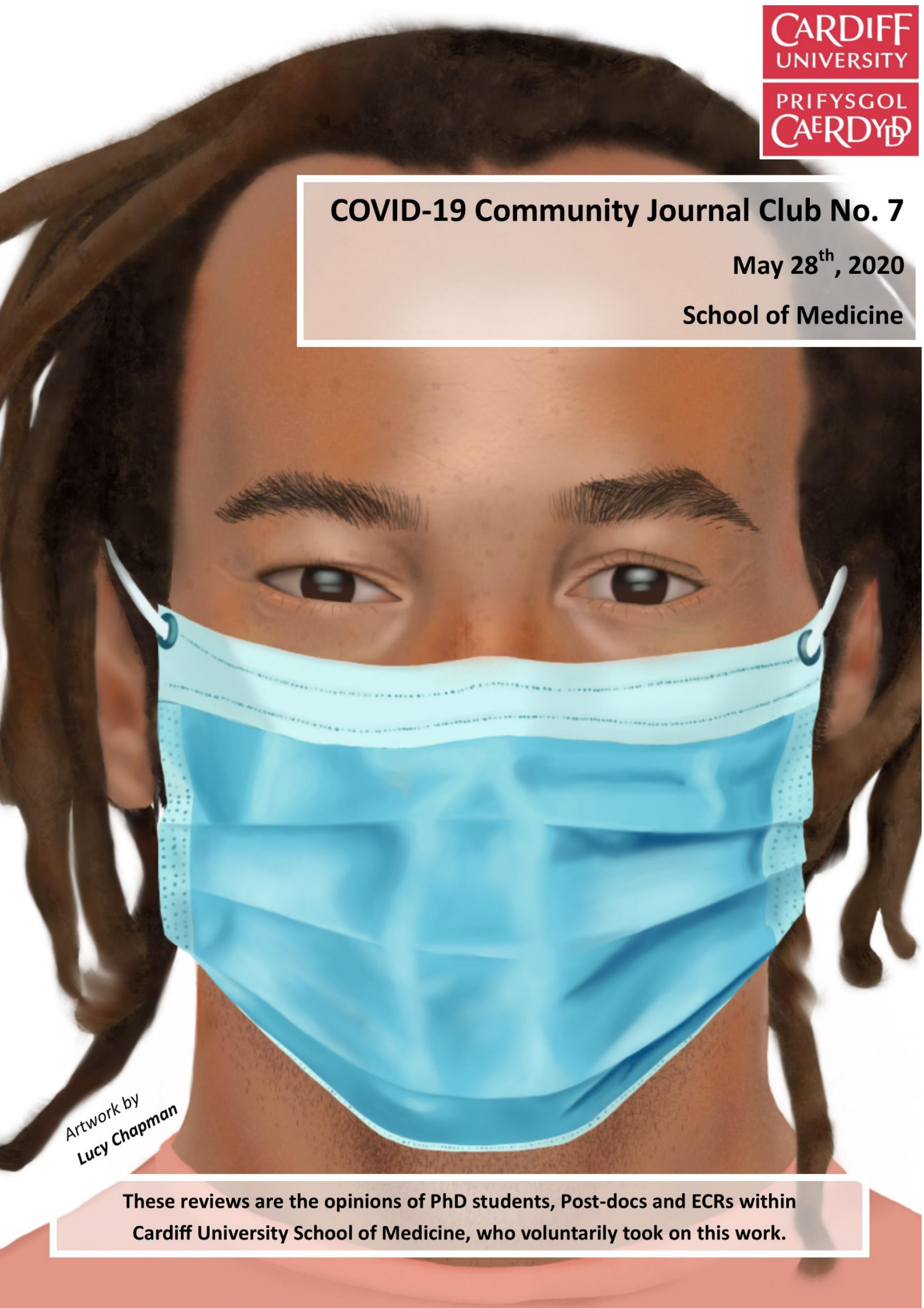

COVID-19 Community Journal Club No. 7

May 28th, 2020

School of Medicine

Artwork by *Lucy Chapman*

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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Please direct any comments or queries to Awen at gallimoream@cardiff.ac.uk

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Link: https://doi.org/10.1016/j.celrep.2020.107725

SARS-CoV-2: Structures and Genetics

Mechanisms of Disease

Vaccines

Epidemiology

Reviews and Perspectives

Developing COVID-19 Vaccines at Pandemic Speed

Lurie, N. *et al*. 2020. *NEJM*
Link: https://www.nejm.org/doi/full/10.1056/NEJMp2005630

An Audio interview with Dr. Lurie is available at the above link

The "Coalition for Epidemic Preparedness Innovation" (CEPI) is a nongovernmental, international organisation that supports rapid vaccine development against current epidemic pathogens, such as SARS-CoV-2, whilst funding technologies to prepare for those in the future.

Rapid vaccine development aims to get from viral sequencing to clinical trials within 16 weeks. To aid this, a new "outbreak paradigm" is proposed whereby developmental and manufacturing phases run in parallel rather than sequentially. However, the risk of financial loss is greatly increased: large scale manufacturing occurs before safety and immunogenicity data is available. DNA- and RNA-based vaccines are the most promising candidates to develop vaccines quickly.

Challenges for SARS-CoV-2 vaccine development

- Optimisation of target antigen (Spike protein) is required
- Duration of immunity from vaccination and number of doses required is still unknown
- Antibody dependent enhancement may occur following SARS-CoV-2 vaccination

Conducting clinical trials with new vaccine candidates during a pandemics poses its own challenges. Trial design may have to be adapted to remove randomisation and placebo-control in a high-mortality situation. Alternatively, various candidates can be compared against one another or the difference between early and late vaccination can be explored. In the months following a pandemics peak it is important that numerous trials do not crowd sites or burden the ethics and regulatory authorities of countries.

Moving forward, a global financing effort is required to combat a pandemic to support a fair vaccine allocation system in which the most vulnerable populations are prioritised. Additionally, storage of promising vaccine candidates that pass phase 2 clinical trials is critical, should a future coronavirus outbreak occur.

Why do some COVID-19 patients infect many others, whereas most don't spread the virus at all?

Kupferschmidt, K. 2020. *Science*
Link: https://doi.org/10.1126/science.abc8931

SARS-CoV-2 "super-spreading" events are common, where one person can infect lots of people.

Social distancing has reduced the SARS-CoV-2 Reproduction number (R), but as we start to come out of lockdown it is important to quantify other factors that might predict transmission.

Epidemiologists also look at the virus dispersion factor (k), a lower k value indicates a small number of people are causing more transmission i.e. super-spreading.

Researchers are still debating the k for COVID-19, though it is likely higher than 0.1 as most infections where k ≤0.1 die out before spreading to other countries.

As SARS-CoV-2 relies aerosol transmission factors impacting viral dispersion may include:

- **The viral load/shedding of an infected individual-** Timing is also key here, as individuals are highly infectious for a short period.
- **Vocal Activity-** singing/shouting are thought to cause more dispersion than talking.
- **Enclosed spaces-** China identified 318 clusters that resulted in 3+ cases only 1 was outdoors. Japan also estimates transmission risk 19x higher indoors than outside.
- **Amount of social contact-** proximity, hugging and handshakes all increase risk.
- **Low temperatures-** may help the virus survive for longer.
- Ignoring preventative methods such as hand washing

As we start to come out of lockdown, it is hoped that more data can be gathered as to what factors result in super-spreading.

However, the collation of this data presents several problems. If asymptomatic infections are missed, then data will be inaccurate. Individuals are also prone to recall bias; people often misremember the size of the event and what happened. There are also concerns collecting this data could impinge on individual's privacy.

Therefore, epidemiologists will have to consider these factors in study design. Clear benefits of studying super-spreading events include identifying "high-risk" activities improving infection control and ensuring future social distancing measures could be tailored rather than a more generic shut-down.

Harnessing the natural anti-glycan immune response to limit the transmission of enveloped viruses such as SARS-CoV-2

Breiman, A. *et al*. 2020. *PLOS Pathogens*
Link: https://doi.org/10.1371/journal.ppat.1008556

Breiman et al. have previously shown that it is possible to specifically block the interaction of SARS-CoV with ACE2 (a host receptor used by SARS-CoV and SARS-CoV-2 for entry into cells) by anti-A blood group antibodies in a dose-dependent manner *in vitro*. Consequently, Breiman et al. hypothesize that as SARS-CoV-2 is produced in cells co-expressing ACE2 and A/B blood group antigens, it will harbour the corresponding glycan epitopes and could be neutralized by anti-A and/or anti-B antibodies. They seem to suggest that individuals with blood group O might be less likely to contract Covid-19 than non-O individuals due to presence of anti-A and anti-B antibodies; ultimately blocking potential transmission from infected individuals with blood groups A, B, or AB (provided anti-A/B titres are high enough). Figure 1 (https://doi.org/10.1371/journal.ppat.1008556.g001) illustrates the potential effect of ABO blood group on viral transmission.

