

The Weekly Covid-19 Literature Round-Up

Edition 3: April 1, 2020

Collated by Emory ID and Medical Microbiology Fellows

“We have to continually be jumping off cliffs and developing our wings on the way down.”

-Kurt Vonnegut

“People don't care how much you know until they know how much you care.”

-Theodore Roosevelt

Epidemiology

Brought to you by: Divya Bhamidipati, MD

McMichael, Temet M. et al. “Epidemiology of Covid-19 in a Long-Term Care Facility in King County, Washington.” *New England Journal of Medicine* 0, no. 0 (March 27, 2020): null.

<https://doi.org/10.1056/NEJMoa2005412>.

- 167 cases linked to Facility A – 101 residents, 50 healthcare personnel, 16 visitors
- Most with mild to moderate respiratory illness, though 7 cases were asymptomatic. 35 deaths noted at Facility A.
- Median age 83yo in residents, 62.5 among visitors, 43.5 in facility personnel; 67% were women
 - Hospitalization rates: 54.5% residents, 50% visitors, 6% staff
 - Case fatality rate: 33.7% residents, 6.2% for visitors, no staff deaths
 - 94.1% with underlying chronic health conditions including hypertension (67.3%), cardiac disease (60.4%), renal disease (40.6%), diabetes mellitus (31.7%), pulmonary disease (31.7%), and obesity (30.7%)
- Total 30 facilities affected with 3 that had clear links to Facility A
- **Conclusion:** Vulnerable population with chronic underlying conditions and staff working in multiple facilities as well as patients transfers potentially introduced COVID-19 into some of these facilities.

Spellberg, Brad et al. “Community Prevalence of SARS-CoV-2 Among Patients With Influenza-like Illnesses Presenting to a Los Angeles Medical Center in March 2020.” *JAMA*, March 31, 2020.

<https://doi.org/10.1001/jama.2020.4958>.

- 131 tested for SARS-CoV-2 with 7 positive results
 - Median age 38yo, 3 males (43%)
 - Median duration of symptoms at time of presentation was 4 days
 - 6/7 patients with fever, 5 with myalgias, 1 with cough – all mild disease
- At same time, percent positive flu tests declining
- Sentinel testing revealed a 3rd spike (later than previous years) in influenza-like illness during the weeks before study even as the percentage of positive influenza tests was declining.

- Conclusion: 5% rate of COVID-19 among patients with mild influenza-like illness without risk factors is concerning as patients had mild illness and could be active in community throughout illness, increasing transmission. This likely is what contributed to late 3rd spike of influenza-like illness late in the season.

Genomic Epidemiology

Brought to you by: Ahmed Babiker, MBBS

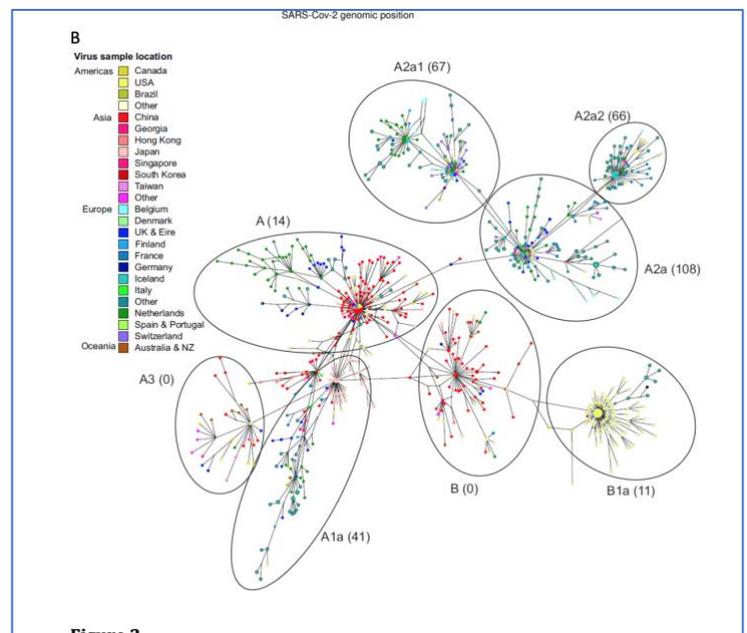
Fauver, Joseph R. et al. "Coast-to-Coast Spread of SARS-CoV-2 in the United States Revealed by Genomic Epidemiology." *MedRxiv*, March 26, 2020, 2020.03.25.20043828.

