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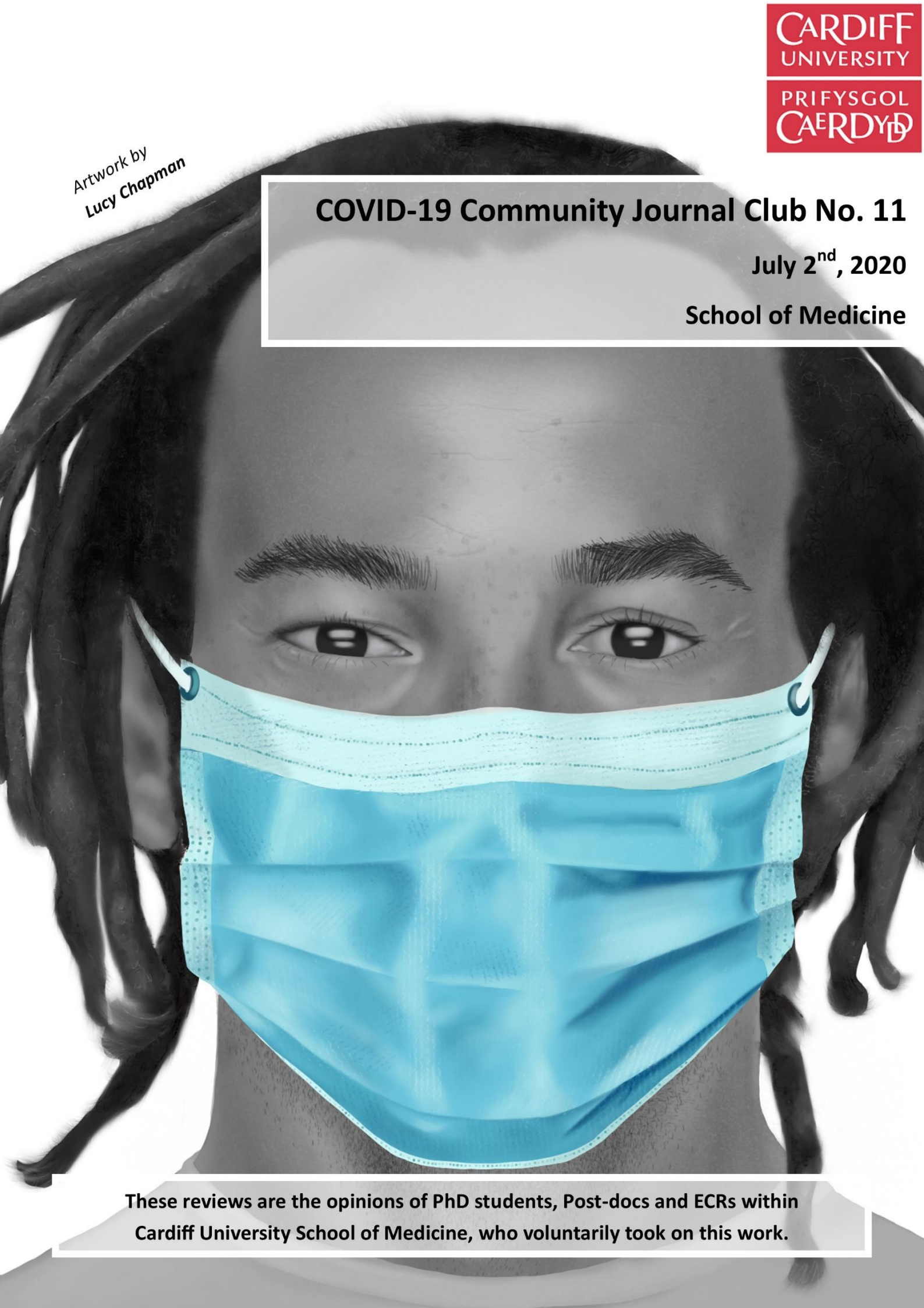

Artwork by
**Lucy Chapman**

# COVID-19 Community Journal Club No. 11

**July 2nd, 2020**

**School of Medicine**

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

Thank you to:

*Elise Moses, Pragati Sabberwal, Sophie Reed, Ruth Jones, Rebecca Bayliss, Ruban Durairaj, Owen Moon, Stephanie Burnell, Camille Rabesahala De Meritens, Kate Liddiard, Sarah Lauder, Patricia Dos Santos Rodrigues, Lorenzo Capitani, Ana Pires, Ellie Pring, Alicia Teijeira Crespo, Stephanie Hanna, Tom Pembroke, Valentina Bart, Sarah Curtis, Freya Shepherd, Stefan Milutinovic, Yuen Chen, Amy Codd, Sarah Gallaway, Shayda Maleki Toyserkani, Sandra Dimonte, and Oliver Scourfield* for producing these reviews.

*Drs Ceri Fielding, Carmen Van den Berg, Luke Davies, Andrew Godkin, Kristin Ladell, Emma Jones and James Matthews* for paper selection.

*Oliver Scourfield* for compiling the digest.

**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

# Radio Shows on COVID-19

The below are radio interviews discussing COVID-19 involving Cardiff University researchers Prof. Ian Humphreys and Dr. Richard Stanton. They are available on BBC sounds for a limited time.

## Finding Cures and Vaccinations

https://www.bbc.co.uk/sounds/play/m000k17d

Ian Humphreys and Richard Stanton appeared on the BBC Radio Wales Science Café, discussing what vaccines are and how they are made, the potential for a successful coronavirus vaccines and the possible impact that the coronavirus pandemic may have on the anti-vaccine movement.

## Coronavirus: what lies ahead

https://www.bbc.co.uk/sounds/play/m000jvdg

Richard Stanton appeared along with colleagues from Cardiff Metropolitan University (Rebecca Aicheler), Swansea University (Simon Williams), Sian Griffiths (Chinese University of Hong Kong), discussing what life is going to be like as we exit lockdown.

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**CAUTIONARY NOTE:**

**SOME REVIEWS ARE OF PAPERS POSTED ONLINE (in *arXiv, bioRxiv and medRxiv)* BEFORE PEER REVIEW.**

# Clinical

## Probability of symptoms and critical disease after SARS-CoV-2 infection

Poletti, P. *et al*. 2020. *arXiv*

**Link:** https://arxiv.org/abs/2006.08471

**Summary:**

The authors investigating the risk of developing symptoms after SARS-CoV-2 infection in Lombardy, Italy. They suggest that over 70% of infected individuals under the age of 60 did not develop symptoms, with the risk of developing symptoms increasing with age.

**Main Findings:**

- Of 5484 individuals in close contact with SARS-CoV-2 infected individuals 51.5% tested positive for SARS-CoV-2 infection. 31% of these infections were symptomatic (≥37.5°C fever or respiratory symptoms), 2.7 % were critical (patient admitted to intensive care unit or deceased with a diagnosis of SARS-CoV-2 infection)
- The probability of developing symptoms increased with age (18.1% of patients < 20 years of age (yoa) , 64.6% of patients > 80 yoa)
- 73.9% of individuals < 60 yoa were asymptomatic
- Critical disease was unlikely in < 60 yoa (0.54% of positive cases) but higher in > 60 yoa (6.6% of positive cases)
- No difference in symptomatic cases were detected between males and females, but females were at lower risk for developing critical disease

**Highlights:**

- Strong age dependency in the risk of symptomatic and critical infection

**Clinical Impact:**

- Low

**Important Methodologies:**

- Close contacts of SARS-CoV-2 infected individuals were defined as living in the same household as a confirmed case or being in close contact with a confirmed case for more than 15 minutes
- SARS-CoV-2 in close cases was detected by RT-PCR and serological testing (IgG)
- contacts were categorised into five groups : (0-19 yoa, 20-39 yoa, 40-59 yoa, 60-79 yoa, 80+ yoa)

**Limitations:**

- Close contacts were initially defined as occurring between14 days before and 14 days symptom onset in the case under consideration. After March 20, close contacts were

defined as contacts occurring between 2 days before and 14 days after the case symptom onset. This alteration could have skewed results.
- Not all close contacts were tested in the same way, some were confirmed by RT-PCR, some by serological testing.
- The study only investigated individuals in Italy, would be interesting to compare cohorts from different countries.