Overall, Breiman et al. propose that 'enhancing innate anti-viral protection conferred by natural anti-glycan antibodies' could potentially help impair viral transmission within the human population. Furthermore, they indicate that this approach might help increase the efficacy of SARS-CoV-2 vaccines; where SARS-CoV-2 spike proteins could be produced in cells expressing enzymes equipped to synthetize A/B antigens, leading to generation of neutralizing anti-spike antibodies as well as anti-A/B responses in 'all cases of ABO incompatible transmissions'. It is important to note that titres of anti-A/B antibodies are highly variable in the human population and may not completely block transmission, especially since SARS-CoV-2 has a strong binding affinity to ACE2. Raising the anti-A/B antibody titres in the whole population could have adverse effects such as septic complications during incompatible platelet transfusions or increased risk of haemolytic disease of the new-born due to ABO incompatibility between mother and foetus/infant. This perspective is largely theoretical/hypothetical and the blocking effect of naturally existing antibodies need to be documented *in vitro* and *in vivo*. Functional assays are required to elucidate the effect of anti-A/B antibodies specifically on the interaction of SARS-CoV-2 and ACE2.

Kidney diseases in the time of COVID-19: major challenges to patient care

Rabb, H. 2020. *J Clin Invest*

Link: https://doi.org/10.1172/JCI138871

This is a viewpoint article outlining a number of kidney-specific aspects of COVID-19 infection and the need for clinical and basic research into COVID-19 and kidney diseases.

Main points raised:

- SARS-CoV-2 can enter kidney epithelial cells via the ACE2 receptor, potentially resulting in injury to the cells, disrupting the whole body fluid, acid-base and electrolyte homeostasis, endocrine production of erythropoietin and vitamin D as well as becoming a viral reservoir. ACE inhibitors and angiotensin receptor blockers are used in renal diseases, it appears that health benefits of these drugs outweigh potential risks in patients infected with SARS-CoV-2, but it is not clear.
- Patients that use dialysis have depressed immune systems, often along with other comorbidities, and must visit the dialysis centre three times a week, which increases chance of infection. Additionally, SARS-CoV-2 infection increases inflammation and D-dimers, which can clot dialysis circuits, it is therefore important to determine how best to dialyse patients.
- Most live kidney transplantations are currently on hold as almost all kidney transplant patients are on immunosuppressive medication, making them more susceptible to infections. New diagnostic techniques are required to screen donors and recipients for COVID-19 and reduction of immunosuppression in stable patients is important.
- In a study carried out on 710 hospitalised patients, 74.2% had indicators of kidney impairment associated with high mortality, possibly due to deleterious lung-kidney crosstalk during COVID-19; therefore sensitive biomarkers of kidney damage are required for SARS-CoV-2 patients.

COVID-19: the vasculature unleashed

Teuwen, L. *et al*. 2020. *Nature Reviews Immunology*
Link: https://doi.org/10.1038/s41577-020-0343-0

Teuwen and colleagues have postulate in this commentary that endothelial cells (ECs) are essential to the initiation and propagation of severe COVID-19, discussing the link between endothelial cells, viral infection and inflammatory changes.
The pulmonary complications developed after infection result from a vascular breach that leads to tissue oedema, endotheliitis, activation of coagulation pathways and deregulated inflammatory cell infiltration. The authors hypothesise that vascular leakage is caused by multiple mechanisms:

1. The virus can directly affect ECs in several organs, and infected ECs are characterised by EC dysfunction, lysis and death.
2. By binding to the ACE2 receptor to enter cells, SARS-CoV-2 impairs the activity of this receptor, indirectly activating the kallikrein-bradykinin pathway and increasing vascular permeability.
3. Activated neutrophils, recruited to pulmonary ECs, produce ROS.
4. Immune cells, inflammatory cytokines and vasoactive molecules lead to enhanced EC contractility and loosening of endothelial junctions, creating inter-endothelial gaps.
5. IL-1β and TNF activate glucuronidases, which degrade the glycocalyx and upregulate the deposition of extracellular matrix, promoting fluid retention.

Together, these mechanisms lead to increased vascular permeability and vascular leakage. This raises the question whether vascular normalisation strategies during the maladapted immune response could be useful. Some clinical trials are ongoing to test this hypothesis.

Journal Reviews

Asymptomatic Carriage: Screening and Transmission

COVID-19: PCR screening of asymptomatic health-care workers at London hospital

Treibel, T.A. *et al*. 2020. *The Lancet*
Link: https://doi.org/10.1016/S0140-6736(20)31100-4

Summary:
The authors present the qPCR results of 400 healthcare workers within Bart's Health NHS trust who self-declared as fit and healthy at the time of enrolment. Nasal and blood samples were taken over 16 weeks (once per week). At the time of lockdown infection in the UK peaked and 7.1 % of healthcare workers were SARS-CoV-2 positive and asymptomatic. This decreased week on week with the number of new patients in London and reached 1.1 % in week 5 post-lockdown.

Main Findings:

- The authors present the qPCR results of 400 fit and healthy healthcare workers within Bart's Health NHS trust and at the time of lockdown 7.1 % of healthcare workers were SARS-CoV-2 positive and asymptomatic. This decreased with the general population's level of infection.

Highlights:

- The timing and trend of asymptomatic infection among healthcare workers matches that of the local general population (London) indicating that transmission was within the general community rather than at the hospital.
- The similarity in trend could allow testing of asymptomatic health care workers to act as a predictor of local prevalence of SARS-CoV-2 within the population.

Clinical Impact:

- Moderate, this could have an impact on healthcare worker testing practises.

Important Methodologies:

- Nasal and blood samples were taken over 16 weeks (once per week) of 400 healthcare workers enrolled in the study.
- qPCR based testing of the enrolled healthcare workers.

Limitations:

- It would be nice to see raw qPCR data relative to positive controls. However, the testing facilities are used clinically, and good practice should be in place.
- The authors suggest: as the prevalence of healthcare workers which are positive for SARS-CoV-2 infection and asymptomatic is low, their effect on transmission is low. There is no data to determine the truth of this here, however Arons et al. reported in the New England Journal of Medicine that pre-symptomatic patients could produce viral cultures 1-6 days before the onset of symptoms. To support their claim, it would have been good to test the saliva of health workers here to determine if non-symptomatic carriers could also produce viral cultures.

Screening of healthcare workers for SARS-CoV-2 highlights the role of asymptomatic carriage in COVID-19 transmission

Rivett, L. *et al*. 2020. *eLife*
Link: https://doi.org/10.7554/eLife.58728

Summary:
Rivett and colleagues report a screening programme of 1032 asymptomatic health care workers from Cambridge University Hospital over a 3 week period in April 2020. 3% of asymptomatic staff were swab positive of these 57% were truly asymptomatic or minimal symptoms. The rate of positive swab increased on wards with known SARS-CoV-2 infection. Clusters of infection were identified on 2 different wards.

Main Findings:

- 5% of swabs were PCR positive. 3% of asymptomatic staff were PCR+
- 21/1270 Individuals had repeat swabs all remained negative
- 14% of symptomatic HCW were PCR +ve
- Wards with known COVID patients had twice the PCR + rate in HCW compared to green wards (6.4 v 3.2%)
- Asymptomatic HCW had a higher PCR cycle threshold indicating a lower viral load
- On 1 ward 3 separate linages of SARS-CoV-2 were found in asymptomatic staff.
- Use of a symptomatic scoring system gave a strong pre-test probability of COVID in the symptomatic group

Highlights

- A significant proportion of asymptomatic staff were PCR+
- COVID wards are associated with increased proportion of PCR+ staff contrary to previous studies indicating non patient facing staff were as likely as patient facing staff to contract SAR-CoV-2. This may reflect the lower community prevalence of COVID in East Anglia

Clinical Impact:

- Argues strongly in favour of routine staff screening is initiated as standard across high and low risk clinical areas to prevent hospital associated infections (particularly in non-COVID areas).

Important Methodologies:

- Use of a symptom scoring system with good pre-test prediction for positive PCR
- SARS-CoV-2 lineage sequencing

Limitations:

- Self-reporting of symptoms
- Self-swabbing for PCR

The airborne lifetime of small speech droplets and their potential importance in SARS-CoV-2 transmission

Stadnytskyi, V. *et al*. 2020. *PNAS*
Link: https://doi.org/10.1073/pnas.2006874117

Summary:
Stadnytskyi et al. describes the size and lifetime of droplets in the air within a closed stagnant air environment produced from speaking using observations from a highly sensitive laser light scattering. They found that loud speech can emit thousands of oral fluid droplets per second. Droplets disappear from the window of view with time constraints in the range of 8-14 mins, corresponding to droplet nuclei of 4 *ca.* 4 µm diameter, or 12- to 21-µm droplets prior to dehydration. These observations confirm that there is a substantial probability that normal speaking causes airborne virus transmission in confined environments.

