2020-04-20

Welcome COVIDers! The newsletter enters its fifth week with a flicker light at the end of the tunnel. It's fitting to start off with a bit of humor courtesy of the Washington Post comics page

Alas, there was no winner of yesterday's prize question. It was a tough one if you are not a big CM fan. The singer was Josh Turner who is also well known for his gospel singing. Here is a fun one for these COVID times, 'Why Don't We Just Dance': https://www.youtube.com/watch?v=IH1Z9DEDqpk Let's move on music fans, why haven't you asked for the 'Chairman of the Board?' What a big gaffe on my part not to have posted a Sinatra song. His young recordings were outstanding, he had the rhythm and technique to really style a song. Here is some good singing from the mid-1960s. Big band and Sinatra in 'Fly me to the Moon:' https://www.youtube.com/watch?v=0YEtYHZXl5M

This came in too late for yesterday's newsletter. Your humble correspondent is always on the lookout for comfort food, and cookies are at the top of the list. Posie at King Arthur Flour sends out a weekly newsletter that is always fun to read and here is a good excerpt from yesterday's, "I've never been able to replicate those underbaked jumbo cookies from my local bakery, but these **soft chocolate chip cookies** come pretty close. Honey and brown sugar gives them their chewy, soft texture; one reviewer said he'd been on a "decades-long search for the perfect chocolate chip cookies" and this was his holy grail! If you want something more rustic, go with our **oatmeal cookie** recipe, which have a touch of spice, crisp edges, and chewy centers. I recommend sprinkling a little sea salt over the top before baking!" **But wait, there's more (channeling Ron Popiel):** why not just go to my COVID-19 website and download the tried and true Alan's Chocolate Chip Cookie recipe!!!! https://agoldhammer.com/covid_19/ What wonderful things you all are learning!!!

Ed Yong is as good a writer on the pandemic as anyone around. Here is his take on what will happen this summer: https://www.theatlantic.com/health/archive/2020/04/pandemic-summer-coronavirus-reopening-back-normal/609940/ Lots of adjusting still left to do. For my good friend and loyal reader Hugh Tilson, this money quote is for you!!! *"Tracking such a pathogen requires a lot of people, but due to chronic underfunding, local U.S. health departments lost more than 55,000 workers from 2008 to 2017. In their absence, a corps of volunteers could be quickly trained in the basics of contact tracing, as Massachusetts Governor Charlie Baker is planning to do. "It might be an opportunity to bring in people who are recently unemployed—a wartime effort where people aren't doing their normal jobs," said Crystal Watson of the Johns Hopkins Center for Health Security."*

I received an email from a colleague who worked with us on some important PhRMA initiatives an has since retired and moved to academia. He alerted me to the details of the large Duke University study of healthcare workers: https://today.duke.edu/2020/04/duke-lead-50-million-study-covid-19-prevention-health-care-workers there is mixed data from the therapeutic clinical trials but maybe hydroxychloroquine has some protective qualities. I also wonder if the HIV drugs are protective. From the Chinese data they do not seem to offer therapeutic benefit. Can the medical records from the NYC outbreak be examined to see what percentage of hospitalized patients taking emtricitabine/tenofovir or lopinavir/ritonavir are? It would be a good project for OHDSI to take on. So many hydroxychloroquine trials, so much weird data, and so little time to solve the question.

I would be remiss not to mention the fine work the Emergent Ventures project at the Mercatus Center (George Mason University) has done in response to the pandemic. https://fastgrants.org/ present the project and lists the recipients. Great to see how individual donors can pool money and fund some needed projects!

MODELING

- This is not a mathematical model but a useful study of ACE2 receptor usage in various animals. The origin and emergence of its causal agent, SARS-CoV-2, in the human population remains mysterious, although bat and pangolin were proposed to be the natural reservoirs. Strikingly, comparing to the SARS-CoV-2-like CoVs identified in bats and pangolins, SARS-CoV-2 harbors a polybasic furin cleavage site in its spike (S) glycoprotein. SARS-CoV-2 uses human ACE2 as its receptor to infect cells. Receptor recognition by the S protein is the major determinant of host range, tissue tropism, and pathogenesis of coronaviruses. In an effort to search for the potential intermediate or amplifying animal hosts of SARS-CoV-2, we examined receptor activity of ACE2 from 14 mammal species and found that ACE2 from multiple species can support the infectious entry of lentiviral particles pseudotyped with the wild-type or furin cleavage site deficient S protein of SARS-CoV-2. ACE2 of human/rhesus monkey and rat/mouse exhibited the highest and lowest receptor activity, respectively. Among the remaining species, ACE2 from rabbit and pangolin strongly bound to the S1 subunit of SARS-CoV-2 S protein and efficiently supported the pseudotyped virus infection. These findings have important implications for understanding potential natural reservoirs, zoonotic transmission, human-to-animal transmission, and use of animal models. https://www.biorxiv.org/content/10.1101/2020.04.19.048710v2
- Using data from the Massachusetts outbreak, a Harvard researcher discusses preventionof resurgence with asymptomatic carriers. As many countries reached the peak of the COVID-19 outbreak, there is debate on how to reopen the economy without causing a significant resurgence. Here we show, using a microsimulation model, that how to reopen safely depends on what percentage of COVID-19 cases can be detected by testing. The higher the detection rate, the less restrictive the reopen plan needs to be. If 70% of cases can be detected, schools and businesses can reopen if 2-layer quarantine is imposed on each confirmed case. Our results suggest that increasing the detection rate is essential to prevent the resurgence of COVID-19. [**note: restating the obvious, more testing is needed!**] https://www.medrxiv.org/content/10.1101/2020.04.16.20067652v1

NEWLY REGISTERED CLINICAL TRIALS

- See the Drug Development section for the important Chinese Vaccine study.
- Hooray for another antiviral mouth rinse trial. This one uses beta-cyclodextrin and citrox, a mixture of citrus bioflavonoids. [**note: I'm unsure whether we can create a DIY gargle based on this study. Citrox appears to only be available from UK sources. Intrepid readers might want to take a look at the article referencing this approach: https://www.mdpi.com/2077-0383/9/4/1126**] NCT04352959
- I haven't the faintest idea whether this approach is any better or worse than hydroxychloroquine but this Spanish group is going to see what the effect of melatonin is with healthcare workers. There is an urgent need to evaluate interventions that can prevent the infection with SARS-CoV 2 of healthcare workers at risk. Melatonin is an inexpensive and safe product with protective effect in both bacterial and viral infections likely due to its anti-inflammatory and anti-oxidative effects. This randomized controlled trial seeks to evaluate is efficacy as a prophylaxis in healthcare workers exposed to the virus in their clinical practice. [**note: please excuse me now, I need to go to the pharmacy and get a couple of bottles. It will be a net positive if it only helps me to sleep at night**] NCT04353128
- I am only including this hydroxychloroquine trial as it is being sponsored by a large managed care company and is being conducted at a top-notch research institution. It also provides more information on what the needed dosage might be. Our hypothesis is that high doses of hydroxychloroquine (HCQ) for at least 2 weeks can be effective antiviral medication both as a treatment in ambulatory patients and prophylaxis/treatment in health care workers because it impairs lysosomal function and reorganizes lipid raft (cholesterol and sphingolipid rich microdomains in the plasma membrane) content in cells, which are both critical determinants of Emerging Viral Disease (EVD) infection. This hypothesis is based on a growing literature linking chloroquine to antiviral activity. We believe there is enough information to launch a clinical trial of hydroxychloroquine for COVID-19. Asymptomatic health care worker prophylaxis. Arm 1: Hydroxychloroquine 600 mg qd (three 200 mg tablets taken once a day) for up to 2 months; Arm 2: Placebo 3 pills qd for up to 2 months; cross-over from placebo to HCQ 600 mg qd is allowed upon confirmatory diagnosis for COVID-19. [**note: looking at the prescribing information of the drug label this appears to be the same dosage given to lupus and RA patients. I hope those folks are still able to get the meds they need. This does not bode well: https://www.fiercepharma.com/manufacturing/bayer-version-covid-19-antimalarial-hopeful-may-come-quality-concerns-report**] NCT04353037

CLINICAL TRIAL RESULTS

- We always need to protect the youngest. Here is a rapid review from China regarding breast feeding by mothers with SARS-CoV-2. We aimed to conduct a rapid review of mother-to-child transmission of COVID-19 during breastfeeding. Methods: We systematically searched Medline, Embase, Web of Science, Cochrane library, China Biology Medicine disc, China National

Knowledge Infrastructure, Wanfang, and preprint articles up to March 2020. We included studies relevant to transmission through milk and respiratory droplets during breastfeeding of mothers with COVID-19, SARS, MERS and influenza. Two reviewers independently screened studies for eligibility, extracted data, assessed risk of bias and used GRADE to assess certainty of evidence. Results: A total of 4481 records were identified in our literature search. Six studies (five case reports and one case series) involving 58 mothers (16 mothers with COVID-19, 42 mothers with influenza) and their infants proved eligible. Five case reports showed that the viral nucleic acid tests for all thirteen collected samples of breast milk from mothers with COVID-19 were negative. A case series of 42 influenza infected postpartum mothers taking precautions (hand hygiene and wearing masks) before breastfeeding showed that no neonates were infected with influenza during one-month of follow-up. Conclusions: *The current evidence indicates that SARS-CoV-2 viral nucleic acid has not been detected in breast milk. The benefits of breastfeeding may outweigh the risk of SARS-CoV-2 infection in infants. Mothers with COVID-19 should take appropriate precautions to reduce the risk of transmission via droplets and close contact during breastfeeding.* [**note: I posted a link yesterday regarding NYC data showing 15% of pregnant women presenting at obstetrics centers were virus positive. Let's hope this study helps out.**] https://www.medrxiv.org/content/10.1101/2020.04.13.20064378v1

DRUG DEVELOPMENT

- The vaccine race heats up!!! Here is a report from China on the rapid development of an inactivated vaccine for SARS-CoV-2. Here we developed a pilot-scale production of a purified inactivated SARS-CoV-2 virus vaccine candidate (PiCoVacc), which induced SARS-CoV-2-specific neutralizing antibodies in mice, rats and non-human primates. These antibodies potently neutralized 10 representative SARS-CoV-2 strains, indicative of a possible broader neutralizing ability against SARS-CoV-2 strains circulating worldwide. Immunization with two different doses (3 μg or 6 μg per dose) provided partial or complete protection in macaques against SARS-CoV-2 challenge, respectively, without any antibody-dependent enhancement of infection. Systematic evaluation of PiCoVacc via monitoring clinical signs, hematological and biochemical index, and histophathological analysis in macaques suggests that it is safe. These data support the rapid clinical development of SARS-CoV-2 vaccines for humans. [**note: This is a well thought out approach and the technology is amenable to scale up.**] https://www.biorxiv.org/content/10.1101/2020.04.17.046375v1 and double WOW!! They have just registered a Phase I/II trial of the vaccine!!! This study is a randomized, double-blinded, single-center, placebo-controlled phase Ⅰ/Ⅱ clinical trial in adults aged 18-59 years. The purpose of this study is to evaluate the immunogenicity and safety of the experimental SARS-CoV-2 inactivated vaccine. The experimental vaccine and placebo were both manufactured by Sinovac Research & Development Co., Ltd. A total of 744 subjects will be enrolled, with 144 at phase Ⅰ, and 600 at phase Ⅱ. Participant will be assigned to receive two doses of experimental vaccine or placebo on the schedule of day 0,14 or day 0,28. NCT04352608

DIAGNOSTIC DEVELOPMENT

- Here is a nice Israeli paper on efficient high throughput SARS-CoV-2 testing to detect asymptomatic carriers. Recent reports suggest that 10-30% of SARS-CoV-2 infected patients are asymptomatic. Other studies report that some subjects have significant viral shedding prior to symptom onset. Since both asymptomatic and pre-symptomatic subjects can spread the disease, identifying such individuals is critical for effective control of the SARS-CoV-2 pandemic. Therefore, there is an urgent need to increase diagnostic testing capabilities in order to also screen asymptomatic carriers. In fact, such tests will be routinely required until a vaccine is developed. Yet, a major bottleneck of managing the COVID-19 pandemic in many countries is diagnostic testing, due to limited laboratory capabilities as well as limited access to genome-extraction and Polymerase Chain Reaction (PCR) reagents. We developed P-BEST - a method for Pooling-Based Efficient SARS-CoV-2 Testing, using a non-adaptive group-testing approach, which significantly reduces the number of tests required to identify all positive subjects within a large set of samples. Instead of testing each sample separately, samples are pooled into groups and each pool is tested for SARS-CoV-2 using the standard clinically approved PCR-based diagnostic assay. Each sample is part of multiple pools, using a combinatorial pooling strategy based on compressed sensing designed for maximizing the ability to identify all positive individuals. We evaluated P-BEST using leftover samples that were previously clinically tested for COVID-19. In our current proof-of-concept study we pooled 384 patient samples into 48 pools providing an 8-fold increase in testing efficiency. Five sets of 384 samples, containing 1-5 positive carriers were screened and all positive carriers in each set were correctly identified. P-BEST provides an efficient and easy-to-implement solution for increasing testing capacity that will work with any clinically approved genome-extraction and PCR-based diagnostic methodologies. [**note: the lead author comes from the same institution where my sister works; she is a professor of Biblical Studies.**] https://www.medrxiv.org/content/10.1101/2020.04.14.20064618v1
- Good stuff again from China identifying what parts of the virus are important in antibody testing. Rapid and accurate tests that detect IgM and IgG antibodies to SARS-CoV-2 proteins are essential in slowing the spread of COVID-19 by identifying patients who are infected with COVID-19. Using a SARS-CoV-2 proteome microarray developed in our lab, we comprehensively profiled both IgM and IgG antibodies in forty patients with early-stage COVID-19, influenza, or non-influenza who had similar symptoms. The results revealed that the SARS-CoV-2 N protein is not an ideal biomarker for COVID-19 diagnosis because of its low immunogenicity, thus tests that rely on this marker alone will have a high false negative rate. Our data further suggest that the S protein subunit 1 receptor binding domain (S1-RBD) might be the optimal antigen for IgM antibody detection, while the S protein extracellular domain (S1+S2ECD) would be the optimal antigen for both IgM and IgG antibody detection. Notably, the combination of all IgM and IgG biomarkers can identify 87% and 73.3% COVID-19 patients, respectively. Finally, the COVID-19-specific antibodies are significantly correlated with the clinical indices of viral infection and acute myocardial injury ($p \leq 0.05$). Our data may help understand the function of anti-SARS-CoV-2 antibodies and improve serology tests for rapid COVID-19 screening. https://www.medrxiv.org/content/10.1101/2020.04.14.20064535v1
- This UK study compares several different primers for genetic testing and finds one that yields the best sensitivity. We aim to test four one-step RT real-time mastermix options for use in SARS-CoV2 real-time PCR, with three primer/probe assays targeting the N gene. The lower limit of detection is determined using a SARS-CoV2 N gene RNA transcript dilution series (to 1

copy/ul) and verified using 74 nose and throat swabs. The N2 assay demonstrates the most sensitive detection of SARS-Cov2 RNA. Three of the four mastermixes performed well, with the Takara One Step PrimeScript III RT-PCR Kit mastermix demonstrating improved performance at the lower limit of detection. https://www.biorxiv.org/content/10.1101/2020.04.17.047118v1

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2020-04-21

I just realized we have not heard anything by Richard Strauss. Let's remedy this right now with a fine rendition of his final composition, The Four Last Songs. IMO, Lucia Popp was one of the most underrated sopranos who sadly departed this world way too early. Here is a wonderful rendition with the Chicago Symphony and Georg Solti: https://www.youtube.com/watch?v=FhXLq3d_6kU 20 minutes of exquisite singing.

It's beginning to look like hydroxychloroquine is becoming the standard of care. Several large-scale trials are now scheduled to look at whether it is protective in healthcare workers and Duke University are establishing a registry to track experience. An infectious disease doc friend of mine said our local hospital is using hydroxychloroquine + azithromycin. Will we be able to do any clinical trials in this country going forward? There is a preprint from VA researches in Clinical Trials Results section that is worth reading (it won't come as a surprise). Iranian researchers did a quick systematic review and meta-analysis of the use of hydroxychloroquine showing no clinical benefit: https://www.medrxiv.org/content/10.1101/2020.04.14.20065276v1 Their country has been hard hit and I wonder what meds they are using in clinical settings.