## Sex differences in immune responses to SARS-CoV-2 that underlie disease outcomes

Takahashi, T. *et al*. 2020. *medRxiv*

**Link:** https://doi.org/10.1101/2020.06.06.20123414

**Summary:**

The global impact of COVID19 means there is great interest in correlating immune and clinical parameters to population-level characteristics, to derive risk factors and inform treatment. This study is the first investigation of sex based immune differences in COVID19 disease. Broadly, the data indicated a more robust innate activation in male patients and greater T cell responses in female patients, with additional sex specific factors impacting prognosis.

**Main Findings:**

- Viral load didn't differ in male or female patients. The antibody titre was greater in patients in comparison to HCW but did not reach significance in male/ female patient comparisons.
- At baseline: CCL5, CCL8 and CXCL10 were upregulated in patients compared to HCW. M-CSF, CXCL9 and TRAIL were upregulated in female patients compared to female HCW. IL-10 was elevated in male patients over male HCW. IL-8 was the only marker that showed sex based differences in the patients (increased in males). CCL5, but not IL-8, was specifically increased in male patients in the longitudinal cohort.
- B cells and monocytes were increased, and T cells were depleted in patients compared to HCW. Sex specific differences were identified in patient monocyte subsets: female patients had greater proportions of $CD14^{+}$ $CD16^{+}$ intermediate monocytes while males had higher amounts of $CD14^{lo}$ $CD16^{+}$ non-classical monocytes, the latter of which correlated with CCL5. The authors suggested chemokine differences could impact monocyte differentiation.
- CD8 T cells expressing activation makers CD38 and HLA-DR were higher in female compared to male patients. Females also tended towards higher frequencies of terminally differentiated (PD-1 and TIM-3 expressing) CD8 T cells (p=0.084). T cell functional cytokine production didn't differ between male/female patients.

**Highlights:**

- Sex-based differences were correlated to COVID19 clinical course: in male patients, deterioration was associated with higher age and BMI. Female patients with stable disease had lower virus RNA and higher anti-S IgG than both female deteriorated patients and males with stable or deteriorated disease. High CCL5, TRAIL, CXCL10 and IL-15 were associated with poor prognosis specifically in female patients. While in females, activated and terminally differentiated T cells didn't differ in the stabilised or deteriorated patients, the frequency of these phenotypes and IFNγ $CD8^+$ T cells correlated with prognosis in male patients. Poor T cell responses also correlated with age in males.

**Clinical Impact:**

- Moderate to high- Sex based differences in the immune response to SARS-CoV-2 could inform clinical practice, although further validation in a larger cohort is needed.

**Important Methodologies:**

- Male and female COVID19 patients were analysed at baseline, with specific exclusion criteria (treatment with immune modifying agents e.g. steroids, tocilizumab) (Cohort A), and longitudinally, consisting of follow up measurements of Cohort A patients and further patients excluded from the baseline measures (Cohort B). These were compared to COVID19 uninfected healthcare workers (HCW) n=103 from the hospital in which the study was conducted.
- Viral RNA in nasopharyngeal and saliva swabs (PCR), anti-Spike IgG and IgM (ELISA).
- Profiling of cytokines and chemokines using Human Cytokine Array/Chemokine Array 71-Plex Panel.
- Immunophenotyping: cell composition, T cell subtypes and functional cytokine production were analysed by flow cytometry (antibodies and clones used provided in good detail).
- Clinical characteristics were scored to derive a disease course and stratify patients into stabilized or deteriorated.

**Limitations:**

- Small study size: 39 patients in Cohort A and a further 54 patients (in addition to repeated measures from Cohort A) in Cohort B. This may have led to underpowered analysis since a number of non-significant trends were observed.
- The study didn't perform any epitope specific analysis but rather stimulated the T cells with a general stimulation cocktail (Cell Stimulation Cocktail, eBioscience).

## ACE2 Expression Is Increased in the Lungs of Patients With Comorbidities Associated With Severe COVID-19

Pinto, B.G. *et al*. 2020. *The Journal of Infectious Diseases*
**Link:** https://doi.org/10.1093/infdis/jiaa332

**Summary:**
Several co-morbid diseases have been identified that increase risk of a more severe COVID-19 disease. Genes associated with these diseases were proposed to be potential therapeutic COVID-19 targets. *IL-6* and *INS* expression was altered in 6 co-morbidities.
Publicly available lung transcriptome data showed *ACE2* was upregulated in disease in 6/7 studies investigating Pulmonary Arterial Hypertension (PAH), Chronic Obstructive Pulmonary Disease (COPD) or smoking. Co-expression analysis identified genes correlating to *ACE2* expression including several histone modifying genes. Human lung ChIP-seq data also suggested that *ACE2* expression may be altered by epigenetic modifications in the lung.

**Main Findings:**

- Authors mined >8,700 abstracts in PubMed to identify genes associated with COVID-19 co-morbidities, listing 26 genes associated with 4 or more diseases.
- *IL-6* and *INS* were associated with all diseases.
- Performed differential gene expression analysis in 7 lung transcriptome studies were identified in Gene Expression Omnibus studying PAH, COPD or smoking.
- *Angiotensin Converting Enzyme 2* (*ACE2*) was upregulated in disease of 6/7 lung transcriptome studies.
- Co-expression analysis of *ACE2* vs other genes identified 544 positive and 173 negatively correlated genes.
- Genes positively correlating to *ACE2* included ADAM10 (regulates ACE2 cleavage), histone modifying genes, and *TLR3* (linked to innate immune response in SARS-CoV/MERS-CoV).
- Pathway enrichment suggested several positively correlating genes were regulated by KDM5B, H3K4me1, H3K4me3 and H3K27ac
- Human lung ChIP-seq data showed H3K4me1, H3K4me3 and H3K27ac peaks near *ACE2* implying that expression may be epigenetically regulated.

**Highlights:**

- Data mining approaches to identify potential COVID-19 therapeutic targets by looking at genes with altered expression in diseases that increase risk of severe COVID-19.

**Clinical Impact:**

- Low, proposed targets still need to be validated in COVID-19 pathology.

**Important Methodologies:**

- Text/data-mining approach to investigating gene expression in published lung tissue transcriptome datasets.

**Limitations:**

- Assuming these gene expression changes relate to biological changes.
- Only two co-morbidities were investigated as others lacked lung transcriptome data.
- No data relating to COVID-19 patients or disease models.

## COVID-19 in children and adolescents in Europe: a multinational, multicentre cohort study

Götzinger, F. *et al*. 2020. *The Lancet Child & Adolescent Health*

**Link:** https://doi.org/10.1016/S2352-4642(20)30177-2

**Summary:**

First multinational, multicentre study of paediatric COVID-19. 582 patients; 82 health care institutions across 25 European countries. Patients were aged 18 and under with confirmed (RT-PCR) SARS-CoV-2 infection, from 1st-24th April 2020. Severe COVID-19 is uncommon in young children, despite incomplete immune maturation and children are less severely affected than older adults. Although uncommon, severe COVID-19 can occur in children and adolescents, and a significant proportion of these require ICU support and mechanical ventilation. Being <1 month, male, having lower respiratory tract infection symptoms and presence of a pre-existing medical condition were associated with increased likelihood of requiring ICU admission.