Main Findings:

- Respiratory viruses can be transmitted via oral fluid droplets that are generated by coughing, sneezing or normal speech.
- High viral loads of SARS-CoV-2 have been detected in oral fluids of COVID-19 patients, including asymptomatic ones.
- Average droplet emission rates of *ca.* 1,000 s^{-1} with peak emission rates as high as 10,000 s^{-1}, with a total integrated volume far higher than in previous reports. The high sensitivity of this method in observing medium-sized (10 µm to 100 µm) droplets, a fraction of which remain airborne for at least 30 s, likely accounts for the large increase in the number of observed droplets.
- Dehydration in the air causes the diameter of the droplets to shrink to about 20-34% of its original size. Range in dehydration due to differential degrees of dehydration in

oral cavity and can be associated with age. Smaller, dehydrated droplets fall at a slower speed.

- For COVID-19, with an oral fluid average virus RNA load of 7×10^6copies per milliliter (maximum of 2.35×10^9 copies per milliliter), the probability that a 50-µm-diameter droplet, prior to dehydration, contains at least one virion is ~37%. For a 10-µm droplet, this probability drops to 0.37%, and the probability that it contains more than one virion, if generated from a homogeneous distribution of oral fluid, is negligible.
- We estimate that 1 min of loud speaking generates at least 1,000 virion-containing droplet nuclei that remain airborne for more than 8 min. These therefore could be inhaled by others potentially trigger a new SARS-CoV-2 infection.
- The smallest droplet nuclei effectively remain airborne indefinitely and have half-lives that are dominated by the ventilation rate, at a saliva viral load of 7×10^6 copies per milliliter, the probability that a 1-µm droplet nucleus (scaled back to its originally hydrated 3-µm size) contains a virion is only 0.01%.

Highlights:

- Oral droplets produced through speaking represent a risk for the spread of SARS-CoV-2.

Clinical Impact:

- Consider risk of spreading disease through speaking and importance of PPE at all times within clinical settings.

Important Methodologies:

- Highly sensitive laser light scattering observations, generated by a 25s burst of repeatedly speaking ‘stay healthy’ in a loud voice.

Limitations:

- Infected individuals have different viral loads in their saliva therefore predicting the risk of virus spread is difficult.
- Droplets of all sizes are unable to be detected by this method.
- Different environmental conditions will affect the likelihood of virus spread through speech.

Clinical

Viral and host factors related to the clinical outcome of COVID-19

Zhang, X. *et al*. 2020. *Nature*
Link: https://doi.org/10.1038/s41586-020-2355-0

Summary:
Zhang et al. analysed viral genomes, and immunological date from 326 confirmed COVID-19 cases in Shanghai. Sequencing suggested 2 different lineages with differential exposure history, but no differences in clinical manifestation and outcomes. Immunological data suggested that lymphocytopenia and high levels of IL6 and IL8 were associated with more severe disease outcome. Overall, the authors suggest that host factors rather than differences in the viral genome affect disease severity.

Main Findings and Highlights:

1. Phylogenetic analysis of viral genomes from 94 cases from Shanghai and 221 cases from the GISAID database revealed two major glades distinguished by two linked variations ORF8: p.84L>S (28144T>C) and ORF1ab: p.2839S (8782C>T).
   Six cases with clear contact history to Huanan Seafood Wholesale Market (suspected initial outbreak site) were all clustered into clade I, while three cases diagnosed at the same period without contact history to Huanan Seafood Market were all clustered into clade II. These two clades likely represent two lineages derived from a common ancestor that independently evolved in early December 2019 in Wuhan, only one of which (Clade I) was spawned within the Huanan Seafood Market.

2. There was no difference in clinical manifestations (lymphocyte count, D-Dimer, CRP, duration of viral shedding) or disease severity between the two clades. This indicates a stable evolution of the virus and that host factors are mainly responsible for disease outcome.

3. Severe and critical cases showed prominent lymphocytopenia. The most suppressed cell type was CD3 T-cells. B cells and NK cells showed less suppression. Furthermore, IL6 and IL8 were negatively correlated with T cell count and high levels of IL 6 were related to disease severity.

Clinical Impact:

- Low. Since there is no difference in disease manifestation between the virus lineages, sequencing of viral genomes is unlikely to predict disease outcome.
- The association of a reduced T cell count and elevated cytokine levels with severe disease is already known.

Important Methodologies:

- Phylogenetic analysis of viral genomes.

Limitations:

- I am not overly familiar with phylogenetic analysis or with the analysis of genome sequencing, so I am not sure about limitations of this paper. I could imagine that a sample size of 326 is relatively low. I also think that further genome analysis has to be done on viral isolates from other parts of the world to track mutations possibly associated with ethnic differences to completely rule out an influence of the viral factors on disease outcome.

Diagnostics and Therapeutics

SARS-CoV-2 detection with CRISPR diagnostics

Guo, L. *et al*. 2020. *Cell Discovery*
Link: https://doi.org/10.1038/s41421-020-0174-y

Summary:
Guo *et al*. present CASdetec (CRISPR assisted detection), a method developed for the detection of SARS-CoV-2 nucleic acid. Following their previously established SARS-CoV-2 detection protocol CDetection (Cas12b mediated DNA detection), CASdetec is optimised for the highly-specific detection of the SARS-CoV-2 target RNA-dependent RNA polymerase (RdRp). Amplification of RdRp in *E.Coli* cells expressing SARS-CoV-2 RdRp coupled with optimised single-guide RNA (sg-RNA) design and enhanced fluorescence signal enables the rapid and specific detection of SARS-CoV-2 with no cross-reactivity to other human coronaviruses.

Main Findings:
- Detection of SARS-CoV-2 nucleic acid at the lower end of the detection limit (5×10^3 copies/mL) was made possible by optimisation of recombinase-aided amplification (RAA) primers matching sgRNA-3
- sgRNA and primers were highly specific to SARS-CoV-2 nucleic acid and not cross-reactive with other human coronaviruses SARS-Co-V, MERS-CoV, CoV-HKU1, CoV-229E, CoV-OC43 and CoV-NL63
- Increased accuracy of SARS-CoV-2 nucleic acid detection by nucleic acid amplification and CDetection in the same tube, reducing aerosolisation, cross contamination and false-positive results
- Limits of nucleic acid detection tested using lentiviruses containing SARS-CoV-2, SARS-Co-V and MERS-CoV RdRp developed to mimic isolation of patient samples in transport buffer. Nucleic acid detected at 5×10^4 copies/mL using lysis buffer and 1×10^4 copies/mL with spin columns. Therefore, spin columns are extraction methods suggested for use in hospitals and lysis buffer for point-of-care testing

Highlights:
- CASdetec represents a strategy than can be adapted for the detection of nucleic acid derived from other viruses

Clinical Impact:
- Moderate

Important Methodologies:

- Optimised design of primers and sg-RNAs for SARS-CoV-2 allows specific detection of SARS-CoV-2 nucleic acid and sensitivity to detect viral nucleic acid at the lower limit of detection
- (7) Poly-T fluorescence quenchers enhance fluorescence signal
- RT-RAA reaction optimised for use in a single tube with CDetection reagents to minimise contamination and false-positive results

Limitations:

- *E. coli* cells bearing Blunt-SARS-CoV-RdRp or Blunt-SARS-CoV-2-RdRp were used for developing assay. Protocol will be required for use in clinical isolates from SARS-CoV-2 patients
- sgRNA3 plot missing from Supplementary Figure S2. This is the sgRNA used for optimisation of the protocol

Artificial intelligence–enabled rapid diagnosis of patients with COVID-19

Mei, X. *et al*. 2020. *Nature Medicine*
Link: https://doi.org/10.1038/s41591-020-0931-3

Summary:
Chest CT scans are an important diagnostic tool for COVID-19, useful before definitive results are obtained by RT-PCR. However, some patients may have normal radiological findings at early disease stages. Mei and colleagues use artificial intelligence (AI) algorithms to integrate chest CT findings with clinical symptoms, exposure history and laboratory testing to rapidly diagnose patients. The AI system had equal sensitivity as compared to a senior thoracic radiologist and improved the detection of patients who were positive for COVID-19 via RT–PCR but presented with normal CT scans. When CT scans and associated clinical history are available, the proposed AI system can help to rapidly diagnose COVID-19 patients.