<https://doi.org/10.1101/2020.03.25.20043828>.

- The first 9 SARS-CoV-2 PCR positive samples (March 6-14) from Connecticut were sequenced using MinION technologies.
- Sequencing turn-around time was approx 14 h from time of receiving the sample.
- SARS-CoV-2 genomes clustered into three distinct phylogenetic clades, indicating multiple independent virus introductions into Connecticut.
- Two of the genomes, clustered primarily with viruses from China and Europe. However, these were sequenced from patients with no travel history.
- Conclusion: A combination of phylogenetic analyses and classic epidemiological investigation revealed that the COVID-19 outbreak in Connecticut was driven by multiple virus introductions from other U.S. states (not international locations).

Gudbjartsson, Daniel F. et al. "Early Spread of SARS-Cov-2 in the Icelandic Population." Preprint. *Epidemiology*, March 30, 2020. <https://doi.org/10.1101/2020.03.26.20044446>.

- Iceland employed two testing strategies:
 - Targeted testing (n=4,551) of high-risk individuals (those with symptoms or high-risk travel/contact history): **11.6%** positivity
 - Open participation in population level (n=5,502) screening: **0.9%** positivity
- Young children and females had lower rates positivity for SARSCoV-2 than adults and males respectively
- SARS-CoV2 was sequenced from 340 individuals
 - SARS-CoV-2 came from eight clades
 - The clade composition differed between the testing groups (targeted vs. population) and changed with time.
 - Virus diversity was higher in population testing vs. targeted testing



- Conclusion: Sequencing revealed the haplotype and diversity composition of the viruses from individuals identified through the population screening was different from those tested in the early targeted

testing. Indicating multiple introductions from different countries and spread within the community outside of high-risk groups.

Transmission/Infection Control

Brought to you by: Jesse O'Shea, MD, MSc

Kimball, Anne. "Asymptomatic and Presymptomatic SARS-CoV-2 Infections in Residents of a Long-Term Care Skilled Nursing Facility — King County, Washington, March 2020." MMWR. Morbidity and Mortality Weekly Report 69 (2020). <https://doi.org/10.15585/mmwr.mm6913e1>.

- CDC epidemiological COVID19 investigation in a SNF in Washington.
- Historical screening for preceding 14 days + active monitoring with twice daily symptom/fever screening of residents and healthcare workers for 1 week (spanning 21 days). NP/OP swabs obtained for all residents.
- Initially, 13/23 PCR positive were asymptomatic, at 7 days, 10 of the 13 developed symptoms and recategorized as pre-symptomatic.
 - High viral quantity by cycle threshold times in all groups suggesting transmission regardless of symptoms.
- Symptom-based screening in SNFs could fail to identify cases.
 - CDC recommends of resident-to-resident interactions and universal use of facemasks for all health care personnel while in SNFs.
- Index patient was a health care provider, may have contributed to rapid spread.
- Limitations: short duration of monitoring and viral cultures were not published yet (still ongoing), unclear if viable virus.

Environmental contamination of the SARS-CoV-2 in healthcare premises: An urgent call for protection for healthcare workers. (Preprint)

<https://www.medrxiv.org/content/10.1101/2020.03.11.20034546v1>

- Goal: investigate extent to which SARs-CoV-2 contaminated healthcare surfaces within Zhongnan Medical Center in Wuhan, China between 02/07-02/27/20.
- 85/626 samples were positive. Most contaminated zones: ICU (n=22/69), obstetrics isolation ward (9/32), and isolation ward for PCR positive patients (11/56).
 - Most contaminated objects included: desktop/keyboard (29/173), doorknob (17/72), telephone (7/56), and self-service printers (2/10). 1/18 of samples from walls/floors were positive.
- Among PPE available: hand sanitizer dispensers (12/59) and gloves (12/78) were most contaminated.
- Hospital environment could potentially be a source of spread. Urgent need for adequate environmental cleaning and strengthening infection prevention programs.
- Limitations: Viral cultures not obtained, unclear bioburden. Did not comment on existing disinfection protocols.