**Main Findings:**

- Median age of the study population was 5 years, ranging from 3 days to 18 years, not-normally distributed. Sex ratio 1.15 males to female. Most common source of infection was a parent (56%) or sibling (4%), remaining 40% was outside of the immediate family or unknown.
- 62% of patients were admitted to hospital and 8% require ICU admission for additional support. Patients that required ICU were younger than those that did not.
- In multivariable analysis, the factors that associated with ICU admission were: being <1 month old (p=0·0035), male (p=0·033), symptoms of lower respiratory tract infection at presentation (p<0·0001), and pre-existing medical conditions (p=0.0015).
- 25% had pre-existing medical conditions, of these the most common were chronic pulmonary disease (asthma and bronchopulmonary dysplasia), malignancy (leukaemia, lymphoma or solid tumour), neurological disorder (epilepsy and cerebral

palsy), congenital heart disease, chromosomal abnormalities (including trisomy 21) and chronic kidney disease. 3% had two or more pre-existing medical conditions.

- Median interval between symptom onset and diagnosis was 2 days (range 0-23 days).
- 34% of patients had chest x-ray, of which 47% showed changes consistent with pneumonia and 5% had changes suggestive of ARDS (required mechanical ventilation).
- Additional viruses were detected in 5% of patients including enterovirus, rhinovirus, influenza, parainfluenza, adenovirus, respiratory syncytial virus, bocavirus and coronavirus (NK63, HKU1 and OC43). Patients with one or more viral co-infections were more likely to require ICU admission, respiratory or inotropic support.
- 13% required oxygen, 5% required continuous positive airway pressure and 4% required mechanical ventilation. One patient was started on extracorporeal membrane oxygenation and 3% required inotropes.
- Most commonly used antiviral drug was hydroxychloroquine followed by remdesivir, Lopinavir–ritonavir and oseltamivir. No patient received chloroquine, favipiravir, zanamivir, or ribavirin. With regard to immunomodulatory medication, patients received systemic corticosteroids, intravenous immunoglobulin, tocilizumab, anakinra or siltuximab. Indicates uncertainties regarding drug treatment options.
- Four patients (>10 years) had fatal outcome. One had cardiorespiratory arrest before arrival at the hospital, one was mechanically ventilated in ICU, the third had undergone human stem cell transplantation 15 months previously and the fourth patient was managed palliatively due to severity of pre-existing medical conditions. 16% never developed clinical symptoms.

**Highlights:**

- Important study investigating the severity of COVID-19 infection in children and adolescents in Europe. Severe infection is uncommon, but being <1 month, male, having lower respiratory tract infection symptoms and presence of a pre-existing medical condition were associated with increased likelihood of requiring ICU admission.

**Clinical Impact:**

- High

**Important Methodologies:**

- Involved a large number of specialist centres across Europe allowing detailed accounts of COVID-19 in children.

**Limitations:**

- This study primarily captured data from children who were seen in the hospital setting; therefore, the study population is likely to represent the more severe end of the disease spectrum. Additionally, at the time the study was conducted, testing capacity was lower than clinical demand; therefore, many children were not tested or diagnosed.

- A variety of in-house and commercial PCR assays were used across different countries, additionally different countries used different thresholds to screen for SARS-CoV-2.
- The number of children receiving antiviral or immunomodulatory treatment was too small to draw meaningful conclusions regarding their effectiveness.

# Transmission: Prevention and the role of Children

## Physical distancing, face masks, and eye protection to prevent person-to-person transmission of SARS-CoV-2 and COVID-19: a systematic review and meta-analysis

Chu, D.K. *et al*. 2020. *The Lancet*

**Link:** https://doi.org/10.1016/S0140-6736(20)31142-9

**Summary:**

The authors present a systematic review of 21 databases and meta-analysis of 172 publications detailing the impact of physical distance and the use of masks and eye protection for transmission of human beta coronavirus infections. Through stringent study selection, assessment of bias and certainty of evidence and additional sensitivity analyses, the authors were able to report robust support for current policies of > 1 metre physical distance in limiting risk of SARS-CoV-2 infection (2-fold risk reduction/m). Additionally, wearing masks and eye protection reduced transmission in both healthcare and community settings, although no interventions were completely protective.

**Main Findings:**

- First comprehensive systematic review and meta-analysis of parameters associated with transmission of SARS-CoV-2, SARS and MERS in both community and healthcare settings – previous analyses have predominantly focussed on randomised trials in influenza infections or healthcare settings.
- Inclusion of 172 observational studies (25697 patients) in 16 countries, with risk of bias and certainty of evidence assessments.
- Important (previously unreported) definition of the optimum physical distance required to reduce viral transmission; 102 fewer infections/1000 with distance > 1m (moderate certainty; n=10736; risk difference -10.2%).
- Additional risk reduction with increasing distance – particularly for high baseline risk groups; 2.02 change in risk reduction/metre, irrespective of virus type and exposure setting.
- Use of face masks confers further protection; 143 fewer infections/1000 with masks (low certainty), although contextual impact is not fully-explored.
- Two clinical trials into face mask use to limit SARS-CoV-2 infections have been registered and will add clarity to these findings.
- Eye protection has not previously been evaluated, but can be effective in reducing viral transmission; 108 fewer infections/1000 (low certainty).
- These findings should guide social policy and the definition of ‘contact’ for track and trace protocols.

**Highlights:**

- Significant reduction in viral transmission with physical distancing >1m (12.8% absolute risk with shorter distance; 2.6% at further distance; relative risk change of 2.02 per m)
- Risk reduction irrespective of virus (SARS, MERS, SARS-CoV-2) and community vs healthcare setting.
- Use of masks had greater protective effect in healthcare than community settings and for N95/respirator masks compared to surgical or re-usable (12-16-layer cotton) masks. However, the absolute risk of infection dropped from 17.4% without face mask to 3.1% with face mask use overall.
- Eye protection (goggles, face shields) could confer additional, hitherto under-appreciated protection (absolute risk with eye protection 5.5% versus 16% without eye protection).
- Stakeholders found physical distancing and the use of face masks and eye protection acceptable, feasible and reassuring, with some concerns for comfort and competition with healthcare providers where PPE is in short supply.
- No interventions are completely protective and must be used in combination with sanitising regimes.

**Clinical Impact:**

- Low-moderate clinical, critical social policy impact (with current status of no vaccine and limited treatment options).
- This study will be of enormous benefit in guiding social policy and contact tracing programs.
- As a corollary, it is anticipated that fewer clinical presentations of COVID-19 will be recorded.

**Important Methodologies:**

- Systematic review and meta-analysis extracting information from 21 standard WHO-specific and COVID-19-specific sources up to 3rd May 2020.
- Detailed explanations of all selection criteria, study parameters, statistical evaluations and adjustments, assessment of bias and certainty of evidence and sensitivity analyses are coherently and transparently presented.
- Supplementary information contains all studies included I analyses.

**Limitations:**

- The strengths and limitations of this research lie in the sheer scope of the undertaking that naturally introduces variables that require recognition and adjusting for, where possible.
- This study was funded and evaluated at final publication stage by WHO, so possible source of bias/influence.
- One contributing author is associated with a registered trial into the impact of face masks in reducing SARS-CoV-2 transmission, so potential skew in focus.
- No randomised trial data included – just observational studies.