Main Findings:

- Patient's age, exposure to SARS-CoV-2, presence of fever, cough with sputum and white blood cell counts were significant features associated with SARS-CoV-2 status
- An AI model trained using only CT imaging data had 83.6% sensitivity and 75.9% specificity
- An AI model trained using only clinical data had 80.6% sensitivity and 68.3% specificity
- Instead, the AI model trained using both CT images and clinical data (joint model) achieved 84.3% sensitivity and 82.8% specificity, comparable to a senior thoracic

radiologist who, using both CT images and clinical data, achieved 74.6% sensitivity and 93.8% specificity.
- The joint model outperformed a thoracic radiology fellow who achieved 56.0% sensitivity and 90.3% specificity
- The joint model correctly diagnosed 17 of 25 patients positive for COVID-19 with a chest CT identified as normal, whereas the senior radiologist fellow identified 0 of 25 as disease positive.

Highlights:
- Combines CT imaging and clinical information to generate an AI algorithm for COVID-19 diagnosis that shows equivalent accuracy to a senior chest radiologist
- Illustrates the potential role for a highly accurate AI algorithm for the rapid identification of COVID-19 patients

Clinical Impact:
- Moderate

Important Methodologies:
- AI models developed using deep convolutional neural network and MLP machine-learning classifiers
- Compares the ability of AI models to diagnosis COVID-19 patients to that of a radiologist with 10 years' experience and to that of a radiology fellow

Limitations:
- Model compared only to the diagnostic abilities of two radiologists
- Limited sample size for model training
- Model trained using CT images from COVID-19 patients, limiting the usefulness of the current model to distinguish COVID-19 from other respiratory failures
- Use of the AI tool would require integration with radiology picture archiving and clinical database systems, something which may not be possible in all hospitals

Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion

Xia, S. *et al*. 2020. *Cell Research*
Link: https://doi.org/10.1038/s41422-020-0305-x

Summary:
Xia, S et al. report the high fusion capacity that SARS-CoV-2 exhibit in comparison with SARS-CoV showing that fusion machinery should be considered as a possible target for the development of coronavirus fusion inhibitors. By solving SARS-CoV-2 6-HB x-ray structure and conjugating the cholesterol molecule to EK1 peptide, they show the inhibitory activity and potency of the EK1C4 lipopeptide against SARS-CoV-2 S-mediated membrane fusion and PsV infection. They also demonstrate its efficiency in vitro and in vivo against other HCoVs such as MERS-CoV and HCoV-OC43. Their results suggest EK1C4 as a possible pan-CoV fusion inhibitor-based therapeutic and prophylactic for treatment and prevention of infection by the currently circulating SARS-CoV-2 and MERS-CoV, as well as future re-emerging SARS-CoV and emerging SARSr-CoVs.

Main Findings:
- S-receptor engagement is necessary for the S-mediated viral fusion and entry. The overall 6-HB conformation is an important and highly conserved component for determinate coronavirus.
- The lipidation of EK1 is a promising strategy to improve its fusion-inhibitory activity against SARS-CoV-2 infection. EK1C4 exhibits the most potent inhibitory activity against membrane fusion mediated by S proteins and entry of coronaviruses.
- Intranasally applied EK1C4 showed strong protection of mice against HCoV-OC43 infection

Highlights
- Found a possible fusion inhibitor against SARS-CoV-2 that could result in a good candidate to treat Covid-19 patients

Clinical Impact:
- High

Important Methodologies:
- Cell-cell fusion assay using Huh-7 and 293T/ACE2 cell as target cells and transfected 293T cells with an S protein expression vector as an effector cell.
- Inhibition of pseudotyped HCoV infection and live HCoV replication. Based on a luciferase assay.
- Circular dichroism spectroscopy
- Expression and purification of fusion protein HR1-L6-HR2 of SARS-CoV-2.
- Crystallization and structure determination.

Limitations:

- Whilst this study identifies EK1C4 as new candidate to be developed against SARS-CoV-2 they use a mouse models. Also they use new-born mice.
- In vivo prophylactic efficacy and therapeutic effect of EK1C4 only has been tested against HCoV-OC43 infected mice.

Efficacy and safety of current therapeutic options for COVID-19 - lessons to be learnt from SARS and MERS epidemic: A systematic review and meta-analysis

Zhong, H. *et al*. 2020. *Pharmacological Research*
Link: https://doi.org/10.1016/j.phrs.2020.104872

Summary:

Zhong et al. undertake a systematic review and meta-analysis to evaluate efficacy and safety of current options of therapies for SARS and MERS alongside COVID-19 with the aim to identify a promising therapy for SARS-CoV-2 patients. They assess 18 studies including 4,941 patients treated with hydroxychloroquine, lopinavir/Ritoavir, Ribavirin, Arbidol. They specifically analysed primary outcomes of mortality, virological eradication and clinical improvement. Secondary outcomes were improvement of symptoms and chest radiography results, incidence of acute respiratory disease syndrome, utilisation of mechanical ventilation and adverse events.

Main Findings:

- Anti-coronary virus interventions significantly reduced mortality (RR 0.65, CI 0.44 – 0.96: I^2 = 81.3%.
- Ameliorate clinical improvement (RR 1.52, 95% CI 1.05 – 2.19) and radio-graphical improvement (RR 1.62, 95% CI 1.11 – 2.36, I^2 = 11.0%.
- No clear effects on virological eradication, incidence of ARDs, intubation and AEs.
- The combination of ribavirin and corticosteroids decreased mortality (RR 0.43, 95% CI 0.27 – 0.68).
- Ribavirin induced more bradycardia, anaemia, and transaminitis.
- Lopinavir/ritonavir based combinations showed superior virological eradication and radio-graphical improvement with a reduced rate or ARDS.
- Hydroxychloroquine improved radio-graphical results but increased AE rate especially diarrhoea.
- Evidence on most outcomes was very low.

Highlights:

- No recommendation to use hydroxychloroquine in place of standard care for SARS-CoV-2 infection.
- Lopinavir/ritonavir combinations might play a role in eradication of SARS-CoV-2.

Clinical Impact:

- Minimal – may help clinicians understand advantages and disadvantages of each anti-coronavirus agent.

Important Methodologies:

- Literature search of main publication databases.
- Analysed data from randomised clinical trials (RCTs), prospective cohort and retrospective cohort studies.
- Summarise relative risks and 95% confidence intervals using random effect models appraised using GRADEpro.

Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis

Mehra, M.R. *et al*. 2020. *The Lancet*
Link: https://doi.org/10.1016/S0140-6736(20)31180-6

Summary:
This paper evaluated the safety and benefit of hydroxychloroquine or chloroquine, with or without macrolide in treatment regimens of COVID-19. The multi-centre study showed Chloroquine and hydroxychloroquine treatment was associated with no evidence of benefit, but was associated with an increase in the risk of ventricular arrhythmias and a greater hazard for in-hospital death with COVID-19.

Main findings:

- 96 032 patients from 671 hospitals in 6 continents were recruited.
- 14 888 patients were in four treatment groups (chloroquine alone, chloroquine with a macrolide, hydroxychloroquine alone, or hydroxychloroquine with a macrolide), and 81 144 were in the control group.
- **Include:** Patients received the treatment within 48 h of diagnosis.
- **Exclude:** Patients received the treatment more than 48 h after diagnosis or while they were on mechanical ventilation, as well as received remdesivir.

	Control group	chloroquine alone	chloroquine with a macrolide	hydroxychloroquine alone	hydroxychloroquine with a macrolide
Patients Number	81 144	1868	3783	3016	6221
Daily Does		765 mg	790 mg	596 mg	597 mg
Mortality	9·3%	16·4%	22·2%	18·0%	23·8%
De-novo ventricular arrhythmia	0·3%	4·3%	6·5%	6·1%	8·1%
ICU length of stay, days	2·6	4·3	4·9	4·3	4·7
Total length of stay, days	11·7	13·2	13·8	13·2	13·8

Highlights:

- Not observed any benefit of hydroxychloroquine or chloroquine on in-hospital outcomes, when initiated early after diagnosis of COVID-19.
- Chloroquine or hydroxychloroquine treatment was associated with an increased hazard for clinically significant occurrence of ventricular arrhythmias and increased risk of in-hospital death with COVID-19.

Clinical Impact:

- Important

Limitations:

- This study did not include all the situations, such as ambulatory, out-of-hospital setting.
- This study only explored the drug treatment regimens with the occurrence of ventricular arrhythmias, but did not measure QT intervals, nor did stratify the arrhythmia pattern.
- The author did not investigate the link between the death rates and patients' cardiovascular risk.
- The author did not conduct dose-response analysis.