Clinical Syndrome

Brought to you by: Alfonso Hernandez, MD, MPH

Shi, Shaobo et al. "Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China." *JAMA Cardiology*, March 25, 2020. <https://doi.org/10.1001/jamacardio.2020.0950>.

- Study designed to examine the association between cardiac injury and mortality among patients with COVID-19 in Wuhan, China, among severe cases.
- Patients without high-sensitivity troponin (hs-tni) or creatinine kinase-myocardial band (CK-MB) were excluded
- Cardiac injury was defined as hs-tni above 99th percentile upper reference limit (>0.04 ng/mL) regardless of abnormality in electrocardiogram (ECG) or echocardiogram
- 416/786 (53%) confirmed COVID-19 cases with cardiac biomarker evaluation
 - 82 (19.7%) with cardiac injury; 334 (80.3%) without cardiac injury; 44 (10.6%) with prior coronary artery disease (CAD); 17 (4.1%) with chronic heart failure (CHF)
- Patients with cardiac injury were older than those without it. Median (IQR): 74 (34-95) vs 60 (21-90)
- As expected patients with cardiac injury were more likely to have co-morbidities including hypertension, diabetes, CAD, CHF.
 - Similarly, patients with cardiac injury were more likely to have higher leukocytes, C-reactive protein, procalcitonin, creatinine, and lower lymphocyte count
- 22 (26.8%) of patients underwent ECG after admission
 - 14/22 were performed during elevation in hs-tni and all were abnormal
- Those with cardiac injury required more non-invasive and invasive mechanical ventilation, received more glucocorticoids, IVIG, and antibiotics, were more likely to develop ARDS and AKI, and were more likely to die (51.2% versus 4.5% for those with and without cardiac injury respectively).
- After adjustment for age, co-morbidities, AKI, NT-BNP level, and ARDS
 - The adjusted hazard ratio for cardiac injury:
 - 4.26 (1.92-9.49) from symptom onset to death
 - 3.41 (1.62-7.16) from admission to death
- **Bottom line:** Troponin is a marker of disease severity and as expected older patients with more co-morbidities and more severe disease had evidence of cardiac injury. We do not know when troponin was measured in relation to different events such as mechanical ventilation and ICU admission making it of limited value as a prognostic factor (troponin was not always drawn at admission but sometimes 5-7 days into the hospital course). There is concern of possible direct myocardial injury from SARS-CoV-2 and future studies are needed to better characterize the presence of myocardial dysfunction during illness with echocardiography.

Giacomelli, Andrea et al. "Self-Reported Olfactory and Taste Disorders in SARS-CoV-2 Patients: A Cross-Sectional Study." *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, March 26, 2020. <https://doi.org/10.1093/cid/ciaa330>.

- Cross-sectional survey of 59 patients Italy
- 20 (34%) reported at least one taste or olfactory disorder
 - 11 (19%) reported both taste and olfactory disorder
 - 12 (20%) reported onset prior to hospital admission
 - 8 (14%) experienced symptoms during the hospital stay

- Taste alterations were more frequent prior to admission whereas after hospitalization taste and olfactory alteration were similar
- Females experienced it more frequently than males (53% vs 25%)
- **Bottom line:** Alterations in taste and smell should be included in review of systems when obtaining a history from PUIs. Studies in non-hospitalized patients will be crucial to assess how common this finding is in the general population.

Bhatraju, Pavan K. et al. "Covid-19 in Critically Ill Patients in the Seattle Region — Case Series." *New England Journal of Medicine* 0, no. 0 (March 30, 2020): null. <https://doi.org/10.1056/NEJMoa2004500>.