- Limited data from community settings –predominantly derived from healthcare settings or households and contacts of confirmed cases.
- Includes both cases confirmed by laboratory testing and probable cases supported by clinical evidence, so may be some inaccuracy and inconsistency.
- Mean ages of cases in included studies within range 30-60 years, so lack of representation from the higher risk >60 years age group and the perceived lower risk <18 years age group. Age and risk stratification would be of value.
- Studies of SARS and MERS transmission comprise 75% patients included and extrapolation to SARS-CoV-2 may not be appropriate.
- By association, studies predominantly describe Asian cohorts, with only a single European publication considered. Exposure, effectiveness and compliance may vary amongst European, African and South American populations.
- No consideration of other confounding variables such as gender, comorbidities or socioeconomic factors.
- Sample numbers in some studies are low, however contribution is weighted and clearly displayed.
- Many studies consider PPE as a composite parameter or bundle interventions so that the impact of individual components may be difficult to dissociate.
- The inclusion of multiple diverse studies introduces variable of inconsistent reporting and context – for example as to whether masks are specifically being selected for use in aerosol-generating procedures, hence contributing to the stronger protective effect of respirator than surgical or reusable masks.
- Duration and nature of exposure (indoor/outdoor etc.) is not recorded or discussed and no clinical associations with physical distances beyond 2m have been reported for validation.
- Additional trials and validation cohorts are required to corroborate findings and improve resolution.

## Airborne SARS-CoV-2 is Rapidly Inactivated by Simulated Sunlight

Schuit, M. *et al*. 2020. *The Journal of Infectious Diseases*

**Link:** https://doi.org/10.1093/infdis/jiaa334

**Summary:**

Schuit *et al* assessed the effect of simulated sunlight, relative humidity and suspension matrix on the stability of SARS-CoV-2 in aerosols. The authors demonstrated that both simulated sunlight and matrix affected the virus decay rate, whilst relative humidity alone did not show an effect. Conditions simulating winter and summer showed that, in simulated saliva, the virus decay at a higher rate in summer-like conditions. These results suggest that the potential

for aerosol transmission of SARS-CoV-2 may be dependent on environmental conditions, especially sunlight.

**Main Findings:**

- Simulated sunlight inactivated the virus in both suspension matrices (simulated saliva vs culture medium), with 90% of the virus inactivated in less than 20 minutes for all levels of sunlight tested;
- A small but significant reduction in decay rate under high-intensity sunlight when the virus was suspended in culture medium compared to simulated saliva;
- Relative humidity alone did not affect the virus, although it contributes to alterations when combined with other factors

**Highlights:**

- Preliminary data suggesting that sunlight may be an important factor influencing the risk of aerosol transmission of the virus.

**Clinical Impact:**

- Minimal

**Important Methodologies:**

- Systems to test viability of SARS-CoV-2 aerosols under different environmental conditions.

**Limitations:**

- The study does not take into account potential variations in initial viral concentration, size of aerosol particles, distance and airflow dynamics between individuals, or the effect of personal protective equipment;
- Length of the tests was shorter when compared to other studies;
- Temperatures were tested in single sets;
- Concentrated viral stock affected properties of simulated saliva, which might not represent expelled particles in infected individuals.

## The role of children in the spread of COVID-19: Using household data from Bnei Brak, Israel, to estimate the relative susceptibility and infectivity of children

Dattner, I. *et al*. 2020. *medRxiv*

**Link:** https://doi.org/10.1101/2020.06.03.20121145

**Summary:**

Dattner et al. developed a dynamic stochastic model to estimate household SARS-CoV-2 transmission in Israel. Data was collected from households (until 02/05/2020) in Bnei Brak, which is one of the most densely populated cities in Israel. All members were tested for COVID-19 using PCR. The study uses a 'simulated likelihood approach' to develop a mathematical model that 'fits' the observational data on COVID-19 collected from infected individuals within households of different sizes. Overall, the formulated mathematical model could fairly assess propagation of SARS-CoV-2 between household members. The results were reported by age with population categorised as children (0-19 including) or adults (20+).

**Main Findings:**

- Overall, 998 out of 1809 adults were COVID-19 positive (55%) compared to 512 out of 1544 children (33%).
- Of all the positive COVID-19 cases in the dataset, 88% of adults and 72% of children reported having symptoms.
- The susceptibility of children to SARS-CoV-2 was estimated to be 45% relative that of adults.
- Model suggests that susceptibility increases with age but only up to age of 20 (after which likelihood of infection does not significantly vary).
- The model estimates that infectivity of children is 85% to that of adults.

**Highlights:**

- Children might be less susceptible to SARS-CoV-2 infection but are almost as infectious as adults.
- Children <1-year-old seem to be more susceptible to SARS-CoV-2 infection than children aged 1-4 years.

**Clinical Impact:**

- Minimal

**Important Methodologies:**

- Designing mathematical model to simulate household transmission of SARS-CoV-2.

**Limitations:**

- Not yet peer reviewed.
- The study is specific to a particular time & place and cannot be generalised.
- Viral load in COVID19 positive children and adults were not measured.

- Data in the study did not include dates of infection or information about 'who infected whom'.
- The study only used '*aggregate numbers of infected individuals in the two age groups in the different households*.'
- Majority of the data used in the study were from household size of 2. Households of 10+ were not shown.
- Only adult-to-adult transmission parameter was estimated.
- Variability among individuals of different households was not reported/explored (e.g. immune status).
- Dataset with higher number of children index cases could provide more information about infectivity of children (including child-child and child-adult transmission).

# Model for COVID-19 Research

## Generation of a Broadly Useful Model for COVID-19 Pathogenesis, Vaccination, and Treatment

Sun, J. *et al*. 2020. *Cell*

**Link:** https://doi.org/10.1016/j.cell.2020.06.010

**Summary:**

Sun *et al.* developed a SARS-CoV-2 sensitive mouse model by transduction with a replication deficient adenovirus containing ACE2 (Ad5-hACE2) via intranasal administration. Upon SARS-CoV-2 infection, these mice developed some key manifestations of disease that have also been observed in COVID-19 patients. In this study, the mice were used to analyse the roles of T cells, IFN-1 and STAT1 in SARS-CoV-2 infection. Additionally, a vaccine candidate, administration of human convalescent plasma and two antiviral therapies (poly I:C and Remdesivir) were assessed. Advantage of this model is that it allows for rapid sensitisation of all mouse strains and genetically modified mice to SARS-CoV-2 infection.

**Highlights:**

- **Development of mice sensitised for SARS-CoV-2 infection.** BALB/c mice transduced intranasally with Ad5-hACE2 and infected with SARS-CoV-2 showed ruffled fur, hunching, difficulty breathing and up to 20% weight loss. High virus titres were found in lung tissue. Similarly treated C57BL/6 mice followed an almost identical course, with 10%–15% weight loss. Both mouse strains showed lung lesions, alveolar oedema, vascular congestion and haemorrhage.
- **IFNs have antiviral and immunomodulatory activity in SARS-CoV-2 infection.** Ad5-hACE2 transduced and SARS-CoV-2 infected IFNAR-/- and STAT1-/- C57BL/6 mice were used to assess the role of type1 IFN in SARS-CoV-2 infection. IFNAR-/- mice showed delayed virus clearance and reduced inflammation, but no changes in weight loss. STAT1-/- mice however showed greater weight loss, increased inflammation and infiltration in the lung and delayed viral clearance. The difference of phenotype between the two mouse strains could be a reflection of the protective effects of IFN-λ. Treatment with Poly I:C - a potent inducer of IFN-1 – resulted in diminished disease and faster clearance of virus from the lungs.
- **Ad5-ACE2-transduced mice reproduced some key manifestations observed in COVID-19 patients.**
  RNA seq. on RNA extracted from lungs of Ad5-Empty and Ad5-ACE2 transduced BALB/c mice at 2 d.p.i. revealed upregulation of genes associated with inflammation pathways as well as CD4 and CD8. TNF, IFN-γ, IL10, IL-15, IL-6, CCL2, and CXCL10 were also upregulated which is in accordance with observations in COVID-19 patients. Additionally, expression of platelet-derived growth factor subunit B (PDGFb) was upregulated.

