The Weekly Covid-19 Literature Round-Up

Edition 4: April 8, 2020

Collated by Emory ID (Adult and Pediatric) and Medical Microbiology Fellows

"An investment in knowledge always pays the best interest."
-Benjamin Franklin

"Wise men ne'er sit and wail their loss,
But cheerly seek how to redress their harms."
-William Shakespeare, Henry VI

Epidemiology

Brought to you by: Daniel Graciaa, MD, MPH

Verity, Robert et al. "Estimates of the Severity of Coronavirus Disease 2019: A Model-Based Analysis." ***The Lancet Infectious Diseases*** **0, no. 0 (March 30, 2020). https://doi.org/10.1016/S1473-3099(20)30243-7.**

- Crude estimates of the case fatality ratio (CFR) early in an epidemic are biased due to multiple factors including time between symptom onset and case/outcome determination, focus on severe cases, and testing characteristics.
- This study used individual COVID-19 cases and aggregate data from China, Hong Kong, Macau and international sites including the *Diamond Princess* cruise ship to adjust for demographic characteristics and possible under-ascertainment of cases
- Found crude CFR in China of 3.67% (95% credible interval 3.56-3.80) but adjusted estimate 1.38% (1.23-1.53) and increased with age:
    - <60yrs 0.32% (0.27-0.38), over 60 yrs 6.4% (1.8-11.1), over 80yrs 13.4% (11.2-15.9).
    - Estimated mean time from symptom onset to death 17.8 days (16.9-19.2).
- Interpretation: These models are consistent with reported data on increasing mortality with age and provide an adjusted overall CFR of 1.38%, with under-ascertainment of mild cases accounting for much of the adjustment from the crude estimate. The denominator matters and estimates will continue to adjust as the epidemic evolves.

Grasselli, Giacomo et al. "Baseline Characteristics and Outcomes of 1591 Patients Infected With SARS-CoV-2 Admitted to ICUs of the Lombardy Region, Italy." ***JAMA*****, April 6, 2020.**
https://doi.org/10.1001/jama.2020.5394.

- Series of 1591 consecutive critically ill patients with nasal and pharyngeal RT-PCR-confirmed SARS-CoV-2 infection admitted Feb 20-Mar 18 to one of 72 hospitals of the ICU coordinating center in Lombardy. Last date of follow up March 25.
- 82% male, median age 63 years (IQR 56-70). 68% (95% CI 65-71) with at least 1 comorbidity, most commonly HTN (49% of 1043 patients with available data). 1287/1300 (99%) of patients with data required respiratory support: 88% invasive and 11% noninvasive. Median PEEP 14 (12-16) cm H_2O and 89% (87-91) required at least 50% FiO_2.

- At end of follow up: 26% (23-28%) had died, 58% (56-61%) remained in ICU, 16% (14-18%) had been transferred from ICU. Median ICU stay of 9 (6-13) days. 240/875 patients with data (27%, 25-31) proned and 5/498 (1%, 0.3-2) required ECMO.
- Conclusions/Limitations: In one of the largest ICU populations published to date, COVID-19 patients in Lombardy had similar age distribution and comorbidities to other locations, with high ICU mortality (26%). Limitations include follow up time, with 58% still in ICU at time of publication, and missing data for some outcomes such as proning and ECMO.

Transmission/Infection Control

Brought to you by: Jane Yoon, MD

Wei, Wycliffe E. "Presymptomatic Transmission of SARS-CoV-2 — Singapore, January 23–March 16, 2020." *MMWR. Morbidity and Mortality Weekly Report* 69 (2020). https://doi.org/10.15585/mmwr.mm6914e1.

- All 243 cases of COVID-19 in Singapore from January 23-March 16 were reviewed.
- 7 clusters were identified in which presymptomatic transmission likely occurred.
    - Transmission occurred 1-3 days before symptom onset in the presymptomatic source patient.
    - 10 cases within these clusters accounted for 6.4% of 157 locally acquired cases.
- Routes of transmission may include respiratory droplets (via speech, singing, and other vocal activities) or indirect transmission via fomites.
- Limitations: possible unknown source, recall bias, under-detection of asymptomatic cases.
- Conclusions: Evidence of presymptomatic transmission of SARS-CoV-2 poses challenges to contact tracing and containment efforts, but reinforces the importance of social distancing in public health efforts to control the COVID-19 pandemic.