- Included adults (>18 years of age) with laboratory confirmed COVID-19 infection (with no evidence of bacterial/viral co-infection) admitted to nine different ICUs in Seattle (n = 24)
- 25% were admitted from a skilled nursing facility, mean age 64y, 58% had DM2
- Mean duration of symptoms prior to admission was 7 days
 - 54% had a known sick contact; 88% had cough and shortness of breath; 50% had fever on ICU admission; 75% had lymphopenia (<1500/mm³) on admission
- All had moderate ARDS based on PaO₂:FiO₂ ratio. 75% required mechanical ventilation: median (IQR) duration 10 (7-12) days.
 - 28% were prone; 39% were paralyzed; 28% received pulmonary vasodilators; 0% received ECMO; 71% received vasopressors; 77% had moderate or thick and purulent secretions
- 50% died in the hospital, 21% were discharged, 29% remained hospitalized
- **Bottom line:** High mortality in ICU patients with COVID-19 with the majority requiring mechanical intubation. The high proportion of patients with moderate or thick purulent secretions suggests that aggressive management of secretions will be important in critically ill patients.

Diagnostics

Brought to you by: Amy Sherman, MD

Broughton, James P et al. "Rapid Detection of 2019 Novel Coronavirus SARS-CoV-2 Using a CRISPR-Based DETECTR Lateral Flow Assay." Preprint. *Infectious Diseases (except HIV/AIDS)*, March 10, 2020. <https://doi.org/10.1101/2020.03.06.20032334>.

- A rapid (less than 30m) CRISPR-Cas12 based lateral flow assay was developed to detect SARS-CoV-2. The development and validation are described by the authors.
- Negative nasopharyngeal swabs from healthy donors and positive nasopharyngeal and oropharyngeal swabs from SARS-CoV-2 positive patients were obtained via UCSF IRB in San Francisco, and RNA extracted.
 - SARS-CoV-2 DETECTR: simultaneous reverse transcription and isothermal amplification using loop-mediated amplification (RT-LAMP), followed by Cas12 detection of specific coronavirus sequences.
 - Primers target the envelope (E) and nucleoprotein (N) genes. These primers overlap WHO assay (E gene region) and US CDC assay (N2 region of N gene).
 - Demonstrated *in vitro* that no cross-reactivity for related coronavirus strains existed for assay.
- Compared to the CDC qRT-PCR: SARS-CoV-2 DETECTR was 90% sensitive and 100% specific for detection in respiratory swab samples. Positive predictive value = 100%. Negative predictive value= 91.7%.

- **Conclusions:** SARS-CoV-2 DETECTR is a more rapid and cost-effective method for diagnosis. Assay sample-to-result time is approx. 45m as compared to 4h of CDC qRT-PCR test, and no bulky instrumentation is required. Of note, limit of detection is 10 copies/microL input for DETECTR, while CDC qRT-PCR is 3.2 copies/microL input. Author CYC is director of UCSF-Abbott Viral Diagnostics and Discovery Center.
- FDA granted emergency use authorization for [Abbott RealTime SARS-CoV-2 EUA test combined with ID NOW](#).

Fatima Amanat et al, "A Serological Assay to Detect SARS-CoV-2 Seroconversion in Humans | MedRxiv."
 Posted March 18, 2020. <https://www.medrxiv.org/content/10.1101/2020.03.17.20037713v1>.

- Although molecular assays are available to diagnosis acute infection, there are no currently approved serological assays to detect SARS-CoV-2 antibodies. Krammer's lab (Icahn School of Medicine at Mount Sinai) developed serological enzyme-linked immunosorbent assays (ELISA) derived from the spike protein of SARS-CoV-2.
 - 2 versions of spike protein were created: (1) full length trimeric and stabilized version of the spike protein and (2) smaller receptor binding domain (RBD) only.
- Banked human serum samples (n=59) with confirmed prior viral infections (e.g. hantavirus, dengue virus, coronavirus NL63) were used to test background reactivity to SARS-CoV-2 spike protein.
- Plasma/serum human samples (4 samples from 3 subjects) from known SARS-CoV-2 positive patients were used to determine reactivity to the 2 versions of the spike protein. Obtained at day 20 (patient 1), day 4 (patient 2), and days 2 and 6 (patient 3) after symptom onset.
 - All COVID-19 samples reacted strongly to both full-length and RBD proteins (stronger reactivity to full-length protein). Other non-COVID-19 samples showed only background reactivity.
 - For the COVID-19 samples, isotyping/subtyping ELISA also completed: Strong reactivity for IgG3, IgM, and IgA.
- **Conclusions:** The assays are promising, and hopefully can be scaled and used to determine seroprevalence in a population and identify prior exposure. This assay may also be used to identify highly reactive human donors to generate convalescent serum for therapeutic options.