Antibody Responses

SARS-CoV-2 infection protects against rechallenge in rhesus macaques

Chandrasheka, A. *et al*. 2020. *Science*
Link: https://doi.org/10.1126/science.abc4776

Summary:
A crucial clinical question during the COVID-19 pandemic, is whether infection with SARS-CoV-2 results in protective immunity. A rhesus macaque model of SARS-Cov-2 infection demonstrated high viral load in both upper and lower respiratory tracts and overall acute viral pneumonia. Humoral and cellular immune responses were also observed. Re-challenge at day 35 found a significant reduction in viral loads in bronchoalveolar lavage (BAL) and induction of anamnestic immune responses. This study provides evidence that SARS-CoV-2 infection produced protective immunity against re-exposure in nonhuman primates.

Main Findings:

- In 9 rhesus macaques infected with SARS-CoV-2, viral RNA peaked at day 2 and was resolved by day 10-14 in BAL, and day 21-28 in nasal swab (NS).
- Subgenomic mRNA (sgmRNA), used as a measure of new viral replication, was also highest at day 2 of infection.
- By day 35, both binding and neutralising antibody responses to SARS-CoV-2 spike (S) protein were detected.
- Antibodies of multiple subclasses were detected against various SARS-CoV-2 antigens with diverse effector functions.
- Both $CD4^+$ and $CD8^+$ T-cell responses against pooled S peptides were found, using IFNγ ELISPOT assays.
- Using autopsy; on day 2 and 4 of infection, high levels of viral RNA were found in respiratory tract tissues with low levels in distant organs (GIST, liver, kidney).
- Day 2 of infection showed evidence of viral pneumonia, as well as multifocal clusters of virus infected cells in areas of acute inflammation with neutrophil infiltration.
- Upon re-challenge on day 35 of infection, median peak viral loads were >5.1 $\log_{10}$ lower in BAL and >1.7$\log_{10}$ lower in NS compared to primary challenge.
- sgmRNA median peak levels were >4.8 $\log_{10}$ lower in NS following re-challenge as compared to primary challenge. Plaque assays in BAL and NS samples also showed no recoverable virus.
- Little to no clinical disease was observed upon re-challenge.
- Neutralising antibody titres were higher on day 14 of re-challenge compared to primary challenge, with overall anamnestic antibody responses observed.

Highlights:

- Successful SARS-CoV-2 model in rhesus macaques used to understand protective immune responses following infection.

Clinical Impact:

- Moderate.

Important Methodologies:

- Histopathology, IHC, RNAscope and cyclic immunofluorescence imaging of tissues.

Limitations:

- Nonhuman primate model did not lead to respiratory failure as seen in humans.

Potent Neutralizing Antibodies against SARS-CoV-2 Identified by High-Throughput Single-Cell Sequencing of Convalescent Patients' B Cells

Cao, Y. *et al*. 2020. *Cell*

Link: https://doi.org/10.1016/j.cell.2020.05.025

Summary:

Cao *et al*., reports on rapid identification of SARS-CoV-2-neutralising antibodies by high-throughput single-cell RNA and VDJ sequencing of antigen-enriched B cells from 60 convalescent patients. From 8,558 antigen-binding $IgG1^+$ clonotypes, 14 potent neutralising antibodies were identified, with the most potent one, BD-368-2, exhibiting an IC_{50} of 1.2 and 15 ng/mL against pseudotyped and authentic SARS-CoV-2, respectively. BD-368-2 also displayed strong therapeutic and prophylactic efficacy in SARS-CoV-2-infected hACE2-transgenic mice. Additionally, the 3.8 A cryo-EM structure of a neutralising antibody in complex with the spike-ectodomain trimer revealed the antibody's epitope overlaps with the ACE2 binding site. They demonstrated that SARS-CoV-2-neutralising antibodies could be directly selected based on similarities of their predicted $CDR3_H$ structures to those of SARS-CoV-neutralising antibodies. Overall, they showed that high-throughput single-cell sequencing could lead to the identification of highly potent neutralising mAbs that have strong therapeutic and prophylactic efficacy, which could greatly assist in the intervention of prevailing and emerging infectious diseases, such as COVID-19.

Highlights:

- 8,558 $IgG1^+$ antigen-binding clonotypes were identified by high-throughput scRNA/VDJ-seq.
- 14 potent SARS-CoV-2 neutralising antibodies were found from 60 convalescent patients.
- BD-368-2 showed high therapeutic and prophylactic efficacy in SARS-CoV-2-infected mice.
- Neutralising antibodies can be directly selected based on predicted CDR3H structures.

Clinical Impact:
Potentially high - Clinical trials using BD-368-2 are underway.

Important Methodologies:
- 10X single-cell 5' mRNA and VDJ sequencing.
- ELISA quantification.
- Pseudovirus neutralisation assay.
- Cryo-EM.

Limitations:
- Small sample size; a total of 64 confirmed COVID-19 patients were enrolled in this study. 12 of these 64 were enrolled in the preliminary study without antigen-enrichment.
- Convalescent patient taken all enrolled from the same hospital in China.
- Patient background information not described e.g. age, gender and ethnicity.
- There may be differences in the genetic profiles of secreted antibodies from diverse ethnic groups.

Cross-reactive antibody response between SARS-CoV-2 and SARS-CoV infections

Lv, H. *et al*. 2020. *Cell Reports*
Link: https://doi.org/10.1016/j.celrep.2020.107725

Summary:
Lv *et al.* studied antigenic differences between SARS-CoV-2 and SARS-CoV, using both human samples and mouse models. They found that while cross-reactivity in antibody binding to the spike (S) protein was common (in both directions), cross neutralization of the live viruses appeared to be rare i.e. there was a non-neutralizing antibody response to conserved epitopes in the spike. This raises the question as to whether a non-neutralizing antibody response could be generated by a vaccine or by infection with SARS-CoV-2. If so, this could lead to antibody-dependent disease enhancement upon future infection with SARS-CoV-2

Main Findings:
Using plasma from SARS-CoV-2 patients:
- From day 11 after onset of symptoms, plasma has antibodies with significant binding to the S ectodomain, S2 subunit and receptor binding domain (RBD) of SARS-CoV-**2**. (Different ELISAs were used to target the whole ectodomain, or specific subunits of it, in order to overcome any conformational blocking of antibody binding).
- Antibodies could also cross-react with the SARS-CoV S ectodomain and RBD

- Except for plasma samples that came from patients with less than 12 days post-symptom onset, all other plasma samples could neutralize the SARS-CoV**-2** virus
- However, only one plasma sample could cross-neutralize SARS-CoV, with very low neutralization activity.

Using plasma from SARS-CoV patients:

- SARS-CoV antibodies have significant cross-reactivity in binding to SARS-CoV**-2** spike, RBD and S2 subunit
- While five of the seven plasma samples from SARS-CoV-convalescent patients could neutralize SARS-CoV none could cross neutralize SARS-CoV-2

Murine model of infection (intranasal) and immunisation (i.p. injection with adjuvant)

- Plasma from mice immunized with SARS-CoV**-2** virus have significant binding to its S ectodomain and RBD
- Plasma from mice immunized with SARS-CoV virus have significant binding to its S ectodomain and RBD
- Plasma from mice infected with SARS-CoV virus could react with its S ectodomain and RBD
- Antibody response from SARS-CoV**-2**-infected mice was not detected, as SARS-CoV**-2** can't replicate in mice
- Some cross-reactivity of plasma from SARS-CoV**-2**-immunized mice to both the SARS-CoV S ectodomain and RBD
- Some cross-reactivity of plasma from SARS-CoV-infected mice to the SARS-CoV**-2** S ectodomain but not to the RBD
- Despite cross reactivity in binding, cross-neutralization activity was not detected in any of the mouse plasma samples

Highlights:

- Cross-reactivity in binding is common between SARS-CoV and SARS-CoV**-2** infections in both directions.
- However cross-neutralization activity may be rare.
- Results from human samples replicated in mouse models

Clinical Impact:

- Implications for vaccine design, as it appears possible to generate a non-neutralising antibody response to SARS-CoV-2

Important Methodologies:

- Production of viral proteins
- Murine models of SARS-CoV-2 immunisation

Limitations:

- Small numbers of samples and suggestion that lack of antibody response in some patients was due to early stage of disease, but not clear why these patients were included in the study

SARS-CoV-2: Structures and Genetics

Structure of replicating SARS-CoV-2 polymerase

Hillen, H.S. *et al*. 2020. *bioRxiv*
Link: https://doi.org/10.1101/2020.04.27.063180

Summary:
Hillen et al. present the cryo-electron microscopic structure of the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) in active form, mimicking the replicating enzyme. The structure comprises three viral proteins (nsp12, nsp8 and nsp7) and two turns of RNA template-product complex. The analysis of this structure enables a better analysis of the inhibitory mechanism of drugs used for the treatment of COVID-19, such as remdesivir.