Santarpia, Joshua L. et al. "Transmission Potential of SARS-CoV-2 in Viral Shedding Observed at the University of Nebraska Medical Center." *MedRxiv*, March 26, 2020, 2020.03.23.20039446. https://doi.org/10.1101/2020.03.23.20039446.

- Air and surface samples were collected from 11 isolation rooms (in 2 isolation units – 1 hospital and 1 residential), where 13 confirmed COVID-19 cases were isolated at UNMC.
    - All patients symptomatic, but most had mild illness not requiring hospitalization.
- Surface samples were taken from common room surfaces, personal items, and toilets.
  Air samples were collected in the isolation room while patients were present (with or without wearing a mask), as well as in the hallways adjacent to rooms.
- 126 of 163 samples (77.3%) had +PCR result for SARS-CoV-2. Viral gene copy concentrations were low and highly variable between samples (0 to 1.75 copies/µl).
    - 76.5% of personal items sampled were positive.
    - 63.2% of room air samples were positive. These did not show evidence of viral replication, though low concentration of virus made interpretation difficult.
- Conclusions: There is significant environmental contamination in rooms of patients with COVID-19, regardless of acuity of illness or symptomatology. This data also suggests that patients aerosolize viral particles even in the absence of cough. The size range of SARS-CoV-2 droplets and particles was not studied, and remains a further area of interest.

Luo, Chao et al. "Possible Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a Public Bath Center in Huai'an, Jiangsu Province, China." *JAMA Network Open* 3, no. 3 (March 2, 2020): e204583–e204583. https://doi.org/10.1001/jamanetworkopen.2020.4583.

- Case series that addressed a cluster-spreading event of 9 patients hospitalized with COVID-19 in Jiangsu Province after bathing at the same public bath center.
    - Bath center characteristics: 300 m^2, temperatures from 25-41°C, humidity 60%.
- Patient 1 had traveled to Wuhan and developed symptoms the day after his visit to the bath. Patients 2-9 visited the bath 1-6 days after patient 1 and began experiencing symptoms within 6-9 days of their visit.
- Conclusions: High temperature and humid conditions may not decrease transmissibility of SARS-CoV-2. Limitations of this study include unknown transmission routes.

Clinical Syndrome

Brought to you by: Aditi Ramakrishnan, MD

Lescure, Francois-Xavier et al. "Clinical and Virological Data of the First Cases of COVID-19 in Europe: A Case Series." *The Lancet Infectious Diseases* 0, no. 0 (March 27, 2020). https://doi.org/10.1016/S1473-3099(20)30200-0.

- Description of patterns of clinical disease and viral load from different samples in 5 patients who had traveled from Hubei Province, China to France
- Patients included:
    - Two women (ages 30 and 46) who were diagnosed within one day of exhibiting mild symptoms
    - Two men (ages 31 and 48) who exhibited mild symptoms with symptomatic worsening after day 10
    - 80-year-old man admitted to the ICU with multi-organ failure resulting in death
- Mildly symptomatic patients, because of high viral loads in upper respiratory tract samples, pose a high risk of viral shedding and disease transmission, which should guide infection control measures.
- Two cases demonstrated a biphasic pattern of illness with secondary respiratory worsening despite decreasing nasopharyngeal viral loads. This pattern suggests that pulmonary disease during the secondary worsening may be more due to pro-inflammatory host response than to viral replication.
- Time to disease worsening in the biphasic pattern above was 10 days

Therapeutics

Brought to you by: Boghuma Kabisen Titanji, MD MSc PhD

Caly, Leon et al. "The FDA-Approved Drug Ivermectin Inhibits the Replication of SARS-CoV-2 in Vitro." *Antiviral Research*, April 3, 2020, 104787. https://doi.org/10.1016/j.antiviral.2020.104787.