- **$CD4^+$ and $CD8^+$ T cell responses are needed for optimal virus clearance.** $CD4^+$ and $CD8^+$ T cell epitopes were predominantly located in the N protein and the S1 region of the S protein, respectively. Ad5-hACE2-sensitized mice also produced neutralizing antibodies in sera after SARS-CoV-2 infection.
- **Ad5-hACE2-sensitized mice are useful in evaluation of vaccines and therapies**
    - Immunization with Venezuelan equine encephalitis replicon particles (VRPs) expressing the SARS-CoV-2 spike (VRP-S) reduced SARS-CoV-2 titres by greater than 3 logs in both BALB/c and C57BL/6 mice by 1 d.p.i.
    - Treatment of Ad5-hACE2-sensitized mice with plasma of patients who overcame SARS-CoV-2 infection prevented weight loss and lung tissue damage after SARS-CoV-2 infection. More rapid clearance of virus was also observed.
    - Treatment with the drug remdesivir resulted in decreased weight loss, significantly accelerated virus clearance, and diminished cellular infiltration of lung tissue in infected Ad5-hACE2-transduced mice

**Clinical Impact:**

- No immediate clinical impact, but potentially very useful tool evaluations of vaccines and therapies in mice.

**Important Methodologies:**

- Transduction of mice with an adenovirus vector to deliver ACE2 into cells and render the mice susceptible to SARS-CoV-2 infection.

**Limitations:**

- Ad5-hACE2 transduced mice do not develop severe disease or manifestations of the disease in organs than the lung.

# Virology

## Emergence of SARS-CoV-2 through recombination and strong purifying selection

Li, X. *et al*. 2020. *Science Advances*

**Link:** https://doi.org/10.1126/sciadv.abb9153

**Summary:**

Li *et al.* show through sequence interrogation that SARS-CoV-2 entire receptor binding motif (RBM) in the spike protein was introduced through recombination with a coronavirus from pangolins. They also uncover evidence of a strong purifying selection (selective removal of deleterious alleles) around RBM and in other viral genes found amongst bat, pangolin and human coronaviruses. Overall they suggest that there are similar evolutionary constraints in different host species and identify a potential evolutionary step in SARS-CoV-2 to enable transmission humans.

**Main Findings:**

- RatG13 (bat, Yunnan 2013) has the closest sequence to SARS-CoV-2 and clusters close to Pan_SL_CoV-GD (Pangolin) in a region that spans ACE2 binding in the spike protein indicating a significant recombination event of the RBM resulting in a cross-species recombination between bat and pangolin CoVs.
- Acquisition of the pangolin RBM enabled a more efficient use of ACE2 for human infection of SARS-CoV-2.
- SARS-CoV-2 was found to possess a unique furin cleavage site (PRRA motif) that results in more efficient cleavage of the S1/S2 protein potentially expanding its tropism and/or enhancing transmissibility within human hosts.
- Evolutionary constraints in many parts of the SARS-CoV-2 genome and functional domains in S gene play a role in cross-species transmission.

**Highlights:**

- Evolutionary recombination and strong purifying selection between coronaviruses from distinct host species is likely to have resulted in a cross-species infection resulting in the transmission of SARS-CoV-2 to a human host.

**Clinical Impact:**

- None

**Important Methodologies:**

- Sequence, recombination and selection analyses.

- Structure modelling of receptor binding

**Limitations:**

- Based on sequence analysis, not proven *in vitro* or *in vivo.*

## Neuropilin-1 is a host factor for SARS-CoV-2 infection

Daly, J.L. *et al.* 2020. *bioRxiv*

**Link:** https://doi.org/10.1101/2020.06.05.134114

**Summary:**

Through the use of immunoprecipitation, site-specific mutagenesis, structural modelling, and antibody blockade, Daly, L. J., and colleagues illustrate how the polybasic sequence motif, RRAR, at the boundary of the spike protein (S) subunits 1 (S1) and 2 (S2) cleaved by the proprotein convertase furin can associate with cell surface neuropilin-1 (NRP1). In cell culture, this interaction enhanced infection of two human cell lines expressing NRP1, an effect that could be reduced by monoclonal antibody blockade. This study highlights NRP1 as a host factor for SARS-CoV-2 infection, providing a novel therapeutic target for COVID- 19.

**Main Findings:**

- The RRAR motif in the S protein conforms to a [R/K]XX[R/K] motif, (aka 'C-end rule' CendR motif), known to bind to NRP1 and NRP2
- GFP-tagged S1 protein and mCherry-tagged NRP1 were shown to associate using confocal microscopy
- Deletion of the furin-cleaved RRAR motif reduced S protein binding to NRP1
- Percentage of infected NRP1-knockout cells was 73% lower compared to wild-type HeLa cells expressing NRP1 (both cell types expressed equal levels of the ACE2 receptor)
- Site-directed mutagenesis by R685D mutation of the S1 subunit and T316R mutation of NRP1 reduced the association by 75% and 80% respectively, highlighting them as key residues in this interaction
- HeLa cells transfected with the T316R mutant of NRP1 were significantly less able to be infected by SARS-CoV-2 compared to cells transfected wild-type NRP1, establishing a role for the interaction of NRP1 and the RRAR motif of the S1 subunit in SARS-CoV-2 infection
- A monoclonal antibody specific for the CendR-binding pocket on NRP1 reduced SARS-CoV-2 infection in two cell lines (Caco-2 and Calu-3) by 38%

**Highlights:**

- The S protein of binds to NRP1 via the CendR motif generated in the S1 subunit following furin cleavage
- This interaction promoted infection in physiologically relevant cell lines

**Clinical Impact:**

- Minimal, but could lay the groundwork for novel COVID-19 therapeutic targets

**Important Methodologies:**

- Immunoprecipitation experiments performed using nanotrap bead-based assays, quantified using western blot analysis
- Cell immunostaining and confocal microscopy
- SARS-CoV-2 in-vitro infection assays

**Limitations:**

- Article is currently a pre-print and has not yet been peer-reviewed
- Interaction not assessed *in-vivo*

## Neuropilin-1 facilitates SARS-CoV-2 cell entry and provides a possible pathway into the central nervous system

Cantuti-Castelvetri, L. *et al*. 2020. *bioRxiv*

**Link:** https://doi.org/10.1101/2020.06.07.137802

**Summary:**

This study highlights how the receptor neuropilin-1 (NRP1) binds the RRAR motif found in the S1 subunit of the SARS-CoV-2 spike protein (S) and significantly potentiates SARS-CoV-2 infectivity. A monoclonal antibody specific for the b1b2 domain of NRP1 blocked this interaction. Additionally, NRP1 was highly expressed in endothelial and epithelial cells of the nasal cavity and human autopsies revealed SARS-CoV-2 infected NRP1-positive cells in the olfactory epithelium and bulb, particularly in NRP1 expressing endothelial cells of small capillaries and medium-sized vessels. Murine studies illustrated NRP1-mediated transport of virus-sized particles into the central nervous system, suggesting a novel mechanism by which SARS-CoV-2 could enter the central nervous system.

