantly higher in severe group than mild but IgM was not significantly different
- 7 days after discharge IgG levels dropped

**Clinical Impact:** Moderate- longer term analysis required

**Important Methodologies:**

- 211 confirmed COVID-19 patients (181 mild and 40 severe/critical)
- Testing every 3 days post symptom onset
- Antibodies detected using magnetic chemiluminescence enzyme immunoassay (MCLIA)

**Limitations:**

- Patients informed consent not obtained
- No details in figure legends with regards to samples, axis, statistics etc
- Figures mislabelled in the text
- Some patients did not test positive for IgG or IgM so RT-PCR testing still required for early diagnosis
- Confusion in discussion- mixing up dates of IgG and IgM peaks. States first antibody tests undertaken at day 13 yet results all show from day 3.

## Potential Implications of Co-infection

**Corona virus activates a stem cell mediated defense mechanism that accelerates activation of dormant tuberculosis: implications for the COVID-19 pandemic.**
Lekhika Pathak et al., BioRxiv, May 2020.
**Link:** https://doi.org/10.1101/2020.05.06.077883

This study proposes that similar to bacteria, adult stem cells may also exhibit altruistic defense mechanism to protect their niche. They provide preliminary data on the altruistic stem cell (ASC) based defense against a mouse corona virus; murine hepatitis virus strain-1 (MHV-1) infection. Here, they demonstrate that MHV-1 infection activates an innate defense mechanism of ASCs by enhancing *M. tuberculosis* (*Mtb)* reactivation. Their results suggest that MHV-1 activates a niche defense mechanism in the stem cells harbouring *Mtb*, which leads to a reduction in the viral load by inducing a mycobacterium load in the lung. The *in vitro* results indicate that, although this ASC mediated defense mechanism reactivates *Mtb*, it also increases the survival percentage of neighbouring lung epithelial cells. Therefore, postulating that this niche defense mechanism could be further explored to develop therapeutics to target corona virus.

**Highlights**

- The mouse model of mesenchymal stem cell (MSC) mediated *Mtb* dormancy, MHV-1 infection in the lung exhibited 20-fold lower viral loads than the healthy control mice, suggesting the potential enhancement of an anti-MHV-1 defense by *Mtb*.
- The ASC based defense against MHV-1 infection involved the *in vivo* expansion and reprogramming of CD271+ MSCs in the lung to ASC phenotype characterised by activation of genes involved in the HIF-2alpha stemness pathway.
- The conditioned media of the ASCs exhibited direct anti-viral activity in an *in vitro* model of MHV-1 induced toxicity to type II alveolar epithelial cells.
- MHV-1 infected *Mtb* harbouring group versus MHV-1 alone group exhibited an 8-fold ($p<0.02$; n=4) higher ASC reprogramming and 5-fold ($p<0.001$; n=3, student t test) higher anti-viral activity.
- However, ASCs facilitated intracellular replication and extracellular release of *Mtb.*
- Data here suggest that MSCs exert an innate defense against MHV-1 by activating the ASC defense mechanism, which might be exploited by dormant *Mtb* to undergo reactivation.
- Findings may provide a novel anti-viral defense mechanism against novel corona virus SARS-Cov2, which could be further utilized to develop vaccine against COVID19.
- Findings also predict a potential increase of tuberculosis in post-COVID19 era.

**Clinical Impact?**

Minimal

**Important Methodologies?**

- Development of stem cell mediated mouse model of *Mtb* reactivation.
- qPCR assay.
- ELISA.

**Limitations?**

- The article is a preprint and has not been peer reviewed.
- Mouse corona virus strain used represents clinically relevant model of human infecting corona virus strain; SARS-CoV-1.
- Low n numbers.