- Small in-vitro study that evaluates the effectiveness of Ivermectin to inhibit SARS-CoV in a cell culture assay system

- Researchers infected Vero/hSLAM with a clinical isolate of SARS-CoV from Australia in the presence of 5uM Ivermectin. Experiments. With serial dilutions of. The drug determined an IC50 of 2.5uM. -2.8uM
    - 93% reduction of viral RNA (indicative of release virus particles) in supernatants of drug treated cultures and a 99.8% reduction in cell-associated viral RNA (indicative of unreleased, unpackaged virions) at 24hours.
    - By 48hours this effect increased to a 5000% reduction in drug treated cell cultures compared to untreated controls.
- Ivermectin is an FDA approved drug for the treatment many parasitic infections. It has been observed to have in-vitro activity against other viruses including zika, dengue, HIV and west Nile but this in-vitro activity is yet to translate to clinical effectiveness for any of these other infections.
- The proposed mechanism of action of this antiviral effect is Ivermectin binds to and destabilizes the Importin α/β1 heterodimer thereby preventing Importin α/β1 from binding to the viral protein and preventing it from entering the nucleus. This likely results in reduced inhibition of the antiviral responses, leading to a normal, more efficient antiviral response
- Limitations: Much like hydroxychloroquine and many other drugs currently being repurposed for clinical use in COVID-19, only a well-designed RCT will determine utility of ivermectin in the treatment of COVID-19.

Molina, Jean Michel et al. "No Evidence of Rapid Antiviral Clearance or Clinical Benefit with the Combination of Hydroxychloroquine and Azithromycin in Patients with Severe COVID-19 Infection." ***Médecine et Maladies Infectieuses*****, March 30, 2020. https://doi.org/10.1016/j.medmal.2020.03.006.**

- Another small study from France assessing prospectively the effectiveness of hydroxychloroquine plus azithromycin for the treatment of severe COVID-19.
- Small prospective series of 11 patients with moderate to severe COVID-19 treated with 10 days of hydroxychloroquine 600mg daily plus Azithromycin 500mg daily for 2-5.
    - Treatment was initiated for all patients on hospital admission.
    - Within 5 days, one patient died, 2 patients were transferred to intensive care, 1 patient discontinued after 4 days dies to prolongation of their QTc.
- Repeated nasopharyngeal swabs were performed on 10/11 patients excluding the one person who died. In 8/10 patients these swabs were still positive for SARS-CoV2 at days 5-6 post treatment initiation.
- Conclusion: A small study without a control group, which adds to the pile of equivocal evidence on the effectiveness or lack thereof of hydroxychloroquine to treat SARS-CoV2. A large RCT is needed to definitively address this question.

Vaccinology

Brought to you by: Amy Sherman, MD

Kim, Eun et al. "Microneedle Array Delivered Recombinant Coronavirus Vaccines: Immunogenicity and Rapid Translational Development." ***EBioMedicine*****, April 2, 2020, 102743. https://doi.org/10.1016/j.ebiom.2020.102743.**

- There are currently no safe and effective vaccines for Covid-19. The spike protein (S) of SARS-CoV-2 is a key target for vaccine development.

- The authors describe development of a novel microneedle array (MNA) that is embedded with SARS-CoV-2 spike protein (a subunit vaccine).
    - In a mouse model, mice were inoculated intracutaneously with MNAs loaded with either SARS-CoV-2-S1 or SARS-CoV-2-S1fRS09 protein. Venous samples were taken at 1 week intervals and ELISA performed to evaluate for antibody to the spike protein. FACS analysis and neutralization assays completed as well.
- By 2 weeks post-immunization, statistically significant increases in antigen-specific antibodies responses were described.
- Conclusions: The authors describe rapid development and testing in vivo of a novel MNA vaccine for SARS-CoV-2, with initial results showing good immunogenicity. MNAs are potentially advantageous since the skin is an immunologically active organ, and a relatively low dose Ag delivery is needed (which improves safety characteristics). Next steps: determining stability of MNA SARS-CoV-2-S1 vaccine, and safety/efficacy studies in humans.