Even the US military are jumping on the hydroxychloroquine bandwagon with this newly registered CT: NCT04343677

The estimable Derek Lowe on the Angiotensin connection (good name for an Indie rock band; I better trademark this ASAP): https://blogs.sciencemag.org/pipeline/archives/2020/04/20/good-news-on-the-coronavirus-angiotensin-connection

Alan

SARS-CoV-2 (AKA, COVID-19): It's here; we know what to do; and we are doing it!

Stay Safe, Mask Up, & Wash Hands

MODELING

- This study pretty much debunks the 'closing off travel from China' claim. Effective public response to a pandemic relies upon accurate measurement of the extent and dynamics of an outbreak. Viral genome sequencing has emerged as a powerful means to link seemingly unrelated cases, and large-scale sequencing surveillance can inform on critical epidemiological parameters. Here, we report analysis of 156 SARS-CoV2 sequences from individuals in the New York City metropolitan area during the initial stages of the 2020 COVID-19 outbreak. The

majority of samples had no recent travel history or known exposure. Comparison to global viral sequences showed that the majority of sequences were most related to samples from Europe. Our data are consistent with numerous seed transmissions and a period of unrecognized community spreading. This work highlights the complementary role of genomic surveillance to traditional epidemiological indicators.
https://www.medrxiv.org/content/10.1101/2020.04.15.20064931v1

- Although I have all but given up on reading modeling papers, this one from the UK is quite interesting. The looked at the effect of inter-city travel restrictions on geographical spread from Wuhan. Background: To contain the spread of COVID-19, a cordon sanitaire was put in place in Wuhan prior to the Lunar New Year, on 23 January 2020, restricting travel to other parts of China. We assess the efficacy of the cordon sanitaire to delay the introduction and onset of local transmission of COVID-19 in other major cities in mainland China. Methods: We estimated the number of infected travellers from Wuhan to other major cities in mainland China from November 2019 to March 2020 using previously estimated COVID-19 prevalence in Wuhan and publicly available mobility data. We focused on Beijing, Chongqing, Hangzhou, and Shenzhen as four representative major cities to identify the potential independent contribution of the cordon sanitaire and holiday travel. To do this, we simulated outbreaks generated by infected arrivals in these destination cities using stochastic branching processes. We also modelled the effect of the cordon sanitaire in combination with reduced transmissibility scenarios representing the effect of local non-pharmaceutical interventions. Findings: In the four cities, given the potentially high prevalence of COVID-19 in Wuhan between Dec 2019 and early Jan 2020, local transmission may have been seeded as early as 2 - 8 January 2020. By the time the cordon sanitaire was imposed, simulated case counts were likely in the hundreds. The cordon sanitaire alone did not substantially affect the epidemic progression in these cities, although it may have had some effect in smaller cities. Interpretation: Our results indicate that the cordon sanitaire may not have prevented COVID-19 spread in major Chinese cities; local non-pharmaceutical interventions were likely more important for this. [**note: I have been worried about intracity transmission as things begin to open up here in the US. I'm not sure this paper gives me complete confidence that this is NOT a concern.**]
https://www.medrxiv.org/content/10.1101/2020.04.16.20067504v1
- This one was too good to pass up!!!! Epidemic spread and drone technology are topics of broad research and public interest (the former heated by the current events, while the latter driven by the latest technological developments in the drone industry). We study the potential of using drones to deliver tests, enabling mass-testing for the infection. We show how the testing intensity, increased with the use of drones, may "flatten the curve" by quarantining test-positive population -- for that, we extend the classical SIR epidemic spread model, introducing the "quarantined" compartment and solving (numerically) differential equations which govern the extended model. [**note to self: time to place an order for a drone or two to see if I can contribute to ending the pandemic!**]
https://www.medrxiv.org/content/10.1101/2020.04.15.20067025v1

NEWLY REGISTERED CLINICAL TRIALS

- Well losartan and valsartan are being studied, why not add telmisartan? This group of Argentine investigators are doing just that! The present study is an open-label randomized phase II clinical trial for the evaluation of telmisartan in COVID-19 patients. Briefly, patients with confirmed diagnosis of SARS-CoV-2, will be randomized to receive 80 mg/12h of telmisartan plus standard care or standard care alone and will be monitored for development of acute respiratory distress syndrome. Other variables regarding lung function, systemic inflammation and cardiovascular function will also be evaluated. NCT04355936
- Well chlorpromazine changes the pH in lysosomes so why not give it a try? That's just what these Egyptian docs are going to do. Chlorpromazine is an antagonist of the dopamine receptor D2 (DRD2) and has been effectively and safely employed for over half a century in the treatment of psychiatric disorders. The potential efficacy of Chlorpromazine as a treatment for COVID-19 depends on the ability of Chlorpromazine to increase the pH of lysosome. This will deprive the COVID-19 to set its genetic material free for further replication. NCT04354805
- Talk about repurposing old drugs! Here is a study of methotrexate embedded in nanoparticles to treat severe SARS-CoV-2 from Brazil. NCT04352465
- Here is a Phase 2 trial of gimsilumab (a monoclonal against GM-CSF) on patients with lung injury or ARDS. NCT04351243
- Here is a first in human trial of a Theravance nebulized pan-JAK inhibitor, TD-0903. It's not clear how fast they can proceed o Phase 2. NCT04350736
- Here is an Egyptian study of isotretinoin. The rationale is retinoic acid which induce FOXP3 and CD8+,CD4+,CD25+,FOXP3+ Tregs which were dramatically reduced in COVID-19 patients to exert its anti-inflammatory effect protecting lung cell and neural cells from the inflammatory and destructive effect of IL-6. In addition to inducing retinoic acid inducible gene-1 (RIG-1) and endosomal toll-like receptor 3 (TLR3) as pathogen-associated molecular patterns. This recognition resulted in the formation of type-1 interferon (IFN1). As an evasion mechanism, virus synthesize proteins that hinder the production of IFN type1 in COVID 2019 infection . NCT04353180

CLINICAL TRIAL RESULTS

- Coming up with good triage approaches for determining which patients are most at risk is imperative. Here is some good work from China. Since the pandemic outbreak of coronavirus disease 2019 (COVID-19), the health system capacity in highly endemic areas has been overwhelmed. Approaches to efficient management are urgently needed. We aimed to develop and validate a score for early prediction of clinical deterioration of COVID-19 patients. Methods: In this retrospective multicenter cohort study, we included 1138 mild to moderate COVID-19 patients admitted to 33 hospitals in Guangdong Province from December 27, 2019 to March 4, 2020 (N =818; training cohort), as well as two hospitals in Hubei Province from January 21 to February 22, 2020 (N =320; validation cohort) in the analysis. Results: The 14-day cumulative incidences of clinical deterioration were 7.9% and 12.1% in the training and validation cohorts, respectively. An Early WArning Score (EWAS) (ranging from 0 to 4.5), comprising of age, underlying chronic disease, neutrophil to lymphocyte ratio, C-reactive protein, and D-dimer levels, was developed (AUROC: 0.857). By applying the EWAS, patients were categorized into low-, medium-, and high risk groups (cut-off values: two and three). The 14-day cumulative

incidence of clinical deterioration in the low-risk group was 1.8%, which was significantly lower than the incidence rates in the medium- (14.4%) and high-risk (40.9%) groups (P <.001). The predictability of EWAS was similar in the validation cohort (AUROC =0.781), patients in the low-, medium-, and high-risk groups had 14-day cumulative incidences of 2.6%, 10.0%, and 25.7%, respectively (P <.001). Conclusion: The EWAS, which is based on five common parameters, can predict COVID-19-related clinical deterioration and may be a useful tool for a rapid triage and establishing a COVID-19 hierarchical management system that will greatly focus clinical management and medical resources to reduce mortality in highly endemic areas. https://www.medrxiv.org/content/10.1101/2020.04.17.20064691v1

- Hydroxychloroquine data starts to trickle in!! Despite limited and conflicting data on the use of hydroxychloroquine in patients with Covid-19, the U.S. Food and Drug Administration has authorized the emergency use of this drug when clinical trials are unavailable or infeasible. Hydroxychloroquine, alone or in combination with azithromycin, is being widely used in Covid-19 therapy based on anecdotal and limited observational evidence. METHODS: We performed a retrospective analysis of data from patients hospitalized with confirmed SARS-CoV-2 infection in all United States Veterans Health Administration medical centers until April 11, 2020. Patients were categorized based on their exposure to hydroxychloroquine alone (HC) or with azithromycin (HC+AZ) as treatments in addition to standard supportive management for Covid-19. The two primary outcomes were death and the need for mechanical ventilation. We determined the association between treatment and the primary outcomes using competing risk hazard regression adjusting for clinical characteristics via propensity scores. Discharge and death were taken into account as competing risks and subdistribution hazard ratios are presented. RESULTS: A total of 368 patients were evaluated (HC, n=97; HC+AZ, n=113; no HC, n=158). Rates of death in the HC, HC+AZ, and no HC groups were 27.8%, 22.1%, 11.4%, respectively. Rates of ventilation in the HC, HC+AZ, and no HC groups were 13.3%, 6.9%, 14.1%, respectively. Compared to the no HC group, the risk of death from any cause was higher in the HC group (adjusted hazard ratio, 2.61; 95% CI, 1.10 to 6.17; P=0.03) but not in the HC+AZ group (adjusted hazard ratio, 1.14; 95% CI, 0.56 to 2.32; P=0.72). The risk of ventilation was similar in the HC group (adjusted hazard ratio, 1.43; 95% CI, 0.53 to 3.79; P=0.48) and in the HC+AZ group (adjusted hazard ratio, 0.43; 95% CI, 0.16 to 1.12; P=0.09), compared to the no HC group. CONCLUSIONS: *In this study, we found no evidence that use of hydroxychloroquine, either with or without azithromycin, reduced the risk of mechanical ventilation in patients hospitalized with Covid-19. An association of increased overall mortality was identified in patients treated with hydroxychloroquine alone.* These findings highlight the importance of awaiting the results of ongoing prospective, randomized, controlled studies before widespread adoption of these drugs. [**sarcastic note to self: It's really a good thing this is becoming the standard of care.**] https://www.medrxiv.org/content/10.1101/2020.04.16.20065920v1
- Here is a small histopathology study from the first epicenter in the US (Seattle) of SARS-CoV-2 infection. To date, documentation of histopathologic features in fatal cases of COVID-19 has been limited due to small sample size and incomplete organ sampling. Methods Post-mortem examinations were performed on 12 fatal COVID-19 cases in Washington State during February-March 2020. Clinical and laboratory data were reviewed. Tissue examination of all major organs was performed by light microscopy and electron microscopy. The presence of viral RNA in sampled tissues was tested by RT-PCR. Results All 12 patients were older with significant

preexisting comorbidities. The major pulmonary finding was diffuse alveolar damage in the acute and/or organizing phases with virus identified in type I and II pneumocytes by electron microscopy. The kidney demonstrated viral particles in the tubular epithelium, endothelium, and podocytes without significant inflammation. Viral particles were also observed in the trachea and large intestines. SARS-CoV-2 RNA was detected in the cardiac tissue of a patient with lymphocytic myocarditis. RT-PCR also detected viral RNA in the subcarinal lymph nodes, liver, spleen, and large intestines. Conclusion SARS-CoV-2 represents the third novel coronavirus to cause widespread human disease since 2002. Similar to SARS and MERS, the primary pathology was diffuse alveolar damage with virus located in the pneumocytes. However, other major organs including the heart and kidneys may be susceptible to viral replication and damage leading to increased mortality in those with disseminated disease. Understanding the pathology of SARS-CoV-2 will be essential to design effective therapies. [**note: I include this for the physicians who read this newsletter and are interested in this stuff.]**
https://www.medrxiv.org/content/10.1101/2020.04.17.20058545v1

- Clinical trial results on safety and efficacy of α-lipoic acid for critically ill patients. Methods: A randomized, single-blind, group sequential, active-controlled trial was performed at JinYinTan Hospital, Wuhan, China. Between February 2020 and March 2020, 17 patients with critically ill COVID-19 were enrolled in our study. Eligible patients were randomly assigned in a 1:1 ratio to receive either ALA (1200 mg/d, intravenous infusion) once daily plus standard care or standard care plus equal volume saline infusion (placebo) for 7 days. All patients were monitored within the 7 days therapy and followed up to day 30 after therapy. The primary outcome of this study was the Sequential Organ Failure Estimate (SOFA) score, and the secondary outcome was the all-cause mortality within 30 days. Result: Nine patients were randomized to placebo group and 8 patients were randomized to ALA group. SOFA score was similar at baseline, increased from 4.3 to 6.0 in the placebo group and increased from 3.8 to 4.0 in the ALA group (P=0.36) after 7 days. The 30-day all-cause mortality tended to be lower in the ALA group (3/8, 37.5%) compared to that in the placebo group (7/9, 77.8%, P=0.09). Conclusion: In our study, ALA use is associated with lower SOFA score increase and lower 30-day all-cause mortality as compared with the placebo group. Although the mortality rate was two-folds higher in placebo group than in ALA group, only borderline statistical difference was evidenced due to the limited patient number. Future studies with larger patient cohort are warranted to validate the role of ALA in critically ill patients with COVID-19. Keywords: Pneumonia; COVID-19; SARS-CoV-2 ; α-Lipoic acid. [**note: one wonders whether this will ever be followed up.**]
https://www.medrxiv.org/content/10.1101/2020.04.15.20066266v1
- My eyes glazed over with this paper: 70 pages and more authors than I could count. It's likely important but out of my wheelhouse. Nonetheless, its included here for those who have a better grasp of this stuff. The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, creates an urgent need for identifying molecular mechanisms that mediate viral entry, propagation, and tissue pathology. Cell membrane bound angiotensin-converting enzyme 2 (ACE2) and associated proteases, transmembrane protease serine 2 (TMPRSS2) and Cathepsin L (CTSL), were previously identified as mediators of SARS-CoV2 cellular entry. Here, we assess the cell type-specific RNA expression of ACE2, TMPRSS2, and CTSL through an integrated analysis of 107 single-cell and single-nucleus RNA-Seq studies, including 22 lung and airways datasets (16 unpublished), and 85 datasets from other diverse organs. Joint expression of ACE2 and the

accessory proteases identifies specific subsets of respiratory epithelial cells as putative targets of viral infection in the nasal passages, airways, and alveoli. Cells that co-express ACE2 and proteases are also identified in cells from other organs, some of which have been associated with COVID-19 transmission or pathology, including gut enterocytes, corneal epithelial cells, cardiomyocytes, heart pericytes, olfactory sustentacular cells, and renal epithelial cells. Performing the first meta-analyses of scRNA-seq studies, we analyzed 1,176,683 cells from 282 nasal, airway, and lung parenchyma samples from 164 donors spanning fetal, childhood, adult, and elderly age groups, associate increased levels of ACE2, TMPRSS2, and CTSL in specific cell types with increasing age, male gender, and smoking, all of which are epidemiologically linked to COVID-19 susceptibility and outcomes. Notably, there was a particularly low expression of ACE2 in the few young pediatric samples in the analysis. Further analysis reveals a gene expression program shared by ACE2+TMPRSS2+ cells in nasal, lung and gut tissues, including genes that may mediate viral entry, subtend key immune functions, and mediate epithelial-macrophage cross-talk. Amongst these are IL6, its receptor and co-receptor, IL1R, TNF response pathways, and complement genes. Cell type specificity in the lung and airways and smoking effects were conserved in mice. Our analyses suggest that differences in the cell type-specific expression of mediators of SARS-CoV-2 viral entry may be responsible for aspects of COVID-19 epidemiology and clinical course, and point to putative molecular pathways involved in disease susceptibility and pathogenesis. https://www.biorxiv.org/content/10.1101/2020.04.19.049254v1