Main Findings:
- The structure of the active RdRp showed engagement with over two turns of duplex RNA, being similar to the free enzyme structure, but additionally revealing a long protruding RNA and extended protein regions in nsp8 (not observed in other viruses).
- Nsp12 binds the first turn of RNA; motif C of the active domain binds the RNA 3'-end and contains the residues required for RNA synthesis; motifs F and G position the RNA template.
- The RNA duplex that exits the RdRp cleft forms the second helical turn. This is flanked by long a-helical extensions (N-terminal regions in the 2 nsp8 subunits). These extensions are positively charged to interact with the RNA backbones, acting as sliding poles that slide along exiting RNA to prevent premature dissociation of the RdRp during replication.
- *In silico* modelling revealed that this RdRp is specific for RNA rather than DNA, and showed consistency with binding of the triphosphorylated form of remdesivir to the NTP site.

Highlights:
- Paves the way for future work on the mechanism of coronavirus replication, transcription and antiviral targeting.

Clinical Impact:
- Minimal

Important Methodologies:
- Cloning, protein expression and purification methodologies.
- Cryo-EM sample preparation, data collection and processing and analysis.

Limitations:

- It is unclear whether replication in infected cells results in RNA duplexes or if RNA strands are separated, as well as when and how RNA strands are separated during the transcription process, limiting the potential of this work.

Controlling the SARS-CoV-2 outbreak, insights from large scale whole genome sequences generated across the world

Phelan, J. *et al*. 2020. *bioRxiv*
Link: https://doi.org/10.1101/2020.04.28.066977

Summary:
This paper analysed the genome sequencing of COVID-19. So far the data showed relatively few evolutionary selection pressures. However, the author also warned with the rapid worldwide expansion into diverse human populations, significant genetic variations are becoming increasingly likely.

Main Findings:

- There are two main clusters (denoted C1 and C2), and six main clades (C1, C1.1, C2, C2.1, C2.1.1, and C2.1.2). C2 is more common in all continents. C1 is the most ancestral cluster.
- They found 14 sites with missense mutations.
  --The strongest signal was for mutation L37F in nsp6, which is involved in the formation of double-membrane vesicles that play a role in restricting autophagosome expansion.
  --The second strongest hit was the L5F mutation in the spike glycoprotein, which could be a result of selection pressure.
- Mutation in the primer binding site were observed, particularly in some countries in Latin America and Africa where >70% of samples carrying a mutation in the primer binding site.
- Mutations in the spike glycoprotein gene were also detected, though currently rare. Some of these mutations present on the interface between spike and ACE2 and could potentially affect vaccine performance if selection pressure drove them to higher frequencies.

Highlights:

- They established a websites to trace new mutations: http://genomics.lshtm.ac.uk/

Clinical Impact:

- Moderate

Important Methodologies:

- Full SARS-CoV-2 genome sequences were downloaded from the GISAID and NCBI
- IQTREE and BEAST software were used to reconstruct the phylogeny tree.
- The SARS-CoV-2 spike glycoprotein structure was downloaded from the PDB (6m0J) and visualised using chimera software.

Limitations:

- Relatively small proportion of sampled sequences (>15,000) compared to infections (>4,000,000).
- Samples are collected early in an outbreak when most genomes are still very similar.

Mechanisms of Disease

Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model

Rockx, B. *et al*. 2020. *Science*
Link: https://doi.org/10.1126/science.abb7314

Summary:
SARS-CoV-2 or MERS and compared the pathogenesis of infection with historical reports of SARS-CoV infection. Following infection SARS-CoV-2 was secreted from both the nose and throat in the absence of overt clinical signs. SARS-CoV-2 infection resulted in greater lung pathology and lesions in comparison to MERS infected animals. Increased age did not affect the disease outcome but did extend viral shedding. SARS-CoV-2 infection of cynomolgus macaques may provide a new model for both vaccine and therapeutic studies.

Main Findings:

- By day 14 post-inoculation (p.i.) all animals had seroconverted, with SARS-CoV-2 specific antibodies against the S1 domain and nucleocapsid proteins of the virus present in the sera.
- Both nasal and throat virus shedding peaked earlier in young animals than aged animals, with the aged group shedding virus for longer.
- Following autopsy at day 4 p.i., viral replication was primarily restricted to the respiratory tract and LN's. Highest viral levels present in the lungs.
- At autopsy of 4 macaques at day 4 p.i., 50% had pulmonary consolidation of the lungs, with 5 – 10% of lung tissue affected.
- Histology of lung lesions demonstrated diffuse alveolar damage (DAD), with characteristic features of; alveolar and bronchiolar epithelial necrosis, alveolar edema, hyaline membrane formation, accumulation of alveolar macrophages and to a lesser extent neutrophils and lymphocytes.
- Areas of advanced lung lesions showed; alveolar wall thickening lined with cuboidal epithelial cells (type II pneumocyte hyperplasia), aggregates of lymphocytes around pulmonary vessels and evidence of multi-nucleated giant cells (syncytia) free in the lumen of bronchioles and alveoli.
- SARS-CoV-2 antigen expression differed between animals but could only be detected in either the lungs or respiratory tract of infected animals.
- Unlike MERS which primarily targets type II pneumocytes, SARS-CoV-2 can also infect type I pneumocytes.

Highlights:

- Provides a model of Covid-19 like disease, with prolonged viral shedding. Viral replication in the upper respiratory tract suggests efficient viral transmission between

hosts, whilst lower respiratory tract viral replication models the development of lung disease.

Clinical Impact:

- Minimal

Important Methodologies:

- Development of a non-human primate model to study Covid-19 disease

Limitations:

- Very small numbers of cynomolgus macaques used in both young and aged adult groups, and only females studied. If macaques are true model of human disease, then a female only study skews towards a milder disease.
- Only one clinical isolate used for infection studies.

Replication of SARS-CoV-2 in human respiratory epithelium

Milewska, A. *et al*. 2020. *Journal of Virology*
Link: https://doi.org/10.1128/JVI.00957-20

Summary:
The Vero E6 cell line has serious limitations for studying SARS-CoV-2. Human coronaviruses use different entry pathways in immortalised cell lines compared to human epithelium; potentially impacting antiviral drug testing and development. A new *ex vivo* culture model has been developed using human airway epithelium (HAE) to study SARS-CoV-2. Efficient replication of the virus was observed in the tissue, with apical release and maximal replication two days post-infection. An Immuno-FISH imaging technique was utilised to visualise viral replication within ciliated cells. HAE *ex vivo* cultures provide a new method for SARS-CoV-2 research, which may better replicate *in vivo* virus infection.

Main Findings:

- Over a 4-day culture, HAE cells show apical infection/release of virus when inoculated with SARS-CoV-2, with maximal replication 2 days post-infection.
- Comparable amounts of virions were released from both HAE and Vero E6 cell cultures.
- SARS-CoV-2 replication was effectively blocked in both HAE and Vero E6 cell cultures by serum from recovered COVID-19 patients.
- RT-qPCR analysis showed nucleocapsid subgenomic mRNAs (sg mRNAs) were abundant in infected HAE cultures; a hallmark of active virus replication.
- Development of an immuno-FISH assay to visualise virus infection of ciliated cells in the respiratory epithelium.

Highlights:

- A new *ex vivo* model for studying SARS-CoV-2.

Clinical Impact:

- Minimal.

Important Methodologies:

- *In vitro* (Vero E6 cells) and *ex vivo* (HAE) cell cultures.
- RT-qPCR.
- Immuno-FISH.

Limitations:

- No sg mRNA analysis of Vero E6 cells.
- No clear evidence to show HAE is better for coronavirus research than Vero E6 cells.

Cigarette smoke exposure and inflammatory signaling increase the expression of the SARS-CoV-2 receptor ACE2 in the respiratory tract

Smith, J.C. *et al*. 2020. *Developmental Cell*
Link: https://doi.org/10.1016/j.devcel.2020.05.012

Summary:
The authors suggest that exposure to cigarette smoke upregulates lung ACE2 receptors in mice and humans by triggering an expansion in the subset of secretory cells that express this receptor. This increase can potentially be reversed by quitting smoking. They further identified ACE2 as an interferon-stimulated gene suggesting that SARS-CoV-2 infections could create positive-feedback loops aiding viral dissemination.