Chen, Chen et al. "SARS-CoV-2–Positive Sputum and Feces After Conversion of Pharyngeal Samples in Patients With COVID-19." *Annals of Internal Medicine*, Letters, March 30, 2020. <https://doi.org/10.7326/M20-0991>.

- Testing of respiratory specimens using RT-qPCR for SARS-CoV-2 has been used to diagnose COVID-19, and to guide clinical decisions regarding isolation/hospital discharge. The authors address the question: Is there value in testing other body sites (e.g. blood, feces, urine)?
- Retrospective analysis of patients admitted to Beijing Ditan Hospital who were (a) diagnosed with COVID and who had (b) paired RT-qPCR tests of pharyngeal swabs with sputum or feces samples.
 - 22 patients identified (age range 2-64yo), all met criteria and had been discharged from the hospital.
 - 545 specimens collected from these 22 patients (pharyngeal swabs: 209; sputum: 262; feces: 74).
- **Results:** Sputum remained positive for SARS-CoV-2 up to 39 days after pharyngeal samples were negative. Fecal samples remained positive for up to 13 days after pharyngeal samples were negative.
- **Conclusions/Limitations:** This study suggests that perhaps patients are not virus-free when pharyngeal specimens alone are negative, which may have implications for continued shedding and transmission. However, note that this was based on a convenience sample, and serial samples were not obtained on a

uniform schedule. Also, PCR may be detecting non-viable virus from the non-pharyngeal sites; thus may not have an effect on transmission dynamics.

Therapeutics

Brought to you by: Max Adelman, MD

“Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: an observational study”. Gautret *et al.* Preprint: <https://www.mediterranee-infection.com/wp-content/uploads/2020/03/COVID-IHU-2-1.pdf>

- Pre-clinical and small, methodologically flawed studies have indicated possible benefit of hydroxychloroquine (HCQ) + azithromycin for treatment of COVID-19; in this two-center, single-arm cohort study in France, the authors of the controversial HCQ+azithro study (<https://doi.org/10.1016/j.ijantimicag.2020.105949>) analyzed data on 80 patients treated with this combination for at least 3 days who had at least 6 days of follow-up data.
- About half of patients had symptoms consistent with lower respiratory tract infection at admission, but vast majority were not critically ill.
 - 92% had low NEWS score; a high NEWS score can predict need for critical care.
- There was no unusual loss to follow-up/patient withdrawal in this study (although the authors do not discuss if there were patients who did not have 6 days of follow up data, perhaps due to death, who would not have been included).
- Overall outcomes were positive: clinically, 1% died (age 86), 4% ICU transfer, and 15% required O2; microbiologically, majority were PCR (NP swab) negative by day 4 and 93% PCR negative by day 8, and only 10% had positive NP culture on day 5 (serial culture is a study strength); few adverse outcomes with HCQ+azithro combo.
- The results are appropriate for study design (with caveat of 6 days of follow up time required, which may have excluded patients who died before this), however authors’ conclusion that HCQ+azithro combo directly led to clinical benefit is overstated given no comparison/control group. However, this preprint study provides more preliminary evidence that, given lack of proven therapies for COVID-19, HCQ+azithro combo deserves study in a rigorous randomized trial.

Shen, Chenguang, Zhaoqin Wang, Fang Zhao, Yang Yang, Jinxiu Li, Jing Yuan, Fuxiang Wang, et al. “Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma.” *JAMA*, March 27, 2020. <https://doi.org/10.1001/jama.2020.4783>.