- Sad cohort analysis from Italy. Design Prospective cohort study performed between February 21th and March 19rd, 2020 Setting Hospital-based study Participants Of 2,217 admitted, 766 consecutive individuals either reporting or presenting with fever, cough or dyspnea, and suspected to carry Covid-19 infection were examined. Intervention All individuals underwent body temperature and pulse oximetry recording, hematological screening, chest X-ray and/or computed tomography (CT) and SARS-COV-2 assay on nasopharyngeal swab. Onset symptoms, course, comorbidities, number of drugs, use of angiotensin converting enzyme inhibitors and angiotensin-II-receptor antagonists, and follow-up swab, clinical, hematological, and radiological exams, treatments, non-invasive respiratory support, ICU admission, and deaths were recorded. Main outcome measures Primary outcomes were non-invasive respiratory support, intensive care unit (ICU) admission, and death. Results Median age of 411 Covid-19 patients was 70.5 years (range 1-99; 66.6% males). CT was positive in 74% and negative in 3.2%. Six patients died within 72 hours; another 66 during hospitalization. Fatality rate was 17.5% (74% males). No death occurred below 60 years. Mortality was 6.6% in 60-69 decade, 21.1% in 70-79, 38.8% in 80-89, and 83.3% above 90 years. Non-invasive respiratory support rate was 27.2%; ICU admission 6.8%. Older age, cough and dyspnea at onset, hypertension, cardiovascular diseases, diabetes, renal insufficiency, >7 drugs intake and positive X-ray at admission were significantly associated with death. Low lymphocyte count, high C-reactive protein, aspartate aminotransferase and lactate dehydrogenase, and low PO2 partial pressure with high lactate at arterial blood gas analysis at admission were also significantly associated with death. Of 32 swab negative patients, 40.6% turned positive at follow-up. Using CT as reference, nasopharyngeal swab had 80% sensitivity. Comorbidity network analysis revealed homogenous distribution of deceased and 60-80 aged patients across diseases. Conclusions Covid-19 caused high mortality among patients older than 70 years and correlated by pre-existing multiorgan impairment irrespective of the age. https://www.medrxiv.org/content/10.1101/2020.04.14.20053090v1

- I believe that I posted an abstract on the use of heparin for treatment of severe cases. Here is another one. Elevated D-dimer and other markers of coagulation disturbances are predictors of severity and mortality in COVID-19 patients and heparin use during hospital stay has been associated to a decreased mortality. Similar findings have been described in other coronaviruses. COVID-19 patient autopsies have revealed thrombi in the microvasculature, suggesting intravascular coagulation as a prominent feature of organ failure and of the acro-ischaemia increasingly reported in these patients. In COVID-19 pulmonary compliance is preserved despite severe hypoxemia, contrarily to classic ARDS, corroborating the hypothesis that perfusion mismatch may play a significant role in the development of respiratory failure in these patients. Based on this rationale, a series of 27 consecutive COVID-19 patients admitted to our hospital were treated with heparin in doses tailored to clinical severity, ranging from 1 - 2 mg/kg of enoxaparin BD. PaO2/FiO2 ratio increased significantly over the 72 hours following the start of anticoagulation, from 254(SD 90) to 325(SD 80), p=0.013, and over half of the patients were discharged home within an average time of 7.3 (SD 4.0) days. Half of mechanically ventilated patients were extubated within 10.3 (SD 1.5) days. The remaining patients showed progressive, albeit slower improvement, and both the mortality and the bleeding complications rate in these patients were absent. Even though this uncontrolled case series does not offer absolute proof of DIC as the underlying mechanism of respiratory failure in COVID-19, as well as patients positive response to tailored dose heparinization, it contributes to the understanding of the physiopathological mechanism of the disease and provides valuable information for the treatment of these very sick patients while we await the results of further prospective controlled studies. https://www.medrxiv.org/content/10.1101/2020.04.15.20067017v1

DRUG DEVELOPMENT

- Pretty cool technology to isolate blocking antibodies. SARS-CoV-2 relies on its spike protein, in particular the receptor binding domain (RBD), to bind human cell receptor angiotensin-converting enzyme 2 (ACE2) for viral entry, and thus targeting RBD holds the promise for preventing SARS-CoV-2 infection. In this work, a competitive biopanning strategy of a phage display antibody library was applied to screen blocking antibodies against RBD. High-affinity antibodies were enriched after the first round using a standard panning process in which RBD-His recombinant protein was immobilized as a bait. At the next two rounds, immobilized ACE2-Fc and free RBD-His proteins were mixed with the enriched phage antibodies. Antibodies binding to RBD at epitopes different from ACE2-binding site were captured by the immobilized ACE2-Fc, forming a "sandwich" complex. Only antibodies competed with ACE2 for recognizing RBD at the same or similar epitopes can bind to the free RBD-His in the supernatant and be subsequently separated by the Ni-NTA magnetic beads. Top 1 lead from the competitive biopanning of a synthetic antibody library, Lib AB1, was produced as the full-length IgG1 format. It was proved to competitively block the binding of RBD to ACE2 protein, and potently inhibit SARS-CoV-2 pseudovirus infection of ACE2-overexpressing Hela cells with IC50 values of 12nM. Nevertheless, top 1 lead from the standard biopanning of Lib AB1, can only bind to RBD in vitro but not have the blocking or neutralization activity. Our strategy can efficiently isolate the blocking antibodies of RBD, and it would speed up the discovery of neutralizing antibodies against SARS-CoV-2. https://www.biorxiv.org/content/10.1101/2020.04.19.049643v1

- In this study, we report our discovery of inhibitors targeting the SARS-CoV-2 main protease (Mpro). Using the FRET-based enzymatic assay, several inhibitors including boceprevir, GC-376, calpain inhibitors II, XII, and MG-132 were identified to have potent activity with single-digit to submicromolar IC50 values in the enzymatic assay. The mechanism of action of the hits was further characterized using enzyme kinetic studies and thermal shift binding assays. Significantly, four compounds (boceprevir, GC-376, calpain inhibitors II and XII) inhibit SARS-CoV-2 viral replication in cell culture with EC50 values ranging from 0.49 to 3.37 micromolar. Notably, boceprevir, calpain inhibitors II and XII represent novel chemotypes that are distinct from known Mpro inhibitors. Overall, the compounds identified herein provide promising starting points for the further development of SARS-CoV-2 therapeutics. [**note: some interesting drug development work**] https://www.biorxiv.org/content/10.1101/2020.04.20.051581v1

DIAGNOSTIC DEVELOPMENT

- Damn!!! If the Wuhan scientists can do this why can't we? The laboratory diagnosis of SARS-CoV-2 infection has relied on nucleic acid tests. However, there are many limitations of nucleic acid tests, including low throughput and high rates of false negatives. More sensitive and accurate tests to effectively identify infected patients are needed. METHODS: This study has developed fully automated chemiluminescent immunoassays (CLIA) to determine IgM and IgG antibodies to SARS-CoV-2 in human serum. The assay performance has been evaluated at 10 hospitals. Clinical specificity was evaluated by measuring 972 hospitalized patients with diseases other than COVID-19, and 586 donors of a normal population. Clinical sensitivity was assessed on 503 confirmed cases of SARS-CoV-2 by RT-PCR and 52 suspected cases. RESULTS: The assays demonstrated satisfied assay precision with coefficient of variation (CV) of less than 4.45%. Inactivation of specimen does not affect assay measurement. SARS-CoV-2 IgM shows clinical specificity of 97.33% and 99.49% for hospitalized patients and normal population respectively. SARS-CoV-2 IgG shows clinical specificity of 97.43% and 99.15% for the hospitalized patients and the normal population respectively. SARS-CoV-2 IgM and IgG show clinical sensitivity of 85.88% and 96.62% respectively for confirmed SARS-Cov-2 infection with RT-PCR, of 73.08% and 86.54% respectively for suspected cases. CONCLUSIONS: we have developed fully automated immunoassays for detecting SARS-CoV-2 IgM and IgG antibodies in human serum. The assays demonstrated high clinical specificity and sensitivity, and add great value to nucleic acid testing in fighting against the global pandemic of the SARS-CoV-2 infection. [**note: do I need to mention massive testing at all?**]
  https://www.medrxiv.org/content/10.1101/2020.04.16.20067231v1
- Good stuff from Singapore, rapid PCR testing using a portable thermocycler. At present, confirmatory diagnosis is by reverse transcription polymerase chain reaction (RT-PCR), typically taking several hours and requiring a molecular laboratory to perform. There is an urgent need for rapid, simplified and cost-effective detection methods. We have developed and analytically validated a protocol for direct rapid extraction-free PCR (DIRECT-PCR) detection of SARS-CoV-2 without the need for nucleic acid purification. As few as 6 RNA copies per reaction of viral nucleocapsid (N) gene from respiratory samples such as sputum and nasal exudate can be detected directly using our one-step inhibitor-resistant assay. The performance of this assay was validated on a commercially available portable PCR thermocycler. Viral lysis, reverse

transcription, amplification and detection are achieved in a single-tube homogeneous reaction within 36 minutes. This minimized hands-on time, reduces turnaround-time for sample-to-result and obviates the need for RNA purification reagents. It could enable wider use of Covid-19 testing for diagnosis, screening and research in countries and regions where laboratory capabilities are limiting. [**note: the portable machine they used came from Ireland!! Who would have guessed? http://www.itislifescience.com/**]
https://www.biorxiv.org/content/10.1101/2020.04.17.042366v1

- Here is a good approach for studying neutralization assays. assays to measure how antibodies and sera affect Spike-mediated viral infection are important for studying immunity. Because SARS-CoV-2 is a biosafety-level-3 virus, one way to simplify such assays is to pseudotype biosafety-level-2 viral particles with Spike. Such pseudotyping has now been described for single-cycle lentiviral, retroviral and VSV particles, but the reagents and protocols are not widely available. Here we detail how to effectively pseudotype lentiviral particles with SARS-CoV-2 Spike and infect 293T cells engineered to express the SARS-CoV-2 receptor, ACE2. We also make all the key experimental reagents available in the BEI Resources repository of ATCC and the NIH. Furthermore, we demonstrate how these pseudotyped lentiviral particles can be used to measure the neutralizing activity of human sera or plasma against SARS-CoV-2 in convenient luciferase-based assays, thereby providing a valuable complement to ELISA-based methods that measure antibody binding rather than neutralization. [**note: sharing of reagents is always a good thing!!**] https://www.biorxiv.org/content/10.1101/2020.04.20.051219v1
- The UK got burned by ordering a lot of lateral flow diagnostic test kits. Here is a summary of the testing experience. The early promise of lateral flow immunoassay (LFIA) devices has been questioned following concerns about sensitivity and specificity. Methods: We used a panel of plasma samples designated SARS-CoV-2 positive (from SARS-CoV-2 RT-PCR-positive individuals; n=40) and negative (samples banked in the UK prior to December-2019 (n=142)). We tested plasma for SARS-Cov-2 IgM and IgG antibodies by ELISA and using nine different commercially available LFIA devices. Results: ELISA detected SARS-CoV-2 IgM or IgG in 34/40 individuals with an RT-PCR-confirmed diagnosis of SARS-CoV-2 infection (sensitivity 85%, 95%CI 70-94%), vs 0/50 pre-pandemic controls (specificity 100% [95%CI 93-100%]). IgG levels were detected in 31/31 RT-PCR-positive individuals tested ≥10 days after symptom onset (sensitivity 100%, 95%CI 89-100%). IgG titres rose during the 3 weeks post symptom onset and began to fall by 8 weeks, but remained above the detection threshold. Point estimates for the sensitivity of LFIA devices ranged from 55-70% versus RT-PCR and 65-85% versus ELISA, with specificity 95-100% and 93-100% respectively. Within the limits of the study size, the performance of most LFIA devices was similar. Conclusions: *The performance of current LFIA devices is inadequate for most individual patient applications.* ELISA can be calibrated to be specific for detecting and quantifying SARS-CoV-2 IgM and IgG and is highly sensitive for IgG from 10 days following symptoms onset.
https://www.medrxiv.org/content/10.1101/2020.04.15.20066407v1

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2020-04-22

WuMo is doing some funny COVID-19 strips these days.

I am fond of piano transcriptions of operas. Franz Liszt was the first one to do this, and regularly toured Europe playing virtuoso pieces. Here is a nice arrangement of Wagner's Götterdämmerung by pianist Nikolay Lugansky: https://www.youtube.com/watch?v=RvbUnf-Fygc

I routinely post a lot of links in the newsletter. I apologize if any of these are behind paywalls. I have subscriptions to several newspapers and magazines and am not able to check this.

Here is an important link. NIH has issued a set of Treatment Guidelines for SARS-CoV-2. Money quotes:

• *The COVID-19 Treatment Guidelines Panel (the Panel)* ***does not recommend*** *the use of any agents for pre-exposure prophylaxis (PrEP) against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outside of the setting of a clinical trial* ***(AIII)****.*

• *The Panel* ***does not recommend*** *the use of any agents for post-exposure prophylaxis (PEP) against SARS-CoV-2 infection outside of the setting of a clinical trial* ***(****AIII****).***

Will this slow down the "standard of care" use of hydroxychloroquine?

This note from the USC group came in too late for yesterday's email: http://createsend.com/t/j-296D9D8CE54262BB2540EF23F30FEDED They did a similar serology surveillance of Los Angeles using the same test kit. Results extrapolate to a much higher infection rate as per Santa Clara county. I'm sure there nitpickers will come out of the woodwork arguing that there is some problem with this surveillance. Even if the test has a small percent of false positives, it does not negate the finding that the level of infection is higher (and maybe much higher) than the reported numbers. While these lateral flow tests may not have the necessary validation to make difficult decisions about who can or cannot return to work, they are useful in doing field epidemiology to get an idea of the background rate of infection!

I am on the email list of the Reagan-Udall Foundation for the FDA. This arrived in my inbox the other day:

> The Reagan-Udall Foundation for the FDA (Foundation) in collaboration with Friends of Cancer Research (*Friends*) today announced the COVID-19 Evidence Accelerator, an expansive public-private partnership combining the efforts of academic, government, and private sector organizations applying data analytics to accelerate the understanding of COVID-19.
>
> "We are honored to be launching this new initiative, working closely with FDA in leveraging real-world evidence to accelerate the pace at which new data can be brought together to ultimately help patients afflicted with COVID-19," said Ellen V. Sigal, Chair of both the Reagan-Udall Foundation and Friends of Cancer Research. "It is inspiring how quickly our Evidence Accelerator partners embraced this opportunity to contribute vital data to help find solutions to this crisis."
>
> Mobilizing major data organizations, government and academic researchers, and health systems, the Evidence Accelerator builds on the innovative work of its partners to maximize the use of real-world data from across the country. Contributing researchers will engage in weekly meetings to present and critically discuss findings from different data sources. In addition, the Evidence Accelerator will facilitate parallel analyses of key questions to rapidly identify patient characteristics, treatment patterns, and management strategies for COVID-19.
>
> "Bringing together experts and stakeholders to respond to COVID-19 is exactly the kind of work that Congress envisioned when establishing the Reagan-Udall Foundation," said Sigal "Additionally, the Evidence Accelerator builds on *Friends'* long history of creating innovative partnerships with all sectors, ensuring patients receive the best treatments in the fastest and safest way possible."

I honestly do not know what to make of this effort. Looking at the link, it appears that they want to reach out to lots of different parties and encouraged data sharing. That is easier said than done. There are a lot of efforts already underway regarding to some of the questions they are asking so it's unclear what this has to offer. Where is the data from all these responses going to be stored and analyzed? Why undertake a public effort on hydroxychloroquine ± azithromycin? OHDSI has already answered the safety issue. There is an interesting summary of a call with lab directors worth reading. [**note: after all these years of using MSFT Word, I finally figured out how to embed weblinks in document. Wow! You can teach an old dog new tricks. I'll make a conscientious effort to provide informative links to drugs going forward.**]

James Hablin has a nice piece in The Atlantic on the immune system response to SARS-CoV-2.

The Santa Clara county coroner's office has identified two early deaths from COVID-19. The earliest death was on February 6 indicating infection in mid to late January.

The New York Times has a great story on molecular epidemiology and how SARS-CoV-2 spread in the Seattle community. I find it amazing that we have these sophisticated tools at our disposal!!

The first clinical data on tocilizumab are out. You will have to scroll down to read the abstract; I'm not spoiling this one as you should read every single abstract that I have laboriously curated!!

MODELING

- Here is a good paper from Ontario analyzing data of SARS-CoV-2 to April 15. When calculated from aggregate data on confirmed cases and deaths, the case-fatality risk (CFR) is a simple ratio between the former and the latter, which is prone to numerous biases. With individual-level data, the CFR can be estimated as a true measure of risk as the proportion of incidence for the disease. We present the first estimates of the CFR for COVID-19 by age and sex based on event history modelling of the risk of dying among confirmed positive individuals in the Canadian province of Ontario, which maintains one of the few individual-level datasets on COVID-19 in the world. [**note: the abstract does not do justice to the paper. There is some good data here and cutting out some of the tables would not work. You can download the paper at the link.**] https://www.medrxiv.org/content/10.1101/2020.04.16.20067751v1

NEWLY REGISTERED CLINICAL TRIALS

[**note**] I did a quick search of the NLM clinical trials data base. 120 of the 795 listed trials involve hydroxychloroquine. It should be noted that the 795 registered trials are not all for pharmaceutical intervention so it's not easy to say what the % of trials hydroxychloroquine represent. However, it is the most commonly used therapy of those I've seen posted. There are 45 trials listed for azithromycin, not all of which are in combination with hydroxychloroquine. All of these are listed as recruiting or not yet recruiting. None are listed as closed for further enrollment. I will let my loyal readers draw whatever conclusion they wish from this data.