Tai, Wanbo et al. "Characterization of the Receptor-Binding Domain (RBD) of 2019 Novel Coronavirus: Implication for Development of RBD Protein as a Viral Attachment Inhibitor and Vaccine." ***Cellular & Molecular Immunology*****, March 19, 2020. https://doi.org/10.1038/s41423-020-0400-4.**

- The authors describe features of the SARS-CoV-2 spike protein (S), and describe how the receptor binding domain (RBD) of the S protein is the most promising target for vaccine development.
    - The S protein is key for viral entry into host cells, and used the RBD to bind to the host receptor.
    - ACE2 is the host receptor that is recognized, and binds to SARS-CoV-2 S protein.
- A recombinant SARS-CoV-2 RBD protein was constructed and purified.
- 4 experiments were conducted to assess the binding between SARS-CoV-2 and the human ACE2 receptor (hACE2).
- Conclusions: The authors showed that the recombinant RBD of SARS-CoV-2 bound strongly to hACE2, and blocked entry of both SARS-CoV-2 and SARS-CoV into host cells; may serve as viral attachment inhibitor. SARS-CoV RBD specific polyclonal antibodies cross-neutralized SARS-CoV-2 pseudovirus infection—this could be the basis of a SARS-CoV RBD-based vaccine to prevent infection of both SARS-CoV-2 and SARS-CoV.

Diagnostics

Brought to you by: Ahmed Babiker, MBBS

Hogan, Catherine A. et al. "Sample Pooling as a Strategy to Detect Community Transmission of SARS-CoV-2." ***JAMA*****, April 6, 2020. https://doi.org/10.1001/jama.2020.5445.**

- Sample pooling is a strategy used for community monitoring of other infectious diseases.
- Nasopharyngeal and bronchoalveolar lavage samples collected between Jan 1st- Feb 26th 2020 which had previously tested negative by routine respiratory virus testing and had not been tested for SARS-CoV-2 were selected.
    - Nine or 10 individual samples were pooled, and screening was performed using reverse transcriptase–polymerase chain reaction targeting the envelope (*E*) gene.

    - Positive pools were deconvoluted and individual samples tested for both *E* and the RNA-dependent RNA polymerase (*RdRp*) gene for confirmation
- A total of 292 pools were screened,
    - 2740 nasopharyngeal samples
    - 148 bronchoalveolar lavage samples
- The confirmed positivity rate for SARS-CoV-2 was 0.07% (2/2888)
    - 1 pool showed a positive *E* signal that was not reproducible with testing of the individual samples of that pool.
- Conclusion: A pooled screening strategy was pursued to increase testing throughput, limit use of reagents, and increase overall testing efficiency at an expected slight loss of sensitivity.

Rodino, Kyle G. et al. "Evaluation of Saline, Phosphate Buffered Saline and Minimum Essential Medium as Potential Alternatives to Viral Transport Media for SARS-CoV-2 Testing." ***Journal of Clinical Microbiology*****, March 30, 2020, JCM.00590-20, jcm;JCM.00590-20v1. https://doi.org/10.1128/JCM.00590-20.**

- The preferred and most commonly collected specimen is a nasopharyngeal (NP) swab placed in viral or universal transport media (VTM).
    - Due to testing demands we face nationwide shortages of all components of testing, including VTM.
- Authors evaluated the total following below medias by spiking in SARS -CoV -2 positive patient material at a concentration of 10,000 copies/mL (n=48)
    - VTM (n=12)
    - Sterile 0.9% saline (n=12)
    - Sterile phosphate buffered saline (PBS) (n=12)
    - Minimum 21 essential media (MEM) (n=12)
- Each media was tested twice without spiking of SARS-CoV-2 to serve as negative controls (n=8)
- All mediums were equivalent (100% qualitative agreement and CT variation < 2 cycles) with VTM over 7 days (Day 0, 1, 3, 7) at both refrigerated and frozen storage conditions.
- Conclusion: The above data support the use of these alternative medium for the testing of COVID-19 patients.