**Main Findings:**

- NRP1 rendered HEK-293T cells (which did not express ACE2) susceptible to infection with lentiviruses pseudotyped with the S protein

- Pre-incubation prior to infection assays with soluble wild-type NRP1 b1b2 domain but not a soluble triple mutant form (S346A, E348A and T349A) reduced SARS-CoV-2 pseudovirus infection, indicating the soluble domain was competitively inhibiting binding to cell surface NRP1 and highlighting these residues as potentially contributing to the interaction
- Pre-incubation with NRP1-specific monoclonal antibody reduced SARS-CoV-2 infection of Caco-2 cells by 40% compared to control antibody
- Assessment of scRNA-seq datasets from COVID-19 patients revealed that *NRP1*, *FURIN* and *TMPRSS11A* were enriched in SARS-CoV-2 infected compared to non-infected cells
- SARS-CoV-2 infected olfactory epithelial cells in the olfactory tract of COVID-19 patients showed high NRP1 expression levels
- Autopsies of COVID-19 patients showed immunoreactivity for the S protein in the olfactory bulb and tracts, which was higher in NRP1 positive cells of small capillaries and medium-sized vessels, suggesting viral entry into the brain may involve the olfactory endothelium
- Murine studies showed uptake of virus-sized nano-particles bound to a CendR peptide (AgNP-CendR) by the olfactory epithelium
- AgNP-CendR particles, but not control particles without a CendR peptide, were detected in the brain following intranasal administration particularly in neuronal cells of the cortex and olfactory bulb, revealing a potential NRP-dependent intranasal pathway for entry into the brain

**Highlights:**

- The NRP1-S1 protein interaction contributes to SARS-CoV-2 infectivity
- Olfactory epithelial cells infected with SARS-CoV-2 display high NRP-1 expression
- Infection of olfactory cells and binding to NRP-1 may enable entry into the brain

**Clinical Impact:**

- Minimal, but could lay the groundwork for novel COVID-19 therapeutic targets

**Important Methodologies:**

- Use of lentivirus pseuotyped with SARS-CoV-2 S protein that drive expression of GFP to measure infection
- Assessment of NRP1 expressing in olfactory epithelium and olfactory bulb from autopsies of COVID-19 patients using immunohistochemistry
- Use of virus-sided nanoparticles coupled to CendR peptides to illustrate transport of the nanoparticles following intranasal administration into the olfactory bulb

**Limitations:**

- Article not yet peer-reviewed
- Limited images of immunohistochemistry for NRP1 and S protein in olfactory epithelium and olfactory bulb of COVID-19 patients; additional images would have strengthened their evidence base

- Despite experiments showing movement of CendR peptides on silver nanopatrticles following intranasal administration into the olfactory bulb, the peptides used did not match the RRAR motif of the S protein. A replicated experiment using a CendR peptide derived from the sequence of SARS-CoV-2 would have strengthened their conclusions

# Antibodies and T cells

## Intrafamilial Exposure to SARS-CoV-2 Induces Cellular Immune Response without Seroconversion

Gallais, F. *et al*. 2020. *medRxiv*

**Link:** https://doi.org/10.1101/2020.06.21.20132449

**Summary:**

Gallais *et al.* report on a small scale study assessing markers of past SARS-CoV-2 exposure within seven families living in the same household with one index patient, i.e. a family member with COVID-19 symptoms. The study looks at levels of cellular and humoral immune responses to SARS-CoV-2 in blood samples taken 47 to 69 days post symptom onset. T-cell responses to SARS-CoV-2 and the presence of SARS-CoV-2 antibodies was assessed in each person relative to ten healthy controls. All index patients developed anti-SARS-CoV-2 antibodies and substantial T-cell responses. However 6/8 contacts who also displayed symptoms of COVID-19 did not possess anti-SARS-CoV-2 antibodies but did develop robust SARS-CoV-2 T-cell responses. Therefore suggesting that T-cell responses to SARS-CoV-2 may be a more sensitive indicator of past SARS-CoV-2 infection compared to antibodies.

**Highlights:**

1. Cellular and humoral responses to SARS-CoV-2 found in patients with known exposure to the virus 47 to 69 days post symptom onset
2. T-cell responses to SARS-CoV-2 found in index patients and their contacts far above those of healthy donors
3. Index patients all developed anti-SARS-CoV-2 antibody responses whilst their contacts did not show seroconversion
4. Cross-reactive T-cell responses were found in index patients, contacts and healthy donors to other coronaviruses (HCoV-229E and HCoV-OC43). However, SARS-CoV-2 specific IFNγ+ T-cells were much higher in index patients and contacts.

**Clinical Impact:**

- Epidemiological data relying upon the detection of SARS-CoV-2 antibodies may lead to an underestimation of prior exposure to the virus. T-cell responses to SARS-CoV-2 should be considered as a marker of past exposure to SARS-CoV-2.

**Important Methodologies:**

- Overlapping 15-mer peptide pools spanning the sequences of the entire SARS-CoV-2 spike glycoprotein, the nucleoprotein, the membrane protein, the envelope small membrane protein and the accessory proteins 3A, 7A, 8 and 9B were used to assess T-cell responses

- T-cell reactivity was confirmed using an IFNγ ELISpot relative to negative controls
- RT-PCR was used to test for SARS-CoV-2 nucleic acid from nasopharyngeal swabs
- Three serological assays were used to determine the presence of anti-SARS-CoV-2 antibodies – i) The Abbott Architect SARS-CoV-2 IgG; ii) The Euroimmun Anti-SARS-CoV-2 Assay; iii) The Biosynex

**Limitations:**

- Small sample size; families (n = 7), index patients (n = 9) and contacts (n = 8).
- More work is required to determine if the prevalence of cellular and humoral responses provide protection against a reinfection

## Different pattern of pre-existing SARS-COV-2 specific T cell immunity in SARS-recovered and uninfected individuals

Le Bert, N. *et al*. 2020. *bioRxiv*

**Link:** https://doi.org/10.1101/2020.05.26.115832

**Summary:**

Le Bert *et al.* report that SARS-CoV-1-recovered patients still possess memory T cells reactive to SARS-CoV-1 NP (nucleocapsid protein), which are cross-reactivity to SARS- CoV-2 NP. Half of unexposed people possess T cells targeting the non-structural proteins NSP7 and 13, which were rarely detected in COVID-19- and SARS-CoV-1-recovered patients. NSP7-specific T cells in unexposed people targeted areas with low homology to "common cold" human coronaviruses but conserved among animal beta-coronaviruses. They hypothesise that infection with related viruses might offer some protection against SARS-CoV-2.

**Main Findings**:

In recovered COVID-19 (SARS-CoV-2) patients

- IFN-γ ELISpots using pools of synthetic peptides covering NP, found reactive T cells in all patients, with most patients having T cells against multiple sites in NP
- NSP7 and NSP13 peptide pools had a T cell response in only 3/24 convalescents
- T cells producing IFN-γ and/or TNF-α were detectable in 7/9 convalescents
- Defined single peptides that were able to activate T cells in 7 patients
- COVID-19 convalescents developed T cells specific to regions that were also targeted by T cells of SARS recovered subjects e.g. NP region 101-120, NP region 321-340

In recovered SARS-CoV-1 patients

- 17 years after infection, virus-specific memory T cells persisted, reacting to SARS-CoV-1 NP and not to the NSPs

- Although at lower frequency, T cells in all 23 individuals tested reacted to SARS-CoV-2 NP (but not NSP 7 and 13) and were able to expand. Suggests SARS-CoV-1 infection can induce T cells able to cross-react against SARS-CoV-2.