Main Findings:

- No age or gender related differences were detected in ACE2 receptor levels in mice and humans across different cohorts
- Smokers expressed 30/55% more ACE2 compared to non-smokers in epithelial lung tissues
- samples from patients who reported smoking the greatest number of pack-years expressed the highest levels of ACE2
- comparing current to former smokers, quitting smoking for at least 12 moths was associated with a 40% reduction in ACE2 expression
- ACE2 expression in the mammalian lung epithelium was restricted to secretory club and goblet cells and alveolar type 2 cells

- Smoking was associated with an increase in both the number of ACE2+ positive cells and in ACE2 expression within ACE2+ cells
- Cells differentiated *in vitro* in the presence of cigarette smoke expressed significantly more ACE2 relative to cells differentiated in clean air
- Exposure of primary lung cells to interferons upregulated ACE2 expression

. **Highlights:**

- Smokers might be at higher risk of severe COVID19 disease due to increased expression of ACE2 by lung cells and quitting smoking could reduce the risk

Clinical Impact:

- Low

Important Methodologies:

- Analysis of ACE2 expression in 1) lung tissue from the Genotype-Tissue Expression project, 2) whole-lung tissue samples from organ donors, 3) pathologically-normal lung tissue from a cohort of patients analysed as part of The Cancer Genome Atlas
- *In vivo* exposure of mice to diluted cigarette smoke
- Analysis of lung epithelial cells sampled from different human cohorts
- single-cell RNA-Seq to identify the cell expressing ACE2 in murine and human lungs
- *in vitro* exposure of primary lung epithelial cells to cigarette smoke and interferons

Limitations:

- other studies have reported age related changes in ACE2 expression
- in human tissues, smoking was correlated with levels of ACE2 mRNA expression but protein expression was not confirmed

Vaccines

Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial

Zhu, F. *et al*. 2020. *The Lancet*
Link: https://doi.org/10.1016/S0140-6736(20)31208-3

Summary:
Zhu et al. report the first in human trial (n=108) of a SARS-CoV-2 vaccine. This is a phase I, non-randomised, dose escalation study. The study primarily aimed to assess adverse reactions. Antibody and T cell responses were also measured. Although some of the immune measures differed significantly between the high and low dose groups, the phase II trial underway is testing the low and middle doses.

Main Findings:

- At least one adverse reaction was common (>80%), but there was no significant difference in adverse effects across treatment groups up to 28 days. High pre-existing immunity to Adv5 (>1:200 titre) was associated with lower incidence of fever post vaccination.
- Antibodies against the spike protein receptor binding domain (RBD) were detected at day 14 in 44-61% of participants receiving low to high vaccine doses, and in >95% of participants in all groups by Day 28.
- At day 28, the neutralising antibody titre was significantly higher in the high vaccine dose group compared to the middle and low doses. The neutralising antibody titre across all groups significantly correlated with anti-spike protein antibody titre but not with the anti-RBD antibody titre. This may suggest an anti-RBD response is not essential to prevent infection/ viral replication and antibodies against other spike protein antigens are sufficient.
- IFNγ production peaked at day 14, with a significant difference in responses between the high/middle and low vaccine dose, but not between the high and middle dose.
- $CD8^+$ T cells were predominantly IFNγ single-positive although, the high vaccine dose tended to induce IFNγ and TNFα double positive cells. There was a dose dependent increase in TNFα production. $CD4^+$ T cells were largely polyfunctional and similarly, TNFα production was significantly higher in the high dose group.
- Both antibody and T cell responses were blunted by pre-existing Adv5 immunity. Neutralising antibody production was affected to a greater extent than T cell responses, across all treatment groups. Antibody responses were also lower in participants aged 40-60.

Highlights:

- Rapid induction of both humoral and cellular responses. The latter is particularly important as the data from previous pandemics suggests T cell responses rather than antibodies confer greater long-term protection.

Clinical Impact:

- High. The next clinical trial phase will explore efficacy and a wider age range (particularly >60 years), which was underexplored in this study.

Important Methodologies:

- Single dose intramuscular injection of non-replicating Adv5 vector expressing SARS-CoV-2 spike protein. Three doses were tested in n=36: $5x10^{10}$, $1x10^{11}$ and $1.5\ x10^{11}$.
- Symptomatic side effects were self-reported, additional toxicities by laboratory testing.
- Humoral responses were assessed by ELISA and neutralising antibody assays using live virus and pseudovirus expressing the SARS-CoV-2 spike protein, respectively.
- Cytokines were measured by ELISPOT (IFNγ) and flow cytometry (IL-2, TNFα and IFNγ from $CD4^+$ and $CD8^+$ T cells) by stimulating PBMC with spike protein overlapping peptide pools.
- Pre-existing immunity to Adv5 was measured in a serum neutralising assay.

Limitations:

- Study size was not designed based on power calculations; therefore, statistical analysis was not comprehensive. This also prevented analysis of an antibody dependent enhancement response to the vaccine, which is a particular safety concern considering the detrimental effects of deregulated immune response in COVID19 patients.
- This is a phase 1 study; efficacy of vaccine in the context of SARS-CoV-2 infection was not tested. The analysis is short term (up to 28 days), however; follow up of the participants for 6 months is planned.
- The reduced immune responses associated with a high pre-existing immunity to Adv5 is a serious concern for the efficacy of the vaccine, particularly since this is common in the population; 44-56% of participants across the three dosage groups had >1:200 anti-Adv5 titre.

DNA vaccine protection against SARS-CoV-2 in rhesus macaques

Yu, J. *et al*. 2020. *Science*

Link: https://doi.org//10.1126/science.abc6284

Summary:

The team developed a series of DNA vaccines consisting of varying truncated forms of the SARS-CoV Spike (S) protein: 1) full-length (S), 2) deletion of the cytoplasmic tail (S.dCT), 3) deletion of the transmembrane domain and cytoplasmic tail reflecting the soluble ectodomain (S.dTM), 4) S1 domain with a foldon trimerization tag (S1), 5) receptor-binding domain with a foldon trimerization tag (RBD), and 6) a prefusion stabilized soluble ectodomain with deletion of the furin cleavage site, two proline mutations, and a foldon trimerization tag (S.dTM.PP). Vaccinated (5mg IM) macaques elicited humoral and cellular immune response. Neutralizing antibody (NAb) titers were similar to convalescent humans infected with SARS-CoV-2. Additionally, after being challenge with SARS-CoV-2, the full-length (S) protein vaccine resulted in >3.1 and >3.7 $\log_{10}$ decrease in viral load medians in bronchoalveolar lavage (BAL) and nasal mucosa (NS) compared to controls.

Main Findings:

- S-specific binding Abs were detected after boost immunization at weeks 5 by ELISA, and NAbs were increased for both live and pseudovirus neutralization assays for all forms of truncation
- NAb titers (median titer 170) from S and S.dCT vaccinated animals were similar to Nab titres in convalescent macaques (median titer 106) and humans (median titer 93)
- S- and RBD-specific Abs elicited a range of effects: antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent complement deposition (ADCD), antibody-dependent monocyte cellular phagocytosis (ADCP), and antibody-dependent NK cell activation
- S-specific IFN+ CD4+ and CD8+ T cell responses observed in the short forms: S1 and RBD – Th1-biased cellular immune response
- Decreased levels of SARS-CoV-2 subgenomic RNA (sgmRNA) levels were detected in the vaccine groups with a >3.1 and >3.7 $\log_{10}$ decrease in viral load medians in BAL and NS compared to controls
    - sgmRNA - believed to reflect viral replication cellular intermediates that are not packaged into virions and thus putative replicating virus in cells

Highlights:

- Demonstrate effective vaccine protection against SARS-CoV-2 in rhesus macaques
- Comparable NAb titers from vaccinated groups to convalescent titers
- The correlation (inverse correlation) between NAbs and effector functions such as S- and RBD-specific ADCD versus $\log_{10}$ sgmRNA levels suggests the primary role of protection against SARS-CoV-2
- Minimal to no protection was seen in the S.dTM group – confirming importance of prefusion ectodomain stabilization. Optimal protection seen with full length S.

- Authors concluded that protection is likely not sterilizing but instead mediated by rapid immunologic control following challenge

Clinical Impact:

- Limited

Important Methodologies:

- ELISA & neutralisation assays
- IFNγ-ELISPOT
- RT-PCR

Limitations:

- Statistics not always shown in the figures and data is quite spread
- Further research needed to investigate durability of protection
- Studies to observe vaccine immunogenicity and protective efficacy in various other age groups is necessary
- Further observations of side-effects would be interesting
- Neutralisation of the virus in both upper and lower respiratory tract would be needed to overcome infection – this study shows more efficient control of lower.

Epidemiology

Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study

Bi, Q. *et al*. 2020. *The Lancet Infectious Diseases*
Link: https://doi.org/10.1016/S1473-3099(20)30287-5

Summary:
Investigations into the epidemiology of SARS-CoV-2 which highlight that isolation and contact tracing reduce the time during which cases are infectious in the community, and the reducing effect this has on R. 391 SARS-CoV-2 cases and 1286 close contacts were analysed. Cases were identified through symptomatic surveillance and contact tracing, and estimated the time from symptom onset to confirmation, isolation, and admission to hospital were compared. Metrics of disease transmission were estimated and factors influencing transmission risk were analysed. Data suggested that children are at a similar risk of infection to the general population, although less likely to have severe symptoms; hence they should be considered in analyses of transmission and control.