- Previous small clinical studies of variable quality have indicated possible benefit of convalescent plasma (CP) for treating respiratory viral infections including SARS (ie SARS-CoV-1); the authors of this study reported findings of 5 critically ill patients in a single center in China treated with CP for COVID-19.
- No data presented on patients who were screened but not enrolled.
 - Regardless, all 5 patients were critically ill (all intubated with ARDS, 1 on ECMO), had persistently positive NP PCRs despite illnesses lasting from 2-3 weeks, and had elevated CRP and IL-6 despite prior anti-virals and steroids.
- After receiving convalescent plasma with high titers of SARS-CoV-2 specific and neutralizing antibodies, all patients had a decrease in inflammatory markers and PCR cycle time and increase in P:F ratio between 3-12 days after transfusion; 3 patients were extubated within 9 days (including patient on ECMO), although 2 remained intubated at study end; all patients had increase in Ab titers

- Interpretation/limitations: There are some intriguing findings here, although without a control group they should be interpreted with caution, and 2 of 5 patients remained intubated at time of publication despite treatment with convalescent plasma. Given paucity of treatments for COVID-19, convalescent plasma requires further study in randomized controlled trials.

“A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing.” Gordon *et al.* Preprint: <https://doi.org/10.1101/2020.03.22.002386>

- To identify potential drug targets, knowledge of how SARS-CoV-2 proteins interact with human proteins is crucial.
- Researchers (led by a team at UCSF) tagged viral proteins and expressed them *in vitro*, then used a technique called affinity purification mass spec to determine which host proteins each of these viral proteins interact with.
- They identified 332 SARS-human protein interactions; a number of the human proteins identified were involved in lipid modification and vesicle tracking as well as innate immune pathways (e.g. TANK-binding kinase 1).
- Overall, they were able to identify 69 known drugs that interact with these human proteins and therefore may be able to interfere with viral functions.
- The researchers make the key point that it is not known if these drugs will have any effect on viral processes, and if they do, if the effect will be beneficial or detrimental. However, this is an important starting point for novel drug development.

Pediatrics

Brought to you by: Madeleine Goldstein, MD

Zeng L, Xia S, Yuan W, et al. Neonatal Early-Onset Infection With SARS-CoV-2 in 33 Neonates Born to Mothers With COVID-19 in Wuhan, China. JAMA Pediatr. Published online March 26, 2020. doi:10.1001/jamapediatrics.2020.0878

- Limited number of neonates born to affected mothers have been investigated, and no information on early-onset infection in newborns has been published previously.
- Cohort study of 33 neonates born to mothers with COVID-19 that identified 3 infected neonates via positive NP and anal PCR swabs (2 full term born via C/S, 1 born preterm at 31 weeks due to fetal distress). All 3 infected neonates were found to have pneumonia. The most common symptom from the 33 neonates was shortness of breath (4 of 33 neonates), radiographic findings were nonspecific, and there were no deaths.
- Neonates with or at risk of COVID-19 have mild clinical symptoms and outcomes were favorable. Vertical maternal-fetal transmission cannot be ruled out, thus, it is crucial to screen pregnant women and implement strict infection control measures including close monitoring of neonates at risk.
- Limitations: Of the 3 neonates with COVID-19, it is unclear if clinical presentation is multifactorial (including prematurity, concurrent bacterial sepsis, asphyxia, Meconium aspiration, etc) vs SARS-CoV-2. Additionally, it is unclear from study if sources of SARS-CoV-2 used for PCR from URT and anuses were maternal in origin.

Dong L, Tian J, He S, et al. Possible Vertical Transmission of SARS-CoV-2 From an Infected Mother to Her Newborn. JAMA. Published online March 26, 2020. doi:10.1001/jama.2020.4621

- It is controversial regarding whether SARS-CoV-2 can be transmitted in utero.
- A neonate born to a mother with confirmed COVID-19 (presented at 34 weeks gestation) was found to have elevated antibodies to SARS-CoV-2 and elevated cytokines (includes IL-6, IL-10) 2 hours after birth. All nasopharyngeal PCR swabs taken from 2 hours old to 16 days of age were negative.
- The elevated IgM level 2 hours after delivery suggests that the neonate was infected in utero (exposed for 23 days from time of maternal dx to delivery)
- Limitations: Single case studied and no PCR testing of maternal amniotic fluid or placenta was performed.