Pediatrics

Brought to you by: Mehgan Teherani, MD, MSGM

CDC MMWR. "Coronavirus Disease 2019 in Children — United States, February 12–April 2, 2020." ***MMWR. Morbidity and Mortality Weekly Report*** **69 (2020). https://doi.org/10.15585/mmwr.mm6914e4.**

Pediatrics – Clinical Syndrome in US children

- From the first US pediatric case reported on March 2, 2020-April 2, 2020, there have been 2,572 (1.7% of total population) cases among children <18 years of age.
- Median age was 11yo, however, 33% were in the 15-17 yo range. 57% males.
- Pediatric patients report less clinical symptoms including 56% with fever, 54% with cough, and 13% with shortness of breath compared to 71%, 80%, and 43% in adults, respectively.

- Similar to studies from China, highest risk of hospitalization were in patients <1 yo (range from 15-62% of hospitalization in pediatric patients).
- 23% of patients reported underlying conditions, most commonly chronic lung disease (including asthma) – 40%, cardiovascular disease – 25%, and immunosuppression -10%.
- Limitations: Characteristics were reported for a small number of total pediatric patients including hospitalization (33%), symptoms (9.4%), and underlying conditions (13%), thus no statistical analyses were able to be performed. Testing practices vary widely from hospital to hospital across the US, often with only hospitalized children receiving testing.

"Lessons After the Early Management of the COVID-19 Outbreak in a Pediatric Transplant and Hemato-Oncology Center Embedded within a COVID-19 Dedicated Hospital in Lombardia, Italy. *Estote Parati.* by Adriana Balduzzi et al. Preprint. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3559560.

Pediatrics—Epi/IP/Transmission in pediatric Oncology and BMT experience in Lombardia, Italy

- Retrospective observational single center study with a pediatric Hematology-oncology unit, transplantation unit, and outpatient pediatrics unit.
- 5 pediatric cancer patients identified in the Lombardia area all had a benign self-limiting course without any specific anti-viral therapy. (none at authors' institution)
- IP procedures in the single hospital included cohorting patients with resp sxs, rescheduling non-urgent patients, postpone transplantation for non-malignant disease, donors having COVID-19 testing on day of donation. Also consider shortages of ICU beds and drug shortage of Toculizumab for CAR-T cell therapy prior to chemo/transplant.
    - Observed a drop in new leukemia diagnoses, which they assume will translate to disease diagnosis at a later stage.
- Theory on to why less severe illness in immunocompromised/peds patients: Drug therapy they are on limits inflammatory responses and these patients also more likely to have already been practicing social distancing. Also, decreased maturity and binding ability of angiotensin converting enzyme II in children compared to adults.
- Limitations: observational study with very few patient observations and none at the authors' institution.

Davanzo, Riccardo et al. "Breastfeeding and Coronavirus Disease-2019. Ad Interim Indications of the Italian Society of Neonatology Endorsed by the Union of European Neonatal & Perinatal Societies." *Maternal & Child Nutrition* n/a, no. n/a (n.d.): e13010. https://doi.org/10.1111/mcn.13010.

Pediatrics—Transmission/Infection in breastfeeding

- Expert consensus statement based on a lit review of 55 articles on maternal/child data and COVID 19 that was endorsed by the Union of European Neonatal & Perinatal Societies.
- Based on recommendations by WHO, UNICEF, ISS, IUOG, RCOG, and ABM.
    - Recommend rooming-in and direct breastfeeding of infant after birth if mother is pauci-symptomatic or asymptomatic.
    - If mother is positive, she should wash hands prior to feeding/handling, wear surgical mask during breastfeeding and skin to skin, no visitors, and baby should be kept 2meters from mother.

    - If mother is ill (fever, cough, SOB) or if infant is requiring ICU support, infant and mother should be separated, but expressed breastmilk should be given as mother can pass antibodies via breastmilk within a few days of disease onset.
- Recommend RT-PCR RNA for COVID-19 on infants weekly until 28 days of age with + mother and hospitalization for 1-week observation after birth is preferable.
- No evidence of transmission of SARS-COV2 through breastmilk to date.
- Limitations: Data continues to change rapidly, expert opinion in China recommends separation and providing formula or donor breastmilk, however, no reasoning was given and benefits of breastmilk were not addressed. Consensus statement<control trial.