In unexposed people

- T cells targeted only a limited area of SARS-CoV-2 NP
- NSP7- and NSP13-specific T cells were present in 9 out of 18 unexposed donors
- Identified CD4 T cells reactive for an epitope comprised within the NP region 101-20 with high degree of homology to the MERS-CoV, OC43 and HKU1 NP
- Identified CD4 T cells specific for the NSP7 region 26-40, and CD8 T cells specific for an epitope comprised within the NSP7 region 37-49
- But homology between these regions in SARS-CoV-1/2 and other "common cold" coronaviruses (OC43, HKU1 NL63 and 229E) is minimal, suggesting that unknown coronaviruses may have triggered the original T cell response

**Highlights:**

- Defines specific epitopes that T cells from recovered patients and unexposed people respond to.
- Hypothesis that "common cold" coronaviruses and other unknown coronaviruses, can induce cross-reactivity to SARS-CoV-2

**Clinical Impact:**

- Adds to evidence that T cells can provide long-lasting protection against COVID-19

**Important Methodologies:**

- Use of peptide pools to allow coverage of diverse genetic backgrounds of donors and individual epitopes to identify specific T cell responses.

**Limitations:**

- Not clear why infection with SARS-CoV-1 or -2 might *decrease* NSP7- and NSP13-specific T cells
- Some uninfected controls were recruited in 2020 on the basis of being negative for RBD neutralizing antibodies and negative in an ELISA for NP IgG

## Neutralization of SARS-CoV-2 by Destruction of the Prefusion Spike

Huo, J. *et al*. 2020. *Cell Press*

**Link:** https://doi.org/10.1016/j.chom.2020.06.010

**Summary:**

Huo *et al.* finds that the monoclonal antibody CR3022 binds the receptor binding domain (RBD) tightly, neutralizing SARS-CoV-2, and reports on the crystal structure of the Fab/RBD

complex. Some crystals are suitable for screening for entry-blocking inhibitors. The structure-stabilizing CR3022 epitope is inaccessible in the prefusion spike, suggesting that CR3022 binding facilitates conversion to the fusion-incompetent post-fusion state. Cryogenic electron microscopy analysis confirms that incubation of spike with CR3022 Fab leads to destruction of the prefusion trimer. Binders at this epitope could be useful in an RBD-based vaccine approach or therapeutically.

**Main Findings:**

- CR3022 antibody (previously reported not to neutralise SARS-CoV-2) binds tightly to the RBD and allosterically perturbs ACE2 binding
- CR3022 neutralised SARS-CoV-2 by reducing viral plaques
- Determined the crystal structure of the RBD-CR3022 complex which offers a promising vehicle for identifying potential therapeutics
- Identified the mechanism of CR3022 driven neutralisation of SARS-CoV2. CR3022 destabilises the prefusion states of the virus spike protein.
- CR3022 neutralizes SARS-CoV-2, but via an unusual mechanism which some assays appear to detect poorly, as observed by Yuan et al., 2020. It is now important to establish how effective this mechanism is at con-trolling viral infection.

**Highlights:**

- CR3022 binds the RBD of SARS-CoV-2 and shows strong neutralization
- Neutralization occurs by destroying the prefusion spike conformation
- CR3022 binds a highly conserved epitope that is inaccessible in prefusion spike protein
- CR3022 could have therapeutic potential alone or in synergy with a receptor blocker

**Clinical Impact:**

- With monoclonal antibodies recognized as potential antivirals, the results suggest that CR3022 could be of immediate utility because the mechanism of neutralisation will be unusually resistant to virus escape.
- With knowledge of the detailed structure of the epitope, a higher affinity version of CR3022 might be engineered.
- An RBD-based vaccine antigen might focus immune responses, conceivably mitigating the immunopathology reported for SARS-CoV-1.

**Important Methodologies:**

- Cloning
- Surface plasmon resonance (SPR)
- Bio-layer Interferometry
- Plaque reduction neutralisation test
- Crystallisation and X-Ray structure determination
- Cryo-EM data collection and processing

**Limitations:**

- The authors highlight apparent disagreements between the data in this paper with previously published work. However, Huo *et al*. discusses how they addressed these issues with further tests and analysis.
- SARS-CoV-2 can bind receptors other than ACE2 which may limit the effectiveness of treatment/vaccine strategies which only interfere with RBD/ACE2 complex.

## Antibody Dependent Enhancement Due to Original Antigenic Sin and the Development of SARS

Fierz, W. and Walz, B. 2020. *Frontiers in Immunology*

**Link:** https://doi.org/10.3389/fimmu.2020.01120

**Summary:**

Fierz, W. and B. Walz propose a hypothesis which would explain two phenomena that have been observed so far in the development of the SARS-CoV-2 pandemic, the absence of clinical signs and early appearance of IgG in certain patient. What they suggest to be the reason that causes this phenomenon is cross-reactivity and it might be confined to spike proteins. Although cross-reactivity do not implicate neutralization, which might be specific for the coronavirus species. These findings lead them to review whether an early IgG response is protective or not and it's importance in vaccine development. Due to cross-reaction to related coronavirus strains from earlier infections, the patient's viral history of coronavirus infection might be crucial to the severity of the course of the current infection with SARSCoV-2.

**Main Findings:**

- Cross- reactive immune memory is confined to spike proteins
- Neutralizing antibody response to RBD is specific for the coronavirus species
- The antibodies that are produced against SARS-CoV spike glycoprotein increase the binding of the virus to FcγRII-receptors
- In children the lack of earlier confrontation with closely related coronaviruses no (immune memory) might also be the reason for the high relative frequency of undocumented infections.

**Highlights:**

- Hypothesize the reason for the relative absence of clinical signs of infections in children, second, the early appearance of IgG in certain patients.

**Clinical Impact:**

- Moderate

**Important Methodologies:**

- Systematic review of observational and experimental studies.

**Limitations:**

- The number of patients considered in some of the studies mention was low.
- Reliability of this review is determined by the studies chosen.

## Systemic and mucosal antibody secretion specific to SARS-CoV-2 during mild versus severe COVID-19

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

**Link:** https://doi.org/10.1101/2020.05.21.108308

**Summary:**

Specific antibody responses to SARS-CoV-2, particular IgA, are unclear. Using a commercially available assay, Cervia *et al*. studied the differences in IgA and IgG levels in two cohorts of patients: confirmed COVID-19 patients and possibly exposed healthcare workers. While IgA responses were transient in mild cases, both antibodies increased with disease duration in severe patients. Antibody responses were also detected sooner in severe cases. Interestingly, vast levels of IgA correlated with severe ARDS. Also, mucosal IgA secretion was found in a number of seronegative healthcare workers, indicating a need to consider mucosal measurements particularly in asymptomatic, PCR-negative cases.

**Main Findings:**

- Severe patients (n=37) had significantly higher serum titres of IgA and IgG than mild patients (n=19).
- Comparing antibody titres based on sampling time since symptom onset, it was found that both serum IgA and IgG titres increase with disease duration. This correlation was slight for mild patients but very strong for severely infected; particularly with IgG.
- Antibody responses were seen sooner in the most severely affected patients.
    - IgA – 8 days mild, 3-4 days severe
    - IgG – 9-10 days mild, 4-5 days severe
- IgA titres peaked at around 3 weeks post symptom onset in mild patients.
- IgA and IgG titres were not seen after 30 days post symptom onset in some mild patients.
- Classifying patients according to the WHO classification criteria, younger patients generally showed milder disease and older patients had severe manifestations.
- Very high levels of IgA (an OD ratio of over 25) correlated with severe ARDS.

- Longitudinal analysis of 2 mild patients showed that serum IgA responses are transient and vary greatly among individuals. Serum IgG responses occurred around 17-20 days post-symptom onset, although the magnitude of response greatly differed.
- In a subgroup of possibly exposed healthcare workers (n=33), those that were symptomatic and tested positive for COVID-19 contained specific IgG in nasal secretions.
- IgA antibodies were detected in the nasal fluids in 15-20% of exposed healthcare workers that were seronegative. A few individuals showed a similar trend with IgG also.
- Mucosal levels of IgA inversely correlated with patient age.