Main Findings:

- Provides evidence which supports extensive contact tracing and highlights that children might be an important target for interventions aimed at reducing transmission, even if they do not get sick.
- 79% of all cases were adults aged 30-69 years.
- 84% of all cases presented with fever at the time of initial assessment and 9% of cases were severe at the time of first clinical assessment.
- In multiple logistic regression, male sex was associated with severe symptoms. The probability of severe symptoms increased slightly with age, although only individuals aged 60–69 years had a significantly increased risk compared with the reference category, individuals aged 50–59 years.
- Median incubation period for COVID-19 was estimated to be 4.8 days.
- Analyses of how cases are detected, and use of data on individuals exposed but not infected, indicate that infection rates in young children are not lower than the population average (even if rates of clinical disease are).
- Notably, it was estimated that a higher proportion of cases taking 14 days or more to develop symptoms (5%) than estimated by Lauer and colleagues in a similar study (1%) [DOI: 10.7326/M20-0504].
- In Shenzhen, SARS-CoV-2 transmission most probably occurred between very close contacts, such as individuals sharing a household. Even between individuals sharing a household the secondary attack rate was 11–15%, and resulting in fewer than one (0.4) onward transmission per primary case.
- These results shed further light on how SARS-CoV-2 is transmitting, how severe it is, and how effective control measures can be in specific contexts.

Highlights:

- Through analysis of COVID-19 patients including their close contacts which aids investigation into progression of the disease and symptoms associated with such.

Clinical Impact:

- Data further supports the understanding of COVID as a disease with a short incubation period (mean 4-6 days) but a long clinical course.

Limitations:

- NA

Epidemiology of Covid-19 in a Long-Term Care Facility in King County, Washington

McMichael, T.M. *et al*. 2020. *NEJM*
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Summary:
This article describes an outbreak of Covid-19 in a care facility in Washington State, USA. The authors discuss tracing the origins of the outbreak to one patient in facility A. The outbreak resulted in 167 confirmed cases in that facility. Furthermore, outbreaks in other care facilities within King County and the neighbouring Snohomish County were found to have originated from the outbreak in facility A.

Highlights:

1. Report demonstrates the importance of 'track and trace' methodology to monitor the spread of Covid-19 cases
2. Data shows how quickly and virulently SARS-CoV-2 can spread within care facilities
3. Report highlights the importance of appropriate viral control measures in care facilities

Clinical Impact:

- Yes – highlights the importance of track and trace upon the identification of a viral outbreak, especially in care facilities. A detailed account of how the outbreak of Covid-19 in care facilities in Kings County was traced, which could be useful for any subsequent outbreaks. Report also highlights the vulnerability of care facilities to mass outbreaks of SARS-CoV-2 and the importance of control measures such as adequate PPE.

Important Methodologies:
The identification of an outbreak in facility A led to the following series of events:

- Arrival of a Centre for Disease Control (CDC) field team

- Interview by telephone of facility residents, visitors and health care personnel to collect information of symptoms, travel history and close contacts
- During the interviews guidance on self-isolation, quarantine and testing was given
- Specimen collection and testing was carried out
- Over 100 care facilities in King County were contacted by email to obtain information on any suspected outbreaks
- Countywide databases that capture all emergency medical transfers from care facilities to acute facilities were monitored daily for evidence of Covid-19 cases

Limitations:

- Authors state that not all residents at Facility A were tested and an accurate record of visitors was not maintained. Therefore the number of cases described in this article may be under reported, particularly when considering some cases are asymptomatic.
- Authors also note that it is difficult to accurately ascertain when the outbreak began due to the incubation time of SARS-CoV-2.

Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: a prospective cohort study

Cummings, M. J. *et al*. 2020. *The Lancet*
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Summary:

This is one of the largest prospective observational cohort studies reported to date in the USA of critically ill patients with acute hypoxaemic respiratory failure with laboratory confirmed COVID-19 infection. Rate of in-hospital death was assessed as well as duration of invasive mechanical ventilation, frequency of vasopressor use and renal replacement therapy, and time to in-hospital clinical deterioration following admission. Cox proportional hazards regression was used to model relation between clinical risk factors biomarkers and in-hospital mortality. Critical illness was common and associated with a high frequency of invasive mechanical ventilation, extrapulmonary organ dysfunction and substantial in-hospital mortality.

Main Findings:

- Study took place at two New York-Presbyterian hospitals affiliated with Columbia University Irving Medical Center in northern Manhattan.
- Patients admitted primarily through emergency department from surrounding neighbourhoods in northern Manhattan and Southern Bronx
- Patients prospectively identified (aged >= 18, RT-PCR COVID-19 confirmed infection, acute hypoxaemic respiratory failure)

- Critically ill patients were receiving either mechanical ventilation or high-level supplemental oxygen
- Concentrations of plasma-based and serum-based biomarkers drawn within 72 h of hospital admission (C-reactive protein, D-dimer, ferritin, high-sensitivity troponin, procalcitonin, and interleukin-6)
- Between March 2 and April 1, 2020, 1150 adults were admitted to both hospitals with of which 257 (22%) were critically ill
- Median age was 62 years (51-72), 171 (67%) Hispanic/Latino, 13 (5%) were health care workers, 212 (82%) had at least one chronic illness
- 119 (46%) were obese (BMI >= 30) including 39 (71%) of 55 patients who were less than 50 years of age
- Median serum creatinine was 1·5 (IQR 1·9–2·4) and 189 (87%) of 218 patients who had a urinalysis performed had proteinuria
- Lymphocytopaenia and mildly elevated concentrations of aspartate aminotransferase were common
- Concentrations of IL-6, high-sensitivity C-reactive protein, ferritin, D-dimer, high-sensitivity troponin, and procalcitonin were elevated in most patients
- 101 (39%) of 257 patients had died following a median of 9 days (IQR 5–15) in the hospital. This included 84 (41%) of 203 patients who received invasive mechanical ventilation (IMV) during hospitalisation
- Death occurred in 20 (41%) of 49 black or African American patients, 61 (38%) of 159 Hispanic or Latino patients, and 15 (47%) of 32 white patients
- Median time to clinical deterioration following admission was 3 days (1–6)
- Most deaths occurred in patients who were at least 50 years of age
- 94 (37%) of 257 patients remained hospitalised with a median duration of hospitalisation of 33 days (29–36)
- 58 (23%) patients were discharged alive, 12 (21%) of which required supplemental oxygen, and four (2%) were transferred to another institution
- 115 (45%) of 257 patients initially received non-invasive respiratory support via non-rebreathing oxygen face mask, 12 (5%) via high-flow nasal cannula, and three (1%) via non-invasive ventilation
- 203 (79%) patients received IMV for a median of 18 days (IQR 9–28)
- Survivors had a median of 27 days (15–32) of IMV and non-survivors had a median of 10 days (4–16)
- 52 (26%) of 203 patients who were extubated alive, median duration of IMV was 14 days (10–21)
- 71 (62%) of 115 patients who initially received non-invasive respiratory support ultimately received IMV after a median of 3 days (1–5)
- 170 (66%) of 257 patients received vasopressors and 79 (31%) received renal replacement therapy (RRT) during hospitalisation
- In the multivariable Cox model, older age (adjusted HR [aHR] 1·31 [95% CI 1·09–1·57] per 10-year increase), chronic cardiac disease (aHR 1·76 [1·08–2·86]), chronic pulmonary disease (aHR 2·94 1·48–5·84]), higher concentrations of IL-6 (aHR 1·11 [1·02–1·20] per decile increase), and higher concentrations of D-dimer (aHR 1·10

[1·01–1·19] per decile increase) were independently associated with in-hospital mortality

Highlights:

- Critical illness among patients hospitalised with COVID-19 in New York City is common and associated with a high frequency of invasive mechanical ventilation, extrapulmonary organ dysfunction, and substantial in-hospital mortality.
- High incidence of critical illness among racial and ethnic minorities in the current epicentre of the COVID-19 pandemic
- Older age, chronic cardiac disease, higher concentrations of Il-6 and D-dimer were independently associated with in-hospital mortality

Clinical Impact:

- High

Important Methodologies:

- Standardised case record form developed by the International Severe Acute Respiratory and Emerging Infection Consortium and WHO was used to record data

Limitations:

- Study took place in two hospitals in same region of New York City thereby limiting generalisability to other areas
- Studies among more racially, ethnically and geographically diverse cohorts are needed to confirm findings due to high proportion of Hispanic/Latino and African American patients used in this cohort