Basic Science/Virology

Brought to you by: Sam Stampfer, MD, PhD

Gao, Ting et al. "Highly Pathogenic Coronavirus N Protein Aggravates Lung Injury by MASP-2-Mediated Complement over-Activation." *MedRxiv*, April 7, 2020, 2020.03.29.20041962. https://doi.org/10.1101/2020.03.29.20041962.

- Excessive immune response aggravates pathogenesis & mortality from COVID-19. This work investigates the interactions between coronaviruses and the complement system to lead to immune hyperactivation and lung injury.
- The authors show that the nucleocapsid (N) proteins of SARS-CoV-1, SARS-CoV-2, and MERS were able to interact with MASP-2, a serine protease that can activate the complement cascade via the lectin pathway. This interaction was dependent on residues 116-124 of N, which are highly conserved between SARS-CoVs & MERS-CoV.
- In vitro, this interaction was found to cause complement hyperactivation by demonstrating that N increases MASP-2-mediated cleavage of C4α.
    - Interestingly, this interaction did not occur in the less-pathogenic human 229E coronavirus.
- Blocking the interaction or complement activation *in vivo* reduced lung injury. In a mouse pneumonia model with adenovirus infection combined with lectin-pathway activation induced by LPS, all ten mice died if exposed to SARS-CoV-1 N. In contrast, when MASP-2 activation was reduced (either by adding an inhibitor or by using N with a Δ116-124 deletion), half or more mice survived to the end of the study. Lung pathology at just 6-hours post infection showed higher inflammatory markers in the SARS-CoV-1 N group.
- To counter this effect *in vivo*, the authors used a recombinant anti-C5a antibody BDB-001 to treat two patients with critical COVID-19 disease and moderate ARDS. Both had rapidly worsening disease and were worsening in spite of antimicrobials and corticosteroids. Following anti-C5a therapy, both had resolution of fever within 24 hours, and improvement in oxygenation over the following days.
- **Summary:** The N-protein from severely pathogenic coronaviruses causes complement hyperactivation, contributing to acute lung injury. This is mediated by activation of MASP-2, a serine protease in the lectin pathway of complement activation. Reducing MASP-2 activation with inhibitors, or by instead using Δ116-124 N-protein, leads to improved survival in a mouse model. There may be a role for targeted inhibitors of the complement pathway in treating severe COVID-19 disease.

Mantlo, Emily K. et al. "Potent Antiviral Activities of Type I Interferons to SARS-CoV-2 Infection." *BioRxiv*, April 5, 2020, 2020.04.02.022764. https://doi.org/10.1101/2020.04.02.022764.

- Type I interferons are part of the innate immune system. They are signaling molecules expressed during viral infection which induce production of multiple antiviral effectors. The authors evaluate the use of interferon α and β (IFN-α and IFN-β) *in vitro* against SARS-CoV-2.
- Experiments were conducted in Vero cells (which naturally express ACE-2, the SARS-CoV-2 receptor). Cell lines were pre-treated 16 hours with the given concentration of interferon before being infected with specified MOIs of SARS-CoV-2 (0.01 or 1).
- IFN-α was able to reduce the viral titer by 3.4 logs when used at 50 IU/ml, while IFN-β was more potent, able to reduce the titer by 4 logs when used at just 10 IU/ml.
- At lower concentrations, the EC_{50} was 1.35 IU/ml for IFN-α, and 0.76 IU/ml for IFN-β.
    - **Criticism:** The authors state that this is much lower than that of SARS-CoV-1, but close review of their cited paper from 2004 shows that cells were incubated an extra day with SARS-CoV-1, so this conclusion (which is in the abstract) cannot be determined.
    - **Criticism**: The authors pre-treated with interferon, instead of first infecting with SARS-CoV-2. Thus, this is not truly a treatment, and higher doses would likely be needed to actually treat viral infection *in vitro*.
- **Summary:** IFN-α and IFN-β are able to dramatically reduce viral infection when cells are pre-treated, before infection. IFN-β is slightly more potent, but both effective drug concentrations would be easily achievable in humans. However, their activity as post-infection therapeutics should be evaluated more rigorously *in vitro* before moving on to studies *in vivo.*