Zeng H, Xu C, Fan J, et al. Antibodies in Infants Born to Mothers With COVID-19 Pneumonia. *JAMA*. Published online March 26, 2020. doi:10.1001/jama.2020.4861

- Previous study by Chen et al (Lancet 2020) of 9 pregnant women and their infants found no maternal-infant transmission of SARS-CoV-2 based on RT-PCR.
- Retrospective cohort analysis of 6 pregnant women with confirmed COVID-19 (symptoms, CT, and + PCR). All had C/S during 3rd trimester in negative pressure isolation rooms with mothers wearing masks; infants immediately isolated post delivery. SARS-CoV-2 was not detected in the serum or throat swab by RT-PCR in any of the newborns. All 6 infants were asymptomatic, but had virus-specific Ab's detected in their serum (IgG detected in 5, IgM detected in 2).
- Virus-specific IgM was detected in 2 infants at time of birth, which could have been produced by the infant if the virus crossed the placenta.
- Limitations: Small sample size. Did not test cord blood, amniotic fluid, or breast milk for SARS-CoV-2.

Basic Science/Virology

Brought to you by: Sam Stampfer, MD, PhD

Ou, Xiuyuan et al. "Characterization of Spike Glycoprotein of SARS-CoV-2 on Virus Entry and Its Immune Cross-Reactivity with SARS-CoV." *Nature Communications* 11, no. 1 (March 27, 2020): 1–12.
<https://doi.org/10.1038/s41467-020-15562-9>.

- Coronavirus spike protein (S) mediates viral entry via fusion of the viral envelope with the cell or endosomal membrane. S is cleaved into two subunits, S1 (which contains the receptor-binding domain RBD), and S2 (which mediates membrane fusion). The authors investigated the SARS-CoV-2 S protein entry mechanism and immunologic reactivity using pseudovirions derived from lentivirus but expressing the coronavirus spike protein as the sole surface protein on the pseudovirion membrane.
- SARS-CoV-2 was shown to enter cells by endocytosis when using human ACE2 as a receptor. When the authors inhibited PIKfyve (a kinase involved in endosomal maturation), pseudovirion entry was inhibited, indicating that membrane fusion occurs in late endosomes, not early—and identifying PIKfyve as a possible drug target.
- In comparison with S protein from SARS-CoV-1, SARS-CoV-2 S is less thermally stable and has a greater ability to induce membrane fusion at the cell surface in the absence of activation signals from the endosome. The authors speculate that this may be the basis for its higher transmission efficiency, as it is easier to trigger fusion and the virus may more readily directly spread from cell to cell via cell-cell fusion.

- Sera from goats immunized with MHV S protein (betacoronavirus lineage A) was able to react with S proteins from SARS-CoV-1, SARS-CoV-2, and MERS-CoV, indicative of conserved epitopes between these proteins (likely in S2).
- Polyclonal rabbit anti-SARS-CoV-1 S1 has almost no cross-reactivity or pseudovirion neutralization with SARS-CoV-2 S in spite of 76% sequence identity, implying that the immunodominant domains of S1 are highly divergent.
- Sera from a human SARS-CoV-1 patient was about >4X better at neutralizing SARS-CoV-1 S pseudovirions than SARS-CoV-2 S pseudovirions, but did neutralize both. In contrast, COVID-19 convalescent sera were only active against SARS-CoV-2 S, not SARS-CoV-1 S.
- **Conclusion:** SARS-CoV-2 S protein has shared epitopes across multiple betacoronavirus species and has high sequence conservation with SARS-CoV-1 S. In spite of this, the immunodominant epitopes are specific to SARS-CoV-2 with limited cross-reactivity even to SARS-CoV-1.

Wrapp, Daniel et al. "Structural Basis for Potent Neutralization of Betacoronaviruses by Single-Domain Camelid Antibodies." *BioRxiv*, March 28, 2020, 2020.03.26.010165. (Preprint)
<https://doi.org/10.1101/2020.03.26.010165>.