**Highlights:**

- Authors suggest 4 grades of antibody responses to SARS-CoV-2, depending on disease severity.
- Potential for serum IgA levels to be used as a biomarker of severe ARDS.

**Clinical Impact:**

- Minimal.

**Important Methodologies:**

- Used commercially available ELISA specific for S1 of Spike protein to detect IgA and IgG in patients.
- An in-house immunoassay specific for S protein extracellular domain, receptor binding domain and nucleocapsid was used to analyse mucosal fluids (tears, nasal, saliva).

**Limitations:**

- Small numbers for analysis, particularly for longitudinal data.

## The receptor-binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients

Premkumar, L. *et al*. 2020. *Science Immunology*

**Link:** https://doi.org/10.1126/sciimmunol.abc8413

**Summary:**

Serological tests to identify individuals infected with SARS-CoV-2 are needed to contain the spread of disease. Using sera from 63 SARS-CoV-2 patients and animals exposed to zoonotic coronaviruses (CoVs), the receptor binding domain (RBD) of SARS-CoV-2 was evaluated as an antigen for detecting antibodies in patients. On day 9 post-symptom onset, RBD antigen was

found to be 98% sensitive and 100% specific for antibodies induced by CoVs. A strong correlation was found between titres of RBD-binding antibodies and SARS-CoV-2 neutralising antibodies in patients. These data suggest the RBD is a useful antigen to be used in serological assays for SARS-CoV-2.

**Main Findings:**

- Most sera collected from individuals before the COVID-19 pandemic had high levels of antibodies (Abs) to the RBD of common cold human coronaviruses (HCoVs) but no Abs against the RBD of SARS-CoV-1 and SARS-CoV-2. This suggests that common HCoVs do not cause false positives in serological SARS-CoV-2 tests, as previously speculated.
- No guinea pigs or pigs vaccinated with various zoonotic CoVs had Abs that cross-reacted with the recombinant SARS-CoV RBDs.
- In 63 patients with PCR-confirmed SARS-CoV-2, sensitivity was 98% and 81% for total Ig and IgM respectively 9 days or more after symptom onset. Sensitivity was only 57% and 43% for total Ig and IgM respectively 7-8 days post symptom onset.
- There was cross reactivity observed between RBD of SARS-CoV-1, in SARS-CoV-2 infected patients.
- Most patients underwent seroconversion between days 7-9 post-symptom onset.
- All SARS-CoV-2 infected individuals had Abs which bound to common HCoVs.
- Total RBD-binding Ab levels strongly correlated with the levels of neutralising Abs in 50 SARS-CoV-2 patients.
- Patients with high IgM levels were found to have higher neutralising Ab titres.
- No patients developed neutralising Abs in the first 8 days of being symptomatic. However, 91% of patients developed them by day 21.
- High Ab binding to the SARS-CoV-2 RBD correlated with robust neutralising Ab titres, suggesting RBD ELISA's can be used as a surrogate for neutralizing potential in SARS-CoV-2 patients.

**Highlights:**

- Evaluates the specificity and sensitivity of SARS-CoV-2 serological assays against other CoVs.

**Clinical Impact:**

- Minimal

**Important Methodologies:**

- Recombinant RBD ELISA.
- Luciferase neutralisation assay.

**Limitations:**

- No clinical data given for SARS-CoV-2 patients in this study. No indication to severity of disease which may affect Ab titres in sera.

# Vaccines

## Development of an Inactivated Vaccine Candidate, BBIBP-CorV, with Potent Protection against SARSCoV-2

Wang, H. *et al*. 2020. *Cell*

**Link:** https://doi.org/10.1016/j.cell.2020.06.008

**Summary:**

An inactivated vaccine generated from a genetically stable patient derived culture was able to induce high titres of neutralizing antibodies across several vaccination programs in several mammalian species. This was apparent in as few as 7 days following first dose administration. Vaccinated rhesus macaques which received a high dose had undetectable viral load in the throat at day 7 following SARS-CoV-2 challenge and lowered viral load relative to low and placebo groups in their anal swabs. In the lung, only the placebo group had viral load or severe pneumonia histopathology. The vaccine appears to be safe in several animal models.

**Main Findings:**

- Identification of a patient derived culture of SARS-CoV-2 with stability across ten *in vitro* passages indicating genetic stability which can be inactivated and used to generate a vaccine with components recognized by patient convalescent sera.
- Detectable neutralising antibody of all dose sizes (2, 4 and 8 ug/dose) 21 days after first administration across several species. Following single administration (rabbits, guinea pigs, rats and mice) and three administrations 7 days apart (cynomolgus monkeys, rabbits, guinea pigs, rats, mice). At all tested doses (in mice) neutralizing antibody titre reached its maximum 7 days after final administration and increased titre if two- and three-doses were given on different dose programs.
- In vaccinated rhesus macaques that received two spaced, little difference was observed in neutralising antibody titres between low and high dose groups (2 and 8 ug/dose) and this increased with time from 7 to 31 days.
- Viral load of throat swabs remained high in the placebo group up to day 7 after virus challenge, in the low dose group 3/4 and in the high dose group 4/4 had undetectable viral load. Interestingly, on day 5 after challenge the load was comparable in placebo and low dose groups but not high dose groups.
- Viral load of anal swabs was comparable for low dose and placebo across all 7 days post challenge, but high dose had lowered viral loads.
- At day 7 in the lung, viral load was present in the left and right lower lung as well as the right accessory lung in the placebo group. None was detected in either groups which received the vaccine.
- All vaccinated groups had either normal or mild interstitial lung pneumonia pathology relative to placebo groups who had severe interstitial pneumonia.

- No differences in weight or histopathology between placebo groups and treated groups of rats (3x dose/rat which is 900-times human dose).
- No evidence of systemic anaphylaxis in Guinea pigs (0.1x, 1x, negative and positive control) by clinical observations or body weight.
- No evidence of long-term vaccine toxicity in Cynomolgus monkeys (2, 4 and 8 ug/monkey) with no cases of death, impending death, or histopathological differences relative to control groups. One exception, mild to severe granulomatous at the site if injection from day 25. Several blood parameters were assessed including lymphocyte subgroup distribution and cytokines (IL-2, IL-4, IL-5 and IL-6) but nothing was found abnormal.

**Highlights:**

- In a SARS-CoV-2 model capable of inducing severe pneumonia, a conventional inactivated vaccine was able to prevent severe pneumonia like ChAdOx1. Unlike ChAdOx1, this vaccine can eliminate viral load in the lung.

**Clinical Impact:**

- High. The authors are progressing to phase I and II clinical trials and if successful this represents a 'conventional' vaccine for SARS-CoV-2 able to induce protective B-cell immunity.

**Important Methodologies:**

- Virus passage in WHO approved Vero cell lines, virus production, inactivation, and vaccine preparation.
- Anti-body neutralisation assays
- Molecular Biology: RT-PCR, Western-blotting
- Intraperitoneal and intramuscular vaccination of several mammalian species (cynomolgus monkeys, rhesus macaques, mice, rats, rabbits, guinea pigs).
- SARS-CoV-2 challenge of vaccinated rhesus macaques.
- Histopathology.
- Biochemical blood tests including cytokine analysis and lymphocyte sub-setting.

**Limitations:**

- Whilst the authors do assess lymphocyte sub-group distributions of cynomolgus monkeys, these T cells (and those of rhesus macaques) are not assessed for specificity for SARS-CoV-2 *ex vivo* or phenotyped for functional exhaustion or activation. As T cell mediated immunity appears important during SARS-CoV-2 infection this would be good to see.