- Here is an inhaled ribavirin trial. This study is a Phase 1, open label, non-randomized, two-arm interventional clinical trial to evaluate the safety and efficacy of Virazole® in hospitalized adult patients who have tested positive for COVID-19 and, as a result, have significant respiratory distress (PaO2/FiO2 ratio <300 mmHg). NCT04356677

- From Italy, a trial of nafamostat to look at lung function. Purpose: SARS-Cov-2 enters the lung cells by binding to ACE-2 and activating the protease TMPRSS2, which, therefore, can be a target for antiviral treatment. Accordingly, TMPRSS2 inhibitors prevent SARS-CoV cell entry in vitro. The most potent such inhibitors, nafamostat is being used as anticoagulant and anti-pancreatitis agent, and is approved for the treatment of cystic fibrosis as its mucolytic action can prevent lung function deterioration by owering airways infections. RACONA study will test the hypothesize that nafamostat is useful in COVID-19 lung involvement because COVID-19 entails activation of the coagulation cascade, pulmonary embolism, and bacterial superinfections. NCT04352400

- I don't know who *The Camelot Foundation* are but they have a bunch of combinations in this proposed trial. Hydroxychloroquine with azithromycin (natch) but also doxycycline and clindamycin. [**note: you really need to go to the trial site to see everything.**] NCT04349410
- This French trial proposes to look at IV almitrine to see if it can reduce the need for mechanical ventilation in patients with hypoxemic acute respiratory failure because of SARS-CoV-2. NCT04357457

- Any port in a storm. This Egyptian investigator is going to see if MMR vaccine can provide protection in healthcare workers. Till now, mortality reports among children below 9 years

remains extremely low despite that the incidence of death toll is high and exceeding 50,000 patients among older population, One speculation for lower SARS infectivity is that cross-protective antibodies against measles vaccine (MV). In mice susceptible to measles virus, recombinant MV induced the highest titers of neutralizing antibodies and fully protected immunized animals from intranasal infectious challenge with SARS-CoV, The primary objective of the present study is to determine the benefit of measles vaccine in health care professional to decrease the incidence of COVID-19. We Hypothesized that, measles vaccine may lower the incidence of serologically proven SARS-CoV-2 infection and reported respiratory illness {**note: what will this mean to the anti-vaxxer community. We know how to make this vaccine at scale!! Glad to be a Merck shareholder and it's time to buy more.**] NCT04357028

- Here is a French trial of imatinib in aged patients. High-throughput screening studies identified Abl kinase inhibitors (including imatinib) as inhibitors of coronaviruses SARS and MERS. The SARS-CoV-2 coronavirus depend on Abl2 kinase activity to fuse and enter into the cells. Pharmacokinetic studies demonstrated that IC50 of imatinib for ABL1, BCR-ABL1 and ABL2 kinase inhibition is less than 1 microM (around 0.3 microM) below the expected trough plasmatic concentrations of imatinib 400 mg/day (1.7 microM). The EC50 of imatinib for the inhibition of the virus is under investigation but we now have a first estimates with EC50 close to 2.5 microM. This plasmatic concentration is achievable with imatinib 800 mg/d. We hypothesize that clinically achievable imatinib concentration will block the first round of cell to cell virus infection and therefore stop or prevent from SARS-CoV-2 infection in human. Based on our 20 years' experience of prescribing imatinib in patients, we expect that most of the adverse events and pharmacological interactions of imatinib can be anticipated and corrected. The eligible population will be aged (>70y) patients hospitalized for a non-severe COVID-19 disease for less than 7 days. Patients will be randomized 1/1 between standard of care and imatinib 800 mg per day during 14 days. The primary endpoint will be the death rate by 30 days. Secondary endpoint will include progression to severe CIVID-19 disease, safety, outcome at 3 months. We plan to randomize 90 patients in order to show a 10% benefit in term of death rate reduction from 16% to 6%. [**note: I remember when the DSMB stopped the leukemia trial as all patients recovered. Novartis CEO Daniel Vassella stunned his production team saying the drug would immediately be made available at no cost to all prior to FDA approval.**] NCT04357613

CLINICAL TRIAL RESULTS

- Is it time to cross glucocorticoids off the list of drugs to use? These Chinese investigators think so. We comprehensively searched electronic databases and supplemented the screening by conducting a manual search. We included RCTs and cohort studies evaluating the effectiveness and safety of glucocorticoids in children and adults with COVID-19, SARS and MERS, and conducted meta-analyses of the main indicators that were identified in the studies. Results: Our search retrieved 23 studies, including one RCT and 22 cohort studies, with a total of 13,815 patients. In adults with COVID-19, the use of systemic glucocorticoid did not reduce mortality (RR=2.00, 95% CI: 0.69 to 5.75, I^2=90.9%) or the duration of lung inflammation (WMD=-1 days, 95% CI: -2.91 to 0.91), while a significant reduction was found in the duration of fever (WMD=-3.23 days, 95% CI: -3.56 to -2.90). In patients with SARS, glucocorticoids also did not reduce the mortality (RR=1.52, 95% CI: 0.89 to 2.60, I^2=84.6%), duration of fever (WMD=0.82 days, 95% CI: -

2.88 to 4.52, I^2=97.9%) or duration of lung inflammation absorption (WMD=0.95 days, 95% CI: -7.57 to 9.48, I^2=94.6%). The use of systemic glucocorticoid therapy prolonged the duration of hospital stay in all patients (COVID-19, SARS and MERS). Conclusions: Glucocorticoid therapy was found to reduce the duration of fever, but not mortality, duration of hospitalization or lung inflammation absorption. Long-term use of high-dose glucocorticoids increased the risk of adverse reactions such as coinfections, so routine use of systemic glucocorticoids for patients with COVID-19 cannot be recommend.
https://www.medrxiv.org/content/10.1101/2020.04.17.20064469v1

- Another *nail in the coffin* for the BCG hypothesis? In the middle of the global COVID-19 pandemic, the BCG hypothesis, the prevalence and severity of the COVID-19 outbreak seems to be correlated with whether a country has a universal coverage of Bacillus-Calmette-Guerin (BCG), a vaccine for tuberculosis disease (TB), has emerged and attracted the attention of scientific community and media outlets. However, all existing claims are based on cross-country correlations that do not exclude the possibility of spurious correlation. We merged country-age-level case statistics with the start/termination years of BCG vaccination policy and conducted a regression discontinuity and difference-in-difference analysis. The results do not support the BCG hypothesis. [**note: there are some clinical trials in healthcare workers going on and maybe that will answer this question.**]
https://www.medrxiv.org/content/10.1101/2020.04.13.20064287v1
- Oh no!! Say it ain't so Joe! I post this because it comes from Emory University. Objective To mine Twitter to quantitatively analyze COVID-19 symptoms self-reported by users, compare symptom distributions against clinical studies, and create a symptom lexicon for the research community. Materials and methods We retrieved tweets using COVID-19-related keywords, and performed several layers of semi-automatic filtering to curate self-reports of positive-tested users. We extracted COVID-19-related symptoms mentioned by the users, mapped them to standard IDs, and compared the distributions with multiple studies conducted in clinical settings. Results We identified 203 positive-tested users who reported 932 symptoms using 598 unique expressions. The most frequently-reported symptoms were fever/pyrexia (65%), cough (56%), body aches/pain (40%), headache (35%), fatigue (35%), and dyspnea (34%) amongst users who reported at least 1 symptom. Mild symptoms, such as anosmia (26%) and ageusia (24%) were frequently reported on Twitter, but not in clinical studies. Conclusion The spectrum of COVID-19 symptoms identified from Twitter may complement those identified in clinical settings. [**note to self: this in no way will entice me to sign up for a Twitter account. I may be the only person left in America that does not have a Twitter or Facebook account. Ooops, I forgot, my wife doesn't have one either.**]
https://www.medrxiv.org/content/10.1101/2020.04.16.20067421v1
- More data on what blood factors lead to poor clinical outcomes. This retrospective study investigated the implications of changes in blood parameters and cellular immune function in patients with 2019-coronavirus infected disease (COVID-19). Methods: Records were reviewed of 85 patients with COVID-19 between February 4 and 16, 2020. The primary outcome was in-hospital mortality at 28 days. Results: Fourteen patients died. The baseline leukocyte count, neutrophil count and hemoglobin was significantly higher in non-survivors compared with survivors, while the reverse was true of lymphocyte count, platelet, PaO2/FiO2, CD3+ count and CD4+ count. The percentage of neutrophil count > 6.3*109/L in death group was significantly

higher than that in survival group, and multivariate logistic regression showed neutrophil count was independently associated with mortality. However, there were not significant difference in IgG, IgM, IgA, C3, C4 and the percentage of IgE > 100 IU/ml between the death group and survival group. Areas under the receiver operating characteristic curves of the following at baseline could significantly predict mortality: leukocyte, neutrophil, lymphocyte, CD3+ and CD4+ counts. Conclusions: For patients with COVID-19, lymphocyte, CD3+ and CD4+ counts that marked decrease suggest a poor outcome. A high neutrophil count is independently associated with mortality. At admission, leukocyte, neutrophil, lymphocyte, CD3+ and CD4+ counts should receive added attention. https://www.medrxiv.org/content/10.1101/2020.04.16.20067587v1

- Following the assumption by Mehta and colleagues who exhorted physicians to screen patients with severe COVID-19 for hyperinflammation and investigate immunomodulatory drugs in this setting, we relate our short-term - yet promising - experience regarding IL6 blockade with tocilizumab in 30 selected patients of less than 80 years of age, >5 days of prior disease duration, severe (i.e. requiring strictly over 6L/min of oxygen therapy) rapidly deteriorating (i.e. increase by more than 3L/min of oxygen flow within the previous 12 hours) COVID-19-related pneumonia. By comparison with a control group of patients (matched for age, gender and disease severity using the inverse probability of treatment weighted methodology) that did not receive tocilizumab. We demonstrate that, in highly selected patients, IL6 blockade could curb the "cytokine storm", prevent ICU admission and the requirement for mechanical ventilation. Notwithstanding the shortcomings of this retrospective small sample-size study, we believe that these preliminary findings support the fostering of research efforts in the fight against COVID-19-induced inflammation, especially before patients require admission to the ICU. [**NOTE: THIS IS REALLY PRELIMINARY AND NEEDS TO BE REPLICATED WITH CONTROLS.**] https://www.medrxiv.org/content/10.1101/2020.04.20.20061861v1
- More clinical information n the immune system from Germany. the role of host immune responses in viral clearance and its involvement in pathogenesis remains unresolved. For SARS-CoV (2002/03), however, CD4+ T cell responses are generally associated with positive outcomes3,4, while cellular immune responses to SARS-CoV-2 have not yet been investigated. Here we describe an assay that allows direct detection and characterization of SARS-CoV-2 spike glycoprotein (S)-reactive CD4+ T cells in peripheral blood. We demonstrate the presence of S-reactive CD4+ T cells in 83% of COVID-19 patients, as well as in 34% of SARS-CoV-2 seronegative healthy donors, albeit at lower frequencies. Strikingly, in COVID-19 patients S-reactive CD4+ T cells equally targeted both N-terminal and C-terminal parts of S whereas in healthy donors S-reactive CD4+ T cells reacted almost exclusively to the Cterminal part that is a) characterized by higher homology to spike glycoprotein of human endemic "common cold" coronaviruses, and b) contains the S2 subunit of S with the cytoplasmic peptide (CP), the fusion peptide (FP), and the transmembrane domain (TM) but not the receptor-binding domain (RBD). S-reactive CD4+ T cells from COVID-19 patients were further distinct to those from healthy donors as they co-expressed higher levels of CD38 and HLA-DR, indicating their recent in vivo activation. Our study is the first to directly measure SARS-CoV-2-reactive T cell responses providing critical tools for large scale testing, in depth epitope mapping and characterization of potential cross-reactive cellular immunity to SARS-CoV-2. The presence of pre-existing SARS-CoV-2-reactive T cells in healthy donors is of high interest but larger scale prospective cohort studies are needed to assess whether their presence is a correlate of protection or pathology. Results of such studies

will be key for a mechanistic understanding of the SARS-CoV-2 pandemic, adaptation of containment methods and to support vaccine development.
https://www.medrxiv.org/content/10.1101/2020.04.17.20061440v1

DRUG DEVELOPMENT

- *Remember the song, "We Are The World*?" (**no I am not posting any Michael Jackson videos so don't ask**) Here is I think the first Pakistani study. Entry of SARS-CoV-2, etiological agent of COVID-19, in the host cell is driven by the interaction of its spike protein with human ACE2 receptor and a serine protease, TMPRSS2. Although complex between SARS-CoV-2 spike protein and ACE2 has been structurally resolved, the molecular details of the SARS-CoV-2 and TMPRSS2 complex are still elusive. TMPRSS2 is responsible for priming of the viral spike protein that entails cleavage of the spike protein at two potential sites, Arg685/Ser686 and Arg815/Ser816. The present study aims to investigate the conformational details of complex between TMPRSS2 and SARS-CoV-2 spike protein, in order to discern the finer details of the priming of viral spike and to point candidate drug targets. Briefly, full length structural model of TMPRSS2 was developed and docked against the resolved structure of SARS-CoV-2 spike protein with directional restraints of both cleavage sites. The docking simulations showed that TMPRSS2 interacts with the two different loops of SARS-CoV-2 spike protein, each containing different cleavage sites. Key functional residues of TMPRSS2 (His296, Ser441 and Ser460) were found to interact with immediate flanking residues of cleavage sites of SARS-CoV-2 spike protein. Compared to the N-terminal cleavage site (Arg685/Ser686), TMPRSS2 region that interact with C-terminal cleavage site (Arg815/Ser816) of the SARS-CoV-2 spike protein was predicted as relatively more druggable. In summary, the present study provide structural characteristics of molecular complex between human TMPRSS2 and SARS-CoV-2 spike protein and points to the candidate drug targets that could further be exploited to direct structure base drug designing. [**note: we need to salute all those researchers from all over who are putting in time and effort.**] https://www.biorxiv.org/content/10.1101/2020.04.21.052639v1
- The goals of this study were to use artificial intelligence (AI) to predict blueprints for designing universal vaccines against SARS-CoV-2, that contain a sufficiently broad repertoire of T-cell epitopes capable of providing coverage and protection across the global population. To help achieve these aims, we profiled the entire SARS-CoV-2 proteome across the most frequent 100 HLA-A, HLA-B and HLA-DR alleles in the human population, using host-infected cell surface antigen presentation and immunogenicity predictors from the NEC Immune Profiler suite of tools, and generated comprehensive epitope maps. We then used these epitope maps as input for a Monte Carlo simulation designed to identify statistically significant epitope hotspot regions in the virus that are most likely to be immunogenic across a broad spectrum of HLA types. We then removed epitope hotspots that shared significant homology with proteins in the human proteome to reduce the chance of inducing off-target autoimmune responses. We also analyzed the antigen presentation and immunogenic landscape of all the nonsynonymous mutations across 3400 different sequences of the virus, to identify a trend whereby SARS-COV-2 mutations are predicted to have reduced potential to be presented by host-infected cells, and consequently detected by the host immune system. A sequence conservation analysis then removed epitope hotspots that occurred in less-conserved regions of the viral proteome. Finally,

we used a database of the HLA genotypes of approximately 22 000 individuals to develop a digital twin type simulation to model how effective different combinations of hotspots would work in a diverse human population, and used the approach to identify an optimal constellation of epitopes hotspots that could provide maximum coverage in the global population. By combining the antigen presentation to the infected-host cell surface and immunogenicity predictions of the NEC Immune Profiler with a robust Monte Carlo and digital twin simulation, we have managed to profile the entire SARS-CoV-2 proteome and identify a subset of epitope hotspots that could be harnessed in a vaccine formulation to provide a broad coverage across the global population. [https://www.biorxiv.org/content/10.1101/2020.04.21.052084v1 [**note: a universal vaccine would be great; getting there is the tough part.**] https://www.biorxiv.org/content/10.1101/2020.04.21.052084v1

- Here is another universal vaccine approach. The current COVID-19 pandemic, caused by a novel coronavirus SARS-CoV-2, poses serious threats to public health and social stability, calling for urgent need for vaccines and therapeutics. SARS-CoV-2 is genetically close to SARS-CoV, thus it is important to define the between antigenic cross-reactivity and neutralization. In this study, we firstly analyzed 20 convalescent serum samples collected from SARS-CoV infected individuals during the 2003 SARS outbreak. All patient sera reacted strongly with the S1 subunit and receptor-binding domain (RBD) of SARS-CoV, cross-reacted with the S ectodomain, S1, RBD, and S2 proteins of SARS-CoV-2, and neutralized both SARS-CoV and SARS-CoV-2 S protein-driven infections. Multiple panels of antisera from mice and rabbits immunized with a full-length S and RBD immunogens of SARS-CoV were also characterized, verifying the cross-reactive neutralization against SARS-CoV-2. Interestingly, we found that a palm civet SARS-CoV-derived RBD elicited more potent cross-neutralizing responses in immunized animals than the RBD from a human SARS-CoV strain, informing a strategy to develop a universe vaccine against emerging CoVs. https://www.biorxiv.org/content/10.1101/2020.04.20.052126v1
- This intriguing paper comes from the UK with the small molecule provided by a Ukraine company! Currently, no vaccine exists against coronavirus infections, including pandemic SARS-CoV-2, the causative agent of the Coronavirus Disease 2019 (COVID-19). To combat these RNA virus infections, alternative antiviral strategies are needed. A key drug target is the viral RNA polymerase, which is responsible for viral RNA synthesis. In January 2020, the World Health Organisation identified enisamium as a candidate therapeutic against SARS-CoV-2. Enisamium is an isonicotinic acid derivative that is an inhibitor of multiple influenza B and A virus strains in cell culture and clinically approved in 11 countries. Here we show using in vitro assays that enisamium and its putative metabolite, VR17-04, inhibit the activity of both the influenza virus RNA polymerase as well as the SARS-CoV-2 RNA polymerase complex. These results suggest that enisamium is a broad-spectrum small molecule inhibitor of RNA virus RNA synthesis, and implicate it as a possible therapeutic option for treating SARS-CoV-2 infection. [**note: I don't know what countries enisamium is approved in. The active metabolite would be treated as a new drug I suppose.**] https://www.biorxiv.org/content/10.1101/2020.04.21.053017v1
- Here is an interesting paper on another possible target for small molecular weight drugs. Virus entry is a multistep process. It initiates when the virus attaches to the host cell and ends when the viral contents reach the cytosol. Genetically unrelated viruses can subvert analogous subcellular mechanisms and use similar trafficking pathways for successful entry. Antiviral strategies targeting early steps of infection are therefore appealing, particularly when the

probability for successful interference through a common step is highest. We describe here potent inhibitory effects on content release and infection by chimeric VSV containing the envelope proteins of EBOV (VSV-EBOV) or SARS-CoV-2 (VSV-SARS-CoV-2) elicited by Apilimod and Vacuolin-1, small molecule inhibitors of the main endosomal Phosphatidylinositol-3-Phosphate/Phosphatidylinositol 5-Kinase, PIKfyve. We also describe potent inhibition of SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020 by Apilimod. These results define new tools for studying the intracellular trafficking of pathogens elicited by inhibition of PIKfyve kinase and suggest the potential for targeting this kinase in developing a small-molecule antiviral against SARS-CoV-2. [**note: apilimod blocks production of IL-12 and IL-23 and was looked at for Crohn's disease and rheumatoid arthritis. Trials were disappointing. Good luck on getting any promising drug in trials right now.**]
https://www.biorxiv.org/content/10.1101/2020.04.21.053058v1

DIAGNOSTIC DEVELOPMENT

- Another example of how to extend PCR screening using a pooling system. Effective public health response to viral outbreaks such as SARS-CoV-2 is often informed by real-time PCR screening of large populations. Pooling samples can increase screening capacity. However, when a traditional pool is tested positive, all samples in the pool need individual retesting, which becomes ineffective at a higher proportion of positive samples. Here, we report a new pooling protocol that mitigates this problem by replicating samples across multiple pools. The resulting pool set allows the sample status to be resolved more often than with traditional pooling. At 2% prevalence and 20 samples per pool, our protocol increases screening capacity by factors of 5 and 2 compared to individual testing and traditional pooling, respectively. The corresponding software to layout and resolve samples is freely available under a BSD license (https://github.com/phiweger/clonepool). [**note: major kudos to them for making the software publicly available. We are all in this together!**]
https://www.medrxiv.org/content/10.1101/2020.04.16.20067603v1
- The Chinese scientists keep inventing!!! Clinical practice has demonstrated that the SARS-CoV-2 S1 specific antibodies and viral antigens can be used as diagnostic and prognostic markers of COVID-19. However, the popular point-of-care biomarker detection technologies, such as the lateral-flow test strips, provide only yes/no information and have very limited sensitivities. Thus, it has a high false-negative rate and cannot be used for the quantitative evaluation of the patient's immune response. Conventional ELISA (enzyme-linked immunosorbent assay), on the other hand, can provide quantitative, accurate, and sensitive results, but it involves complicated and expensive instruments and long assay time. In addition, samples need to be sent to centralized labs, which significantly increases the turn-around time. Here, we present a microfluidic ELISA technology for rapid (15-20 minutes), quantitative, sensitive detection of SARS-CoV-2 biomarkers using SARS-CoV-2 specific IgG and viral antigen - S protein in serum. We also characterized various humanized monoclonal IgG, and identified a candidate with a high binding affinity towards SARS-CoV-2 S1 protein that can serve as the calibration standard of anti-SARS-CoV-2 S1 IgG in serological analyses. Furthermore, we demonstrated that our microfluidic ELISA platform can be used for rapid affinity evaluation of monoclonal anti-S1 antibodies. The microfluidic ELISA device is highly portable and requires less than 10 µL of

samples for each channel. Therefore, our technology will greatly facilitate rapid and quantitative analysis of COVID-19 patients and vaccine recipients at point-of-care. [**note: this would be a great tool for massive testing and maybe more precise than the lateral flow sticks**] https://www.biorxiv.org/content/10.1101/2020.04.20.052233v1

- Here, we aimed to develop a more quantitative and sensitive serological test for COVID-19 diagnosis, monitoring and clinical investigation, based on the detection of antigen-specific IgA as well as IgM and IgG in blood in response to SARS-CoV-2 infection. Methods In this investigation, we report the development of a set of validated diagnostic kits for detecting serum IgA, IgM, and IgG specific to SARS-CoV-2 nucleocapsid protein (NP) and receptor-binding domain (RBD) of the spike protein by chemi-luminescence immuno-analysis. The kits were tested with a cohort of 216 sera from 87 laboratory-confirmed COVID-19 patients, and 483 sera from SARS-CoV-2 negative or healthy individuals as negative controls. A standard receiver operating characteristic (ROC) analysis was conducted to evaluate the diagnostic accuracy. Using the kits, serum levels of IgA, IgM, and IgG were analyzed, in response to SARS-CoV-2 infection and COVID-19 pathogenesis. Findings The diagnostic kits based on the RBD antigen outperformed those based on the NP. RBD-specific IgA, IgM, and IgG detection kits showed sensitivities of 98.6%, 96.8%, and 96.8%, and specificities of 98.1%, 92.3%, and 99.8%, respectively. In addition, using purified RBD-specific immunoglobulins from a serum pool of COVID-19 patients as standards, the serum concentrations of RBD-specific IgA, IgM, and IgG proteins were determined. The concentrations varied widely among different patients. Median concentration of IgA and IgM reached peaks at 16-20 days after illness onset at 8.84 μg/mL and 7.25 μg/mL, respectively, while median concentration of IgG peaked during 21-25 days after illness onset at 16.47 μg/mL. Furthermore, the serum IgA level positively correlates with COVID-19 severity. Interpretation Our immunoassay of measuring SARS-CoV-2 specific antibodies IgA, IgM, and IgG in serum provides a better serological testing with improved sensitivity and specificity. Data of IgA, IgM, and IgG responses in blood of COVID-19 patients may provide novel insight for the monitoring and treatments of COVID-19. The kits are also suitable for epidemiological studies and vaccine validations. [**note: look at the sensitivity and specificity numbers! It can be done!!!**] https://www.medrxiv.org/content/10.1101/2020.04.17.20064907v1
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2020-04-23

More joy from WuMo

What???? You didn't watch 'Gossip Girl?' For shame; it's now time to binge watch, so go ahead and cue up Netflix but not until you have finished reading this newsletter! If 'Gossip Girl' is not your cup of tea, there is David Simon's HBO series 'The Wire;' IMO the best television series of all time. "You come at the king, you best not miss," – Omar Little. Enough television for now, let's get on with the music selection and the main show for today.

Yes, it is a little late for Easter but I came across this new video of The Messiah (I've sung this several time as a chorister and even learned the opening tenor solo!). It's always stirring to hear this sung well and this fits the bill: https://www.youtube.com/watch?v=lFjQ77ol2DI I found it interesting that they split the opening solo between two tenors. I really liked 'Every Valley', great tone and breath control. This is the first video I've seen that has a fundraising link (I enjoyed the performance so much I donated). We should remember that lots of musicians rely on public performances for income. The pandemic has taken this away. I ask my loyal readers to give back to arts communities as appropriate (hey, you are getting a great newsletter for free!). As a double treat today, here is a delightful COVID-19 kitchen recital by German-Slav soprano Patricia Janeĉková singing a devilishly difficult Rossini song: https://youtu.be/Xy4bFpaGnII She's only 21 and there are a number of wonderful YouTube clips of her singing.

The Washington Post does a very good job of describing how blood clots are wreaking havoc following infection. There are some clinical references in the article and it will be very interesting to do an observational study on hospitalized patients who are already on blood thinner meds to see what their illness progression is (I wonder if anyone is prescribed warfarin these days with the approval of safer alternatives. I also wonder if there will be run to the doc to get Rx scrips for apixaban, dabiatran, or rivaroxaban; god forbid we start seeing television commentators boosting this line of drugs.). There are already some clinical studies that I've referenced about the use of heparin in treatment of severe pneumonia cases.

What would a newsletter day be without a hydroxychloroquine day? The estimable Derek Lowe weighs in. Also worth reading and linked in Derek's blog is this piece by my old friend Steve Usdin on the FDA Experimental Use Authorization of hydroxychloroquine. As Usdin points out and all of us drug regulatory affairs folks well know, FDA did NOT have to do this. Docs can always prescribe for off label use. Perhaps this FDA move gave hydroxychloroquine the cachet needed to make it almost the standard of care (don't forget to add some zinc and azithromycin for max effect!). While I would love to see this drug work, the post-mortem if it does not will get very ugly, very fast. It even looks like Fox News is not

trumpeting the drug much these days; oh wonder of wonders. To add fuel to the fire, here a systematic review by Indian researchers who caution against widespread use of hydroxychloroquine: https://www.medrxiv.org/content/10.1101/2020.04.16.20068205v1 Money quote, "Scarcity of safety and efficacy data warrants medical communities, health care agencies and governments across the world against the widespread use of HCQ in COVID-19 prophylaxis and treatment, until robust evidence becomes available."

Yikes, I hate to be a 'Debbie Downer' but parents are not taking their kids in for routine immunizations. There is a safe way to do these kinds of peditrician visits and parents need to realize that getting the needed vaccinations is important with a capital 'I.'

I've referred to Noah Feldman's 'Deep Background' podcast before. All my readers should have this queued up in their podcast app (I use Stitcher but disclose no financial conflict of interest only that it works quite well). The interview yesterday is with Omai Garner, the director of clinical microbiology testing at UCLA Health, explains why more Americans have not been tested for COVID-19. Well worth the ½ hour listen [**note: you need to make sure you are listening to the correct podcast as a new one, equally interesting, dropped this morning**].

There is another **special treat** for all you bakers at the COVID-19 resource page! Yes, we have a great peanut butter cookie recipe for everyone to try out (providing you can find flour on the grocery shelf if you haven't already hoarded enough). I baked a batch yesterday to help carry me through to the weekend. We were also surprised by a ½ pound box of Sees assorted chocolates that our oldest daughter sent to us. Time to hit the road and work out for sure!

MODELING

- Yes, I know I 'promised' no more modeling papers. However, this one from China is nice in that they estimate the asymptomatic infection in the country. Background: Mounting evidence suggests that there is an undetected pool of COVID-19 asymptomatic but infectious cases. Estimating the number of asymptomatic infections has been crucial to understand the virus and contain its spread, which is, however, hard to be accurately counted. Methods: We propose an approach of machine learning based fine-grained simulator (MLSim), which integrates multiple practical factors including disease progress in the incubation period, cross-region population movement, undetected asymptomatic patients, and prevention and containment strength. The interactions among these factors are modeled by virtual transmission dynamics with several undetermined parameters, which are determined from epidemic data by machine learning techniques. When MLSim learns to match the real data closely, it also models the number of asymptomatic patients. MLSim is learned from the open Chinese global epidemic data. Findings: MLSim showed better forecast accuracy than the SEIR and LSTM-based prediction models. The MLSim learned from the data of China's mainland reveals that there could have been 150,408 (142,178-157,417) asymptomatic and had self-healed patients, which is 65% (64% - 65%) of the inferred total infections including undetected ones. The numbers of asymptomatic but infectious patients on April 15, 2020, were inferred as, Italy: 41,387 (29,037 - 57,151), Germany: 21,118 (11,484 - 41,646), USA: 354,657 (277,641 - 495,128), France: 40,379 (10,807 - 186,878), and UK: 144,424 (127,215 - 171,930). To control the virus transmission, the containment

measures taken by the government were crucial. The learned MLSim also reveals that if the date of containment measures in China's mainland was postponed for 1, 3, 5, and 7 days later than Jan. 23, there would be 109,039 (129%), 183,930 (218%), 313,342 (371%), 537,555 (637%) confirmed cases on June 12. Conclusions: Machine learning based fine-grained simulators can better model the complex real-world disease transmission process, and thus can help decision-making of balanced containment measures. The simulator also revealed the potential great number of undetected asymptomatic infections, which poses a great risk to the virus containment. https://www.medrxiv.org/content/10.1101/2020.04.19.20068072v1

- Here is a cluster analysis from a hard hit area in France. The Oise department in France has been heavily affected by COVID-19 in early 2020. Methods: Between 30 March and 4 April 2020, we conducted a retrospective closed cohort study among pupils, their parents and siblings, as well as teachers and non-teaching staff of a high-school located in Oise. Participants completed a questionnaire that covered history of fever and/or respiratory symptoms since 13 January 2020 and had blood tested for the presence of anti-SARS-CoV-2 antibodies. The infection attack rate (IAR) was defined as the proportion of participants with confirmed SARS-CoV-2 infection based on antibody detection. Blood samples from two blood donor centres collected between 23 and 27 March 2020 in the Oise department were also tested for presence of anti-SARS-CoV-2 antibodies. Findings: Of the 661 participants (median age: 37 years), 171 participants had anti-SARS-CoV-2 antibodies. The overall IAR was 25.9% (95% confidence interval (CI) = 22.6-29.4), and the infection fatality rate was 0% (one-sided 97.5% CI = 0-2.1). Nine of the ten participants hospitalised since mid-January were in the infected group, giving a hospitalisation rate of 5.3% (95% CI = 2.4-9.8). Anosmia and ageusia had high positive predictive values for SARS-CoV-2 infection (84.7% and 88.1%, respectively). Smokers had a lower IAR compared to non-smokers (7.2% versus 28.0%, P <0.001). The proportion of infected individuals who had no symptoms during the study period was 17.0% (95% CI = 11.2-23.4). The proportion of donors with anti-SARS-CoV-2 antibodies in two nearby blood banks of the Oise department was 3.0% (95% CI = 1.1-6.4). Interpretation: The relatively low IAR observed in an area where SARS-CoV-2 actively circulated weeks before confinement measures indicates that establishing herd immunity will take time, and that lifting these measures in France will be long and complex. [**note: the lower IAR among smokers is curious and I am not advocating that any of you rush out to buy cigarettes thinking it confer protection.**]
  https://www.medrxiv.org/content/10.1101/2020.04.18.20071134v1

NEWLY REGISTERED CLINICAL TRIALS

- Here is a multicenter trial of alteplase for respiratory failure in SARS-CoV-2 patients. The dominant pathologic feature of viral-induced ARDS is fibrin accumulation in the microvasculature and airspaces. Substantial preclinical work suggests antifibrinolytic therapy attenuates infection provoked ARDS. In 2001, a phase I trial 7 demonstrated the urokinase and streptokinase were effective in patients with terminal ARDS, markedly improving oxygen delivery and reducing an expected mortality in that specific patient cohort from 100% to 70%. A more contemporary approach to thrombolytic therapy is tissue plasminogen activator (tPA) due to its higher efficacy of clot lysis with comparable bleeding risk 8. We therefore propose a phase IIa clinical trial with two intravenous (IV) tPA treatment arms and a control arm to test the

efficacy and safety of IV tPA in improving respiratory function and oxygenation, and consequently, successful extubation, duration of mechanical ventilation and survival. NCT04357730

CLINICAL TRIAL RESULTS

- I don't know what the safety of the BCG vaccine is and I'm pretty sure I never received it. It may be time to pull the plug on this theory and until I see some actual clinical data, this will be the last post on this topic (I've posted a couple of clinical trial links in past newsletters). The goal of this paper is to showcase that the COVID-19 disease pattern is evolving and to study the relationship between mandatory BCG policy and caseload/million or death/per million. We analyze seven recent publications on the impact of BCG vaccinations on the development of COVID19 illness and extend presented findings using the latest data from April 10, 2020. We analyze data from 98 countries and we extend existing models by adding the dimension of COVID-19-related testing conducted by the analyzed countries. Similarly to prior studies, we find that COVID-19 attributable case and death incidences across countries share a relationship with the BCG vaccination inclusion in the national immunization program of a country when testing is not taken into consideration. However, this relationship vanishes when we add the dimension of testing. We observe that case and death incidences conditional on testing do not get affected by the BCG vaccination inclusion in the national immunization program of a country. Therefore, we show that there is no statistical evidence to support the assertion that inclusion of BCG vaccination in national immunization program (NIP) has any impact of COVID 19 infections (cases) or mortality. https://www.medrxiv.org/content/10.1101/2020.04.18.20071142v1
- Some really nice aggregated data on the clinical characteristics of a large patient cohort in China. Coronavirus disease 2019 (COVID-19) is a global pandemic and has been widely reported; however, a comprehensive systemic review and meta-analysis has not been conducted. We systematically investigated the clinical characteristics of COVID-19 in mainland China to guide diagnosis and treatment. We searched the PubMed, Embase, Scopus, Web of Science, Cochrane Library, bioRxiv, medRxiv, and SSRN databases for studies related to COVID-19 published or preprinted in English or Chinese from January 1 to March 15, 2020. Clinical studies on COVID-19 performed in mainland China were included. We collected primary outcomes including signs and symptoms, chest CT imaging, laboratory tests, and treatments. Study selection, data extraction, and risk of bias assessment were performed by two independent reviewers. Qualitative and quantitative synthesis was conducted, and random-effects models were applied to pooled estimates. This study is registered with PROSPERO (number CRD42020171606). Of the 3624 records identified, 147 studies (20,662 patients) were analyzed. The mean age of patients with COVID-19 was 49.40 years, 53.45% were male, and 38.52% had at least one comorbidity. Fever and cough were the most common symptoms, followed by fatigue, expectoration, and shortness of breath. Most patients with COVID-19 had abnormal chest CT findings with ground glass opacity (70.70%) or consolidation (29.91%). Laboratory findings shown lymphopenia, increased lactate dehydrogenase, increased infection-related indicators, and fibrinolytic hyperactivity. Antiviral therapy, antibiotic therapy, and corticosteroids were administered to 89.75%, 79.13%, and 35.64% of patients, respectively. Most clinical characteristics of COVID-19 are non-specific. Patients with suspected should be evaluated by virological assays and clinically

treated. [**note: the clinicians in the audience might be interested in downloading the article to take a look at the full data**]
https://www.medrxiv.org/content/10.1101/2020.04.18.20070565v1

- And coming up nest, a study of patients in NYC, though the number is not as large as in the above China study. Demographic, clinical, and outcomes data for patients admitted to five Mount Sinai Health System hospitals with confirmed Covid-19 between February 27 and April 2, 2020 were identified through institutional electronic health records. We conducted a descriptive study of patients who had in-hospital mortality or were discharged alive. Results A total of 2,199 patients with Covid-19 were hospitalized during the study period. As of April 2nd, 1,121 (51%) patients remained hospitalized, and 1,078 (49%) completed their hospital course. Of the latter, the overall mortality was 29%, and 36% required intensive care. The median age was 65 years overall and 75 years in those who died. Pre-existing conditions were present in 65% of those who died and 46% of those discharged. In those who died, the admission median lymphocyte percentage was 11.7%, D-dimer was 2.4 ug/ml, C-reactive protein was 162 mg/L, and procalcitonin was 0.44 ng/mL. In those discharged, the admission median lymphocyte percentage was 16.6%, D-dimer was 0.93 ug/ml, C-reactive protein was 79 mg/L, and procalcitonin was 0.09 ng/mL. Conclusions This is the largest and most diverse case series of hospitalized patients with Covid-19 in the United States to date. Requirement of intensive care and mortality were high. Patients who died typically had pre-existing conditions and severe perturbations in inflammatory markers.
https://www.medrxiv.org/content/10.1101/2020.04.19.20062117v1
- And now augment curation of unstructured clinical nots from a massive EHR system courtesy of the Mayo Clinic. Understanding the temporal dynamics of COVID-19 patient phenotypes is necessary to derive fine-grained resolution of the pathophysiology. Here we use state-of-the-art deep neural networks over an institution-wide machine intelligence platform for the augmented curation of 8.2 million clinical notes from 14,967 patients subjected to COVID-19 PCR diagnostic testing. By contrasting the Electronic Health Record (EHR)-derived clinical phenotypes of COVID-19-positive (COVIDpos, n=272) versus COVID-19-negative (COVIDneg, n=14,695) patients over each day of the week preceding the PCR testing date, we identify diarrhea (2.8-fold), change in appetite (2-fold), anosmia/dysgeusia (28.6-fold), and respiratory failure (2.1-fold) as significantly amplified in COVIDpos over COVIDneg patients. The specific combination of cough and diarrhea has a 4-fold amplification in COVIDpos patients during the week prior to PCR testing, and along with anosmia/dysgeusia, constitutes the earliest EHR-derived signature of COVID-19 (4-7 days prior to typical PCR testing date). This study introduces an Augmented Intelligence platform for the real-time synthesis of institutional knowledge captured in EHRs. The platform holds tremendous potential for scaling up curation throughput, with minimal need for training underlying neural networks, thus promising EHR-powered early diagnosis for a broad spectrum of diseases. [**note to self: if I start to run for the Pepto Bismol while coughing, I need to be really concerned. (sorry for the black humor here)**]
https://www.medrxiv.org/content/10.1101/2020.04.19.20067660v1
- But wait, there's more to paraphrase Ron Popiel. An Italian study provides more evidence on the linkage with GI symptoms. [**note to self: I'm getting a funny feeling that I might have had a mild case of SARS-CoV-2 based on some symptoms from three weeks ago. I need an antibody test STAT**] https://www.medrxiv.org/content/10.1101/2020.04.20.20064873v1

- We aim to help inform the choice of estimand (i.e., target of inference) and analysis method to be used in future COVID-19 treatment trials. To this end, we describe estimands for outcome types of particular interest in these trials (ordinal and time-to-event). When the outcome is ordinal, the estimands that we consider are the difference between study arms in the mean outcome, the Mann-Whitney (rank--based) estimand, and the average of the cumulative log odds ratios over the levels of the outcome. For time-to-event outcomes, we consider the difference between arms in the restricted mean survival time, the difference between arms in the cumulative incidence, and the relative risk. Advantageously, the interpretability of these estimands does not rely on a proportional odds or proportional hazards assumptions. For each estimand, we evaluate the potential value added by using estimators that leverage information in baseline variables to improve precision and reduce the required sample size to achieve a desired power. These are called covariate adjusted estimators. To evaluate the performance of the covariate adjusted and unadjusted estimators that we present, we simulate two-arm, randomized trials comparing a hypothetical COVID-19 treatment versus standard of care, where the primary outcome is ordinal or time-to-event. Our simulated distributions are derived from two sources: longitudinal data on over 500 patients hospitalized at Weill Cornell Medicine New York Presbyterian Hospital prior to March 28, 2020, and a CDC preliminary description of 2449 cases reported to the CDC from February 12 to March 16, 2020. We focus on hospitalized, COVID-19 positive patients and consider the following outcomes: intubation, ventilator use, and death. We conduct simulations using all three estimands when the outcome is ordinal, but only evaluate the restricted mean survival time when the outcome is time to event. Our simulations showed that, in trials with at least 200 participants, the precision gains due to covariate adjustment are equivalent to requiring 10-20% fewer participants to achieve the same power as a trial that uses the unadjusted estimator; this was the case for each outcome and estimand that we considered. [**I'm just a drug regulatory guy but still found this paper to be interesting.**] https://www.medrxiv.org/content/10.1101/2020.04.19.20069922v1
- Decent data from France on the putative role of interferon type 1 response. We performed an integrated immune analysis that included in-depth phenotypical profiling of immune cells, whole-blood transcriptomic and cytokine quantification on a cohort of fifty Covid19 patients with a spectrum of disease severity. All patient were tested 8 to 12 days following first symptoms and in absence of anti-inflammatory therapy. Results: A unique phenotype in severe and critically ill patients was identified. It consists in a profoundly impaired interferon (IFN) type I response characterized by a low interferon production and activity, with consequent downregulation of interferon-stimulated genes. This was associated with a persistent blood virus load and an exacerbated inflammatory response that was partially driven by the transcriptional factor NFκB. It was also characterized by increased tumor necrosis factor (TNF)-α and interleukin (IL)-6 production and signaling as well as increased innate immune chemokines. Conclusion: We propose that type-I IFN deficiency in the blood is a hallmark of severe Covid-19 and could identify and define a high-risk population. Our study provides a rationale for testing IFN administration combined with adapted anti-inflammatory therapy targeting IL-6 or TNF-α in most severe patients. These data also raise concern for utilization of drugs that interfere with the IFN pathway. https://www.medrxiv.org/content/10.1101/2020.04.19.20068015v1

DRUG DEVELOPMENT

- Well this is interesting; I linked to an Italian clinical trial of nafamostat just yesterday and here is some *in vitro* data on inhibition. We previously found that nafamostat mesylate, an existing drug used for disseminated intravascular coagulation (DIC), effectively blocked MERS-CoV S protein-initiated cell fusion by targeting TMPRSS2, and inhibited MERS-CoV infection of human lung epithelium-derived Calu-3 cells. Here we established a quantitative fusion assay dependent on SARS-CoV-2 S protein, ACE2 and TMPRSS2, and found that nafamostat mesylate potently inhibited the fusion while camostat mesylate was about 10-fold less active. Furthermore, nafamostat mesylate blocked SARS-CoV-2 infection of Calu-3 cells with an EC50 around 10 nM, which is below its average blood concentration after intravenous administration through continuous infusion. These findings, together with accumulated clinical data regarding its safety, make nafamostat a likely candidate drug to treat COVID-19. [**note: it's clinical use is as a short duration anti-coagulant and I'm curious whether it would help out in that regard to control micro-clotting in the lungs.**] https://www.biorxiv.org/content/10.1101/2020.04.22.054981v1
- A lot of the drug development papers I've read over the past month ignore the real world of pharmacokinetics and whether there is a realistic chance of achieving the right concentration in the body. Here is a good paper addressing this. Many papers are emerging that describe the in vitro antiviral activity of drugs that may be repurposed for therapy or chemoprophylaxis against SARS-CoV-2. However, no comprehensive evaluation of these molecules in the context of the achievable plasma pharmacokinetics after administration of approved doses and schedules to humans has been conducted. Moreover, most publications have focussed on 50% maximum effective concentrations (EC50), which may be an insufficiently robust indicator of antiviral activity because of marked differences in the slope of the concentration-response curve between drugs. Accordingly, in vitro anti-SARS-CoV-2 activity data was digitised from all available publications up to 13th April 2020 and used to recalculate an EC90 value for each drug. EC90 values were then expressed as a ratio to the achievable maximum plasma concentrations (Cmax) reported for each drug after administration of the approved dose to humans (Cmax/EC90 ratio). Only 14 of the analysed drugs achieved a Cmax/EC90 ratio above 1 meaning that plasma Cmax concentrations exceeded those necessary to inhibit 90% of SARS-CoV-2 replication. A more in-depth assessment of these drugs demonstrated that only nitazoxanide, nelfinavir, tipranavir (boosted with ritonavir) and sulfadoxine achieved plasma concentrations above their anti-SARS-CoV-2 activity across their entire approved dosing interval at their approved human dose. For all drugs reported, the unbound lung to plasma tissue partition coefficient (KpUlung) was also simulated and used along with reported Cmax and fraction unbound in plasma to derive a lung Cmax/EC50 as a better indicator of potential human efficacy (lung Cmax/EC90 ratio was also calculable for a limited number of drugs). Using this parameter hydroxychloroquine, chloroquine, mefloquine, atazanavir (boosted with ritonavir), tipranavir (boosted with ritonavir), ivermectin, azithromycin and lopinavir (boosted with ritonavir) were all predicted to achieve lung concentrations over 10-fold higher than their reported EC50. This analysis was not possible for nelfinavir because insufficient data were available to calculate KpUlung but nitozoxanide and sulfadoxine were also predicted to exceed their reported EC50 by 3.1- and 1.5-fold in lung, respectively. The antiviral activity data reported to date is of variable quality and conducted under different conditions by different investigators. However, this analysis has prioritised candidates with the best chance for success in therapy or

chemoprevention of Covid-19 based upon the currently available in vitro activity and human plasma pharmacokinetic data. Future studies should focus on EC90 values and discuss findings in the context of achievable exposures in humans, especially within target compartments such as the lung, in order to maximise the potential for success of proposed human clinical trials. [**note: one needs to read this paper. There are a number of carefully worded caveats not reflected in the abstract. IMO, this is one of the better thought out manuscripts in drug development.**] https://www.medrxiv.org/content/10.1101/2020.04.16.20068379v1

DIAGNOSTIC DEVELOPMENT

- Good to see some Qatari scientists stepping up to the plate. To circumvent the limited availability of RNA extraction reagents, we developed a protocol for direct RT-qPCR to detect SARS-CoV-2 in nasopharyngeal swabs. Incubation of specimens at 65oC for 10 minutes along with the use of TaqPath™ 1-Step RT-qPCR Master Mix provides higher analytical sensitivity for detection of SARS-CoV-2 RNA than many other commercial and laboratory-developed methods. In 132 specimens submitted for SARS-CoV-2 testing, the sensitivity, specificity and accuracy of our optimized approach were 95%, 99% and 98.5%, respectively, with reference to results obtained by a standard approach involving RNA extraction. Also, the RT-qPCR CT values obtained by the two methods were highly correlated. https://www.medrxiv.org/content/10.1101/2020.04.18.20070755v1
- "No test is better than a bad test" at least that's what these Liverpool researchers state. Background: The cessation of lock-down measures will require an effective testing strategy. Much focus at the beginning of the UK's Covid-19 epidemic was directed to deficiencies in the national testing capacity. The quantity of tests may seem an important focus, but other characteristics are likely more germane. False positive tests are more probable than positive tests when the overall population has a low prevalence of the disease, even with highly accurate tests. Methods: We modify an SIR model to include quarantines states and test performance using publicly accessible estimates for the current situation. Three scenarios for cessation of lock-down measures are explored: (1) immediate end of lock-down measures, (2) continued lock-down with antibody testing based immunity passports, and (3) incremental relaxation of lock-down measures with active viral testing. Sensitivity, specifcity, prevalence and test capacity are modified for both active viral and antibody testing to determine their population level effect on the continuing epidemic. Findings: Diagnostic uncertainty can have a large effect on the epidemic dynamics of Covid-19 within the UK. The dynamics of the epidemic are more sensitive to test performance and targeting than test capacity. The quantity of tests is not a substitute for an effective strategy. Poorly targeted testing has the propensity to exacerbate the peak in infections. Interpretation: The assessment that 'no test is better than a bad test' is broadly supported by the present analysis. Antibody testing is unlikely to be a solution to the lock-down, regardless of test quality or capacity. A well designed active viral testing strategy combined with incremental relaxation of the lock-down measures is shown to be a potential strategy to restore some social activity whilst continuing to keep infections low. [**note: I completely agree with this but with a major caveat. I less that precise test that is easy to administer is still useful for the type of field epidemiology that was done in Santa Clara County and Los Angeles. Those studies were meant to look at disease prevalence. For policy decisions about reopening, a**

high accuracy test is necessary. It's good to see a paper from Liverpool but my heart aches to think that we will not see a conclusion to the English Premier League soccer competition and the Liverpool 'championship' will always carry an asterisk.]
https://www.medrxiv.org/content/10.1101/2020.04.16.20067884v1

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2020-04-24

I am glad my girls are grown up and out of the house!!!

You were all thinking what's up with this Goldhammer guy. He gives us indie rock, pop rock, and even country music. Where is Vladimir Horowitz? Why these dumb opera transcriptions, surely there must be a fine concert performance of the great Horowitz. Not to worry, of course there is Horowitz and here he is in a 1987 Vienna concert: https://www.youtube.com/watch?v=8ELwCdgGQLQ Here's a nice anecdote. My wife's college roommate lived across the street from him in New York. She always knew when he was about to give a concert at the piano movers were on the street to hoist his piano out and move it to Carnegie Hall. I don't know if he had it shipped over for the Vienna concert. He was 84 years old at the time of this concert that was his next to last public performance.

I have tried to stay apolitical in this newsletter and it really helps not to have a Twitter account and face the barrage of endless debates. I will only offer the following observation, when a post-mortem is done and I hope it will be sooner rather than later, there are a lot of people who will not come through that process with reputations intact. There will be a lot of others who scramble to delete Tweets that showed their woeful lack of intelligence.

OK, now on to the news of the day or in this first case yesterday. New York reported out an antibody study of NYC. Estimates are that 1 in 5 have been infected by SARS-CoV-2. OK, this is not as high a multiple as was found in Santa Clara County by the Stanford group and I don't have much more details than was in the NYT article that is linked. I do know that Mt. Sinai have a FDA approved diagnostic of high quality (they were one of the first to publish specifications of their test). I assume that information has been shared with the state lab in Albany. As more testing gets done in other regions we can get

closer to really understanding what the case report mortality (CRF) is for this virus. Based on current number for NYC, the mortality rate for those diagnosed is 7.7% a very high number. If we take the survey number of 2.3M infected via antibody testing, this drops down to 0.5%, higher than the 1957-58 Asian Flu epidemic. I'll let the epidemiologists play with the number and do age-related and co-morbidity analyses which we know are big factors in mortality. I'm still betting that it's higher than seasonal flu, maybe approaching 0.3% but awaits better numbers across the country. I wish all the armchair statisticians and epidemiologists would just shut up about these studies and realize that, yes, there are many more infected people than those with RT-PCR confirmed cases!

As my loyal readers know, I have been praising the OHDSI group because of my connection in getting the effort started while at PhRMA back in 2005. They have announced a pretty big cohort study and here is the email from Patrick Ryan (he was one of the key company people who worked on the PhRMA Observational Medical Outcomes Partnership (OMOP) which morphed into OHDSI:

Dear colleagues,

We have been thinking, even before we launched our Study-A-Thon, of the potential to apply our OHDSI tools across the OHDSI network to study the safety and anti-viral effectiveness of all emerging therapies for the treatment of COVID19. Potential therapies against COVID19 are mentioned every day to all of us based on in vitro studies, previous SARS experience and/or experiments, or just clinical experience by colleagues working in different specialties.

Many of us have seen the publication of small observational studies and their impact on clinical practice with great concern. We clearly do not want to contribute to a large amount of research that will be seen in the future as wrong, or even harmful Hence the reason we hadn't declared our intention to conduct such studies in the OHDSI community. On the other side, it feels uncomfortable to do nothing if there's a chance we can positively contribute when so many treatments being actively investigated with limited evidence of any sort about their real-world effects in patients with COVID-19. But much as Odysseus was forced to choose between Scylla and Charybdis on his voyage home, we now need to decide among the lesser of two evils. And following our protagonist's lead, we also choose Scylla.

We're announcing here our intention to set forth on another journey, to collaboratively design and implement a population-level effect estimation study to examine the comparative effects of COVID-19 treatments. We're calling this effort: ***Project Sc(y)lla: SARS-Cov-2 Large-scale Longitudinal Analyses*** *.*

Akin to prior OHDSI LEGEND efforts, our aim is to avoid the pitfalls of cherry-picking specific hypotheses about a particular candidate treatment. Instead, we will create a systematic approach to evaluate all alternative treatments for a large constellation of outcomes of interest in a consistent and reproducible manner. SCYLLA will allow for evidence to accumulate across our network at observational data on COVID-positive patients accrue and additional data partners join the journey to execute our open-source analysis package and share aggregate summary statistics.

Like the Odyssey itself, this will be all about the journey. We recognize the threats and challenges ahead, and we are planning to conduct robust methodological research to test the robustness of our methods while we implement them. These are some of the foreseen difficulties:

- *Source-specific outcome/s definition and phenotyping*

- *Relatively small sample size (by OHDSI standards…)*
- *Confounding by indication in the midst of continuously emerging therapies*
- *Prospective data collection*

We have prepared a brief slide set (uploaded in the MSTeams page) that will hopefully inspire you to engage with us in the process of designing SCYLLA. We are now writing a first draft study protocol that we will aim to share with you for feedback in the coming few days. Please stay tuned if you are willing to contribute.

Let us start this journey together…

Cheers,

Patrick on behalf of Daniel Prieto Alhambra, Marc Suchard, George Hripcsak, Martijn Schuemie, Martijn, Kristin Kostka, Kristin, Jennifer Lane, Jamie Weaver, Talita Duarte-Salles, Albert Prats-Uribe, Ed Burn and Peter Rijnbeek

I joined the group to get back in the game!! They have identified pretty much all the drugs that have been discussed in this newsletter including the anticoagulants that I mentioned yesterday. This is really great stuff and will provide some outstanding information from a multinational research team effort!

Derek Lowe takes a huge burden off of my shoulders with is nice overview articles. Today he covers vaccine development. This is complicated stuff and I think there were some missed opportunities to pilot new vaccine platforms against seasonal flu to get an idea of neutralizing antibody levels as well as safety.

The power of the Internet works in mysterious ways. I saw a news clip on the WaPo website last night about Christina Cuomo's (wife of CNN's Chris) protocol for treating SARS-CoV-2 that involved bathing in dilute bleach solution (even though I own Clorox stock I am appalled at this approach); I thought no way this is true. So I invoked Google and come up with her protocol that involves a lot more than bleach baths. I'm sure that this isn't much better than the new clinical trial protocol that I list below.

Following along in the amazing story category we had the statement by President Trump at yesterday's COVID-2 Task Force briefing about the possibility of injecting household disinfectants and using powerful UV lamps (I am happy I decide two weeks ago to forego watching this stuff). I kid you not!!! The maker of Lysol immediately put out a press release stating :…under no circumstance should its disinfectant products be administered into the human body, through injection, ingestion or any other route!" See also this from The Guardian. [**note: while I am generally in favor of DIY projects and small trials, I do draw the line at what is really ridiculous. I'll continue with my fexofenadine and AREDS-2 protocol which seems to be working quite well. It's OK for the rest of you to go to your local pharmacies now and purchase these; I'm stocked up for the foreseeable future.**] It's also not clear that the finding that higher temperatures and UV light that we see during summer months will have an effect. I've linked to previous studies that provide mixed evidence here.

That's it for today, I need to do some work on the OHDSI project.

MODELING

- Here is a good tool to help track the path of SARS-CoV-2. We describe fifteen major mutation events from 2,058 high-quality SARS-CoV-2 genomes deposited up to March 31st, 2020. These events define five major clades (G, I, S, D and V) of globally-circulating viral populations, representing 85.7% of all sequenced cases, which we can identify using a 10 nucleotide genetic classifier or barcode. We applied this barcode to 4,000 additional genomes deposited between March 31st and April 15th and classified successfully 95.6% of the clades demonstrating the utility of this approach. An analysis of amino acid variation in SARS-CoV-2 ORFs provided evidence of substitution events in the viral proteins involved in both host-entry and genome replication. The systematic monitoring of dynamic changes in the SARS-CoV-2 genomes of circulating virus populations over time can guide therapeutic and prophylactic strategies to manage and contain the virus and, also, with available efficacious antivirals and vaccines, aid in the monitoring of circulating genetic diversity as we proceed towards elimination of the agent. The barcode will add the necessary genetic resolution to facilitate tracking and monitoring of infection clusters to distinguish imported and indigenous cases and thereby aid public health measures seeking to interrupt transmission chains without the requirement for real-time complete genomes sequencing. [**note: I remember the first time I saw grocery store bar code check out in action and was awed by the advancement in technology!! At PhRMA we had a nice project that read drug package bar codes and pulled up the product label for pharmacists. Neat stuff though I don't think the bar code app on my phone will allow me to track this virus.**] https://www.biorxiv.org/content/10.1101/2020.04.21.054221v1
- The coronavirus family is looking to be more ubiquitous in nature. Coronaviruses can become zoonotic as in the case of COVID-19, and hunting, sale, and consumption of wild animals in Southeast Asia facilitates an increased risk for such incidents. We sampled and tested rodents (851) and other mammals, and found Betacoronavirus RNA in 12 rodents. The sequences belong to two separate genetic clusters, and relate closely to known rodent coronaviruses detected in the region, and distantly to human coronaviruses OC43 and HKU1. Considering close human-wildlife contact with many species in and beyond the region, a better understanding of virus diversity is urgently needed for the mitigation of future risks. [**note: this falls into the category that 'everything is everywhere.'**]
  https://www.biorxiv.org/content/10.1101/2020.04.22.056218v1

NEWLY REGISTERED CLINICAL TRIALS

- This French study will look at low dose IL-2 for respiratory distress. The purpose is to demonstrate the efficacy of low-dose interleukin 2 (Ld-IL2) administration in improving clinical course and oxygenation parameters in patients with SARS-CoV2-related ARDS. NCT04357444
- As an atheist this trial is definitely not for me but it might provide solace to many others. This is a multicenter; double blind randomized controlled study investigating the role of remote intercessory multi-denominational prayer on clinical outcomes in COVID-19 + patients in the

intensive care unit. All patients enrolled will be randomized to use of prayer vs. no prayer in a 1:1 ratio. Each patient randomized to the prayer arm will receive a "universal" prayer offered by 5 religious denominations (Christianity, Hinduism, Islam, Judaism and Buddhism) in addition to standard of care. Whereas the patients randomized to the control arm will receive standard of care outlined by their medical teams. During ICU stay, patients will have serial assessment of multi-organ function and APACHE-II/SOFA scores serial evaluation performed on a daily basis until discharge. Data assessed include those listed below. [**note: I include this simply because faith plays a big role in many people's lives.**] NCT04361838

- AstraZeneca will be studying its diabetes drug dapagliflozin against SARS-CoV-2, NCT04350593

CLINICAL TRIAL RESULTS

- From Lombardy here is an observational study of the impact of anti-androgenic therapies on SARS-CoV-2 infection. There are gender differences in susceptibility and vulnerability to the Coronavirus disease 2019 (COVID-19). The S protein of coronaviruses facilitates viral entry into target cells and employs the host cellular serine protease TMPRSS2 for S protein priming. The TMPRSS2 gene expression is responsive to androgen stimulation and it could partially explain gender differences. We tested the hypothesis that men who received 5-Alpha reductase inhibitors (5ARIs) or androgen deprivation therapy (ADT) for prostate cancer could have a different susceptibility to COVID-19. We carried out an observational study on patients who were referred to our COVID-19 regional centre in Lombardy from 1st to 31st March 2020. Data from 421 patients, 137 women (32.54%) and 284 men (67.44%) with laboratory-confirmed COVID-19, were included in this report. Overall 84 patients died: 28 women (33.33%) and 56 men (66.67%). Among men, 12 patients (4.22%) reported assuming 5ARI treatment, and 6 were under ADT. Over 12 patients under 5ARIs, 3 (25%) died; 2 deaths (33%) were reported in patients under ADT. Our findings showed that only 4.22% of the overall population received 5ARI anti-androgen therapy, a percentage, which revealed to be significantly lower ($P<0.0001$) than what observed in Italian men aged more than 40 years (14.97%). https://www.medrxiv.org/content/10.1101/2020.04.20.20068056v1
- From Hubei Province, epidemiological characteristics of neurosurgery departments. We prospectively collected epidemiological data on medical staff members who are working in neurosurgery departments in 107 hospitals in Hubei province through self-reported questionnaires or telephone interviews. Data of medical staff members with laboratory-confirmed coronavirus disease 2019 (COVID-19) were analysed. The final follow-up date was 1 March 2020. Findings A total of 5,442 neurosurgery department medical staff members were surveyed. One hundred and twenty cases, involving 54 doctors and 66 nurses, were found to have been infected with SARS-CoV-2. The overall incidence was 2.2%. These cases were concentrated in 26 centres, 16 of which had admitted a total of 59 patients with COVID-19 complicated by craniocerebral disease. Medical staff members in centres receiving COVID-19 patients had a higher risk of contracting infection than those in centres not receiving COVID-19 patients (relative risk: 19.6; 95% confidence interval: 12.6-30.6). Contact with either COVID-19 patients (62.5%, 75/120) or infected colleagues (30.8%, 37/120) was the most common mode of transmission. About 78.3% (94/120) of the infected cases wore surgical masks, whereas 20.8% (25/120) failed to use protection when exposed to the source of infection. Severe infections

were observed in 11.7% (14/120) of the cases, with one death (0.8%, 1/120). All the infected medical staff members had been discharged from the hospital. A total of 1,287 medical staff members were dispatched to participate in the frontline response to COVID-19 under level 2 protection of whom one was infected. Medical staff members who took inadequate protection had a higher risk of contracting infection than those using level 2 protection (relative risk: 36.9; 95% confidence interval: 5.2-263.6). Interpretation Neurosurgical staff members in Hubei province were seriously affected by COVID-19. Level 2 protection and strengthening of protective measures are likely to be effective in preventing medical workers from being infected. https://www.medrxiv.org/content/10.1101/2020.04.20.20064899v1

- Further information from China on antibody production following infection and recovery. Test results for SARS-CoV-2 IgM and IgG antibodies of 221 confirmed COVID-19 patients were retrospectively examined, and their clinical data were collected and analyzed based on various subgroups. SARS-CoV-2 IgM and IgG antibodies were determined with the chemiluminescence method. Findings: The concentration (S/CO) of SARS-CoV-2 IgM and IgG antibodies peaked on day 19-21 after symptom onset, with a median of 17.38 (IQR 4.39-36.4) for IgM and 5.59 (IQR 0.73-13.65) for IgG. Detection rates reached highest on day 16-18 and day 19-21 for IgM and IgG, which were 73.6% and 98.6%, respectively, with significantly higher concentration of IgG in critically ill patients than in those with mild to moderate disease (P=0.027). The concentration of the antibodies on day 16-21 is not correlated with the course or outcome of the disease (Spearman $r < 0.20$, $P > 0.05$). Nasopharyngeal swabs revealed positive SARS-CoV-2 RNA in up to 52.7% of recovered patients after discharge, whose IgG proved to be significantly lower than that of those with negative RNA results (P = 0.009). IgG and IgM were tested twice within 14 days after discharge with a 7-day interval, and the second testing of these antibodies displayed a decrease in concentration of 21.2% (IQR, 11.2%, 34.48%) for IgG and 23.05% (IQR, -27.96%, 46.13%) for IgM, without statistical significance between the patients with re-detectable positive RNA results and those with negative RNA results after discharge. However, those with positive results experienced a count decrease in lymphocyte subsets. Interpretation: The concentration of SARS-CoV-2 IgM and IgG antibodies peaked on day 19-21 after symptom onset, and antibody testing on day 16-21 is associated with increased detection rates, but the antibody concentration does not affect the course and outcome of the infection. Recovering patients with re-detectable positive SARS-CoV-2 RNA displayed lower concentration of IgG, but the downward trend of IgG during recovery indicated its limited duration of protection, and the protective effect of IgG remains to be investigated. [**note: The question of longlasting immunity to SARS-CoV-2 is a thorny one and as noted in this study remains to be fully investigated. I may be that we will be faced with ongoing exposures but maybe this turns out to be like other coronaviruses, mild infection with low mortality.**]
  https://www.medrxiv.org/content/10.1101/2020.04.20.20065953v1
- Here is a serological study from France. Here, we performed a pilot study to assess the levels of anti-SARS-CoV-2 antibodies in samples taken from 491 pre- epidemic individuals, 51 patients from Hopital Bichat (Paris), 209 pauci-symptomatic individuals in the French Oise region and 200 contemporary Oise blood donors. Two in-house ELISA assays, that recognize the full-length nucleoprotein (N) or trimeric Spike (S) ectodomain were implemented. We also developed two novel assays: the S-Flow assay, which is based on the recognition of S at the cell surface by flow-cytometry, and the LIPS assay that recognizes diverse antigens (including S1 or N C- terminal

domain) by immunoprecipitation. Overall, the results obtained with the four assays were similar, with differences in sensitivity that can be attributed to the technique and the antigen in use. High antibody titers were associated with neutralisation activity, assessed using infectious SARS-CoV- 2 or lentiviral-S pseudotypes. In hospitalized patients, seroconversion and neutralisation occurred on 5-14 days post symptom onset, confirming previous studies. Seropositivity was detected in 29% of pauci-symptomatic individuals within 15 days post-symptoms and 3 % of blood of healthy donors collected in the area of a cluster of COVID cases. Altogether, our assays allow for a broad evaluation of SARS-CoV2 seroprevalence and antibody profiling in different population subsets.
https://www.medrxiv.org/content/10.1101/2020.04.21.20068858v1

- These Dutch researchers developed an algorithm for rapid identification of SARS-CoV-2 infected patients at ERs. Detection of SARS-CoV-2 is based on an RT-PCR of nasopharyngeal swab material. However, RT-PCR testing is time-consuming and many hospitals deal with a shortage of testing materials. Therefore, we aimed to develop an algorithm to rapidly evaluate an individual's risk of SARS-CoV-2 infection at the ED. Methods: In this multicenter retrospective study, routine laboratory parameters (C-reactive protein, lactate dehydrogenase, ferritin, absolute neutrophil and lymphocyte counts), demographic data and the chest X-ray/CT result from 967 patients entering the ED with respiratory symptoms were gathered. Using these parameters, an easy-to-use point-based algorithm, called the corona-score, was developed to discriminate between patients that tested positive for SARS-CoV-2 by RT-PCR and those testing negative. Computational sampling was used to optimize the corona-score. Validation of the model was performed using data from 592 patients. Results: The corona-score model yielded an area under the receiver operating characteristic curve of 0.91 in the validation population. Patients testing negative for SARS-CoV-2 showed a median corona-score of 3 versus 11 (scale 0-14) in patients testing positive for SARS-CoV-2 ($p<0.001$). Using cut-off values of 4 and 11 the model has a sensitivity and specificity of 96% and 95%, respectively. Conclusion: The corona-score effectively predicts SARS-CoV-2 RT-PCR outcome based on routine parameters. This algorithm provides the means for medical professionals to rapidly evaluate SARS-CoV-2 infection status of patients presenting at the ED with respiratory symptoms.
https://www.medrxiv.org/content/10.1101/2020.04.20.20067512v1
- Here is one for the pathologists in the audience and I am certainly not one of them!! The goal of an autopsy is to discover the cause of death (COD) using a macro/microscopic investigation. Traditionally, the intact organs are carefully removed, inspected, and weighed. Because lung weight is often affected by the cause of death and the last breath occurs very near if not at the moments of death, the evaluation of the lungs is one of the starting points of any COD investigation[3]. Method A comprehensive search was performed to systematically review all reported autopsy findings in COVID-19 patients in order to better understand the underlying disease mechanisms resulting in death. We then compared these findings with the results of a targeted literature review of hyaluronan in relationship to acute respiratory distress syndrome (ARDS). Results In total, data from 181 autopsies were identified. From this group, 6 autopsies of COVID-19 patients were selected for a detailed review and statistical analysis. The average lung weight of those who were determined to have died as a result of SARS-CoV-2 was 2196g-approximately 2.5x normal lung weight. Hyaline membranes were consistently identified on histologist sections. A review of the literature reveals that hyaluronan has been associated with

the pathophysiology of ARDS since 1967. However, its key role in driving the morbidity and mortality of the condition has heretofore not been fully recognized. Conclusions We propose that the induced hyaluronan storm syndrome or IHS, is the model that best addresses the heretofore perplexing respiratory failure that is the proximal cause of death in a minority, but ever rising number, of patients. In addition to treating and preventing IHS in currently infected individuals now; an aggressive research effort should be undertaken to discover why the majority of individuals who are exposed to the virus are either minimally or asymptomatic, while a minority of high-risk individuals rapidly progress to respiratory failure and death. https://www.medrxiv.org/content/10.1101/2020.04.19.20071647v1

- First paper from Sudan!!! These researcher add to the body of knowledge regarding possible markers for disease progression. Yes, it's a meta-analysis but kudos to them for getting involved in the research. This review aims to investigate the association of lymphocyte count, CRP, LDH, and D-Dimer with the severe form of COVID-19. This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The databases of MEDLINE/PubMed, WHO-Virtual Health Library (VHL), and ScienceDirect were used for the systematic search. Random effects model was used to estimate the pooled standardized mean differences (SMD) with the corresponding 95% confidence interval (CI), using OpenMeta Analyst software. A total of 11 studies, with 2437 COVID-19 patients, which fulfilled the eligibility criteria were included in the meta-analysis. The analysis revealed that lymphocyte count was significantly lower in patients with the severe form of COVID-19 (SMD = - 1.025, P value <.001). Also, the analysis of SMD showed that patients with severe COVID-19 have a significantly higher serum levels of CRP (SMD = 3.363, P value <.001), D-Dimer (SMD = 1.073, P value <.001), and LDH (SMD = 3.345, P value <.001). Low lymphocyte count and high levels of CRP, LDH, and D-Dimer are associated with severe COVID-19. These laboratory markers could be used as clinical indicators of worsening illness and poor prognosis of COVID-19. https://www.medrxiv.org/content/10.1101/2020.04.20.20072801v1
- Not necessarily a clinical study but important to those of us wearing homemade masks! The U.S. Centers for Disease Control and Prevention (CDC) has therefore recently recommended home-made cloth face coverings for use by the general public in areas of significant community-based transmission. Because medical masks and N95 respirators are in short supply, these are to be reserved for healthcare workers. There is, however, little information on the effectiveness of home-made face coverings in reducing droplet dissemination. Here, we ascertained the performance of ten different fabrics, ranging from cotton to silk, in blocking high velocity droplets, using a 3-layered commercial medical mask as a benchmark material. We also assessed their breathability and ability to soak water. We reason that the materials should be as breathable as possible, without compromising blocking efficiency, to reduce air flow through the sides of the mask since such flow would defeat the purpose of the mask. We found that most home fabrics substantially block droplets, even as a single layer. With two layers, blocking performance can reach that of surgical mask without significantly compromising breathability. Furthermore, we observed that home fabrics are hydrophilic to varying degrees, and hence soak water. In contrast, medical masks are hydrophobic, and tend to repel water. Incoming droplets are thus soaked and 'held back' by home fabrics, which might offer an as of yet untapped and understudied advantage of home-made cloth masks. Overall, our study suggests that most double-layered cloth face coverings may help reduce droplet transmission of respiratory

infections. [**note: homemade masks can be a fashion statement as well. I'm going to contact my supplier and see if she makes a matching bow tie that I can wear in one of the many media appearances I expect to do.**]
https://www.medrxiv.org/content/10.1101/2020.04.19.20071779v1

- This may explain some of the GI symptoms of SARS-CoV-2. Both gastrointestinal symptoms and fecal shedding of SARS-CoV-2 RNA have been frequently observed in COVID-19 patients. However, whether SARS-CoV-2 replicate in the human intestine and its clinical relevance to potential fecal-oral transmission remain unclear. Here, we demonstrate productive infection of SARS-CoV-2 in ACE2+ mature enterocytes in human small intestinal enteroids. In addition to TMPRSS2, another mucosa-specific serine protease, TMPRSS4, also enhanced SARS-CoV-2 spike fusogenic activity and mediated viral entry into host cells. However, newly synthesized viruses released into the intestinal lumen were rapidly inactivated by human colonic fluids and no infectious virus was recovered from the stool specimens of COVID-19 patients. Our results highlight the intestine as a potential site of SARS-CoV-2 replication, which may contribute to local and systemic illness and overall disease progression.
https://www.biorxiv.org/content/10.1101/2020.04.21.054015v1

DRUG DEVELOPMENT

- Another paper that the folks at PETA won't like. This Belgian team finds that hamsters may be a good pre-clinical model for drug testing. Here, we show that productive SARS-CoV-2 infection in the lungs of mice is limited and restricted by early type I interferon responses. In contrast, we show that Syrian hamsters are highly permissive to SARS-CoV-2. In wild-type hamsters, SARS-CoV-2 infection triggers bronchopneumonia and a strong inflammatory response in the lungs with neutrophil infiltration and edema. We further assess SARS-CoV-2-induced lung pathology in hamsters by micro-CT alike used in clinical practice. Finally, we identify an exuberant innate response as key player in immune pathogenesis, in which STAT2 signaling plays a double-edged role, driving severe lung injury on the one hand, yet restricting systemic virus dissemination on the other. Our results endorse hamsters as pre-clinical model to rationalize and assess the therapeutic benefit of new antivirals or immune modulators for the treatment of COVID-19 patients https://www.biorxiv.org/content/10.1101/2020.04.23.056838v1
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DIAGNOSTIC DEVELOPMENT

- More good work from Seattle, showing a way that RT-qPCR can be scaled up by home collection. The urgent need for massively scaled clinical or surveillance testing for SARS-CoV-2 has necessitated a reconsideration of the methods by which respiratory samples are collected, transported, processed and tested. Conventional testing for SARS-CoV-2 involves collection of a clinical specimen with a nasopharyngeal swab, storage of the swab during transport in universal transport medium (UTM), extraction of RNA, and quantitative reverse transcription PCR (RT-qPCR). As testing has scaled across the world, supply chain challenges have emerged across this entire workflow. Here we sought to evaluate how eliminating the UTM storage and RNA

extraction steps would impact the results of molecular testing. Using paired mid-turbinate swabs self-collected by 11 individuals with previously established SARS-CoV-2 positivity, we performed a comparison of conventional (swab → UTM → RNA extraction → RT-qPCR) vs. simplified (direct elution from dry swab → RT-qPCR) protocols. Our results suggest that dry swabs eluted directly into a simple buffered solution (TE) can support molecular detection of SARS-CoV-2 via endpoint RT-qPCR without substantially compromising sensitivity. Although further confirmation with a larger sample size and variation of other parameters is necessary, these results are encouraging for the possibility of a simplified workflow that could support massively scaled testing for COVID-19 control.
https://www.biorxiv.org/content/10.1101/2020.04.22.056283v1

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2020-04-25

Some social distancing approaches are better than others!!!

You knew that he had to make an appearance. Yes, I was present at the famous May 1970 performance of the LA Philharmonic and Frank Zappa's Mothers of Invention. It was memorable in all kinds of ways that would take too long to explain. Zappa was an iconoclastic musician and composer and there are a number classical groups who continue performing chamber music he wrote. I told you my taste in music is very eclectic. Here is the classic Montana: https://www.youtube.com/watch?v=DmcYTShN4Fk and here is one of his finer chamber pieces 'G-Spot Tornado' with accompanying dancers; it was the last time he conducted, passing away nine months after this performance at the age of 52. [**Contest Alert!!!! What was the name of the leader of the do-wop band inspired by Frank Zappa?? This is a tough one but a nice prize will go to the winner!**]

This opinion piece 'Against Pandemic Research Exceptionalism' is well worth reading. As I have commented in the past, there is a waste of clinical trial resources with lots of duplicative studies, particularly with hydroxychloroquine. It may be too late in the game to change the behavior of the biomedical research community but consideration of how to do things better has to be a part of any post-mortem.

Here is a very good article from The New Yorker on the quest to develop a drug treatment. Fascinating discussion of all the possible targets.

Here is the full story on he putative effect of summer weather on SARS-CoV-2 that was covered at Thursday's White House briefing.

STOP THE PRESSES. Paging all you pharmaco-vigilantes, flash alert from the FDA. Don't recklessly take hydroxychloroquine!!!! Question for Dr. Hahn, why did the FDA issue an experimental use authorization for this drug? It just encouraged more use. Do you understand that this drug has been flying off of pharmacy shelves and that lupus and RA patients cannot find it? Do you think community physicians are paying any attention to this warning?

MODELING

- Here is a exit strategy for the UK: https://gbohner.github.io/coexist/ Note that this contradicts another paper from the UK that I posted on Thursday. These researches note, the number of daily tests carried out is much more important than their sensitivity, for the success of a case-isolation based strategy. [**note: can we have our cake and eat it too?**]
- Here is the preprint covered by the New York Times and others about modeling of SARS-CoV-2 based on seasonality. The virus causing COVID-19 has spread rapidly worldwide and threatens millions of lives. It remains unknown if summer weather will reduce its continued spread, thereby alleviating strains on hospitals and providing time for vaccine development. Early insights from laboratory studies of related coronaviruses predicted that COVID-19 would decline at higher temperatures, humidity, and ultraviolet light. Using current, fine-scaled weather data and global reports of infection we developed a model that explained 36% of variation in early growth rates before intervention, with 17% based on weather or demography and 19% based on country-specific effects. We found that ultraviolet light was most strongly associated with lower COVID-19 growth rates. Projections suggest that, in the absence of intervention, COVID-19 will decrease temporarily during summer, rebound by autumn, and peak next winter. However, uncertainty remains high and the probability of a weekly doubling rate remained >20% throughout the summer in the absence of control. Consequently, aggressive policy interventions will likely be needed in spite of seasonal trends. [**note: bottom line – this meets the Rumsfeld threshold of knowns/unknowns.}**
https://www.medrxiv.org/content/10.1101/2020.04.19.20071951v1
- I don't know where to put papers such as this one. Interesting stuff from Italy about ACE2 polymorphisms and individual susceptibility. The current SARS covid-19 epidemic spread appears to be influenced by ethnical, geographical and sex-related factors that may involve genetic susceptibility to diseases. Similar to SARS-CoV, SARS-CoV-2 exploits angiotensin-converting enzyme 2 (ACE2) as a receptor to invade cells, notably type II alveolar epithelial cells. Importantly, ACE2 gene is highly polymorphic. Here we have used in silico tools to analyze the possible impact of ACE2 single-nucleotide polymorphisms (SNPs) on the interaction with SARS-CoV-2 spike glycoprotein. We found that S19P (common in African people) and K26R (common in European people) were, among the most diffused SNPs worldwide, the only two SNPs that were able to potentially affect the interaction of ACE2 with SARS-CoV-2 spike. FireDock

simulations demonstrated that while S19P may decrease, K26R might increase the ACE2 affinity for SARS-CoV-2 Spike. This finding suggests that the S19P may genetically protect, and K26R may predispose to more severe SARS-CoV-2 disease. [**note: molecular epidemiologists are going to be very busy looking at a lot of genetic data to see what the correlations are.**] https://www.biorxiv.org/content/10.1101/2020.04.23.057042v1

- Well, this modeling study was too good to pass up!!! I have always had a fondness for biker movies going back to 'Easy Rider' so this is particularly important to me!!! In this paper, we present a mathematical model of trigonometric type for transmissibility and deaths as a result of COVID-19. In the model, we analyze the spread of COVID-19 by considering a new parameter, the motorcycle as a means of public transport, which has not been considered in several other models for COVID-19. We use the mathematical model to predict the spread and deaths and we suggest strategies that can be put in place to prevent the spread caused by motorcycle as a means of public transport. https://www.medrxiv.org/content/10.1101/2020.04.18.20070797v1
- More good molecular epidemiology. At cellular level, virus infection initiates with binding of viral particles to the host surface cellular receptor angiotensin converting enzyme 2 (ACE2). SARS-CoV-2 engages ACE2 as the entry receptor and employs the cellular serine protease 2 (TMPRSS2) for S protein priming. TMPRSS2 activity is essential for viral spread and pathogenesis in the infected host. Understanding how TMPRSS2 protein expression in the lung varies in the population could reveal important insights into differential susceptibility to influenza and coronavirus infections. Here, we systematically analyzed coding-region variants in TMPRSS2 and the eQTL variants, which may affect the gene expression, to compare the genomic characteristics of TMPRSS2 among different populations. Our findings suggest that the lung-specific eQTL variants may confer different susceptibility or response