- Camelids (such as llamas) produce heavy-chain only antibodies (VHHs) with a single variable domain. They have similar specificity as regular antibodies but typically have enhanced thermal stability, and multiple VHHs can be combined in a single construct. An anti-SARS-CoV-2 multivalent VHH construct could be used as a therapeutic during this pandemic, and may even be compatible with administration by an inhaled route (as has been done with other VHHs).
- A llama was immunized with a combination of spike proteins from SARS-CoV-1 and MERS-CoV. Strongly-binding VHHs were isolated and tested for neutralization; all neutralizing VHHs bound only to the receptor-binding domain (RBD) on the spike protein. Two VHHs were found to block binding of the RBD to its cellular receptor, and were characterized further by co-crystallization with the RBDs from SARS-CoV-1 and MERS-CoV spike proteins.
 - The structures show that both VHHs would prevent the RBD from binding to its cellular receptor, and would likely also trap the RBD in an alternate "up" conformation. They speculate that this may result in S protein being prematurely triggered for fusion, and thus irreversibly inactivated.
- The SARS-CoV-1 monovalent VHH did not neutralize pseudovirions bearing SARS-CoV-2 spike protein. To improve avidity, they engineered a bivalent VHH construct fused to an Fc domain, and this construct did show neutralizing activity.
- No VHHs were isolated that bound both MERS-CoV and SARS-CoV-1 S proteins.
- **Conclusion:** VHHs from a llama immunized with coronavirus spike proteins show potent neutralizing activity and likely irreversibly inactivate S protein. One specially-engineered bivalent VHH construct has potential therapeutic use for COVID-19.

Zhu, Yuanmei, Danwei Yu, Hongxia Yan, Huihui Chong, and Yuxian He. "Design of Potent Membrane Fusion Inhibitors against SARS-CoV-2, an Emerging Coronavirus with High Fusogenic Activity." *BioRxiv*, March 28, 2020, 2020.03.26.009233. <https://doi.org/10.1101/2020.03.26.009233>.

- Coronavirus spike (S) proteins mediate viral entry by fusing the viral membrane with the cell membrane. This process involves a major conformational change in S which brings together two regions: heptad repeat 1 (HR1) and heptad repeat 2 (HR2). HR1 & HR2 are structurally conserved in many viral fusion proteins, and peptides derived from them often have antiviral activity by blocking this interaction. This has been shown for both SARS-CoV-1 and MERS-CoV.
- Established a system to evaluate S protein function by expressing S protein on cells and evaluating cell-cell fusion. Interestingly, SARS-CoV-2 S protein was noted to have increased and more rapid fusion activity compared to SARS-CoV-1 S.
 - They note that HR2 is conserved fully between SARS-CoV-2 and SARS-CoV-1, but that HR1 has 8 substitutions. They synthesized peptides corresponding to HR1 from both viruses and the conserved HR2 and evaluated them.
- The SARS-CoV-2 HR1 peptide was more thermally stable than that of SARS-CoV-1, and bound the HR2 peptide more stably, implying a stronger HR1-HR2 interaction in SARS-CoV-2.
- They conjugated a cholesterol group to their HR2 peptide to form their inhibitor IPB02; this had increased stability and better binding to HR1 from both SARS-CoVs compared to the unconjugated HR2 peptide.
 - IPB02 potently inhibited cell-cell fusion with SARS-CoV-2 S (IC_{50} 25 nanomolar), as well as SARS-CoV-2 S-typed pseudovirion entry with an IC_{50} of 80 nanomolar. SARS-CoV-1 S-typed pseudovirions were inhibited with an IC_{50} of 251 nanomolar.
- Conclusion: The authors successfully developed a lipopeptide inhibitor of SARS-CoV-2 with nanomolar activity. It mimics a segment of the spike protein and prevents membrane fusion, and also has activity against SARS-CoV-1.

Disclaimer: The above references were selected and summarized by amazing Emory ID fellows. We have tried to put together an accurate list and summary, but please know that this is not intended to be 100% comprehensive! Also, it is impossible to keep completely up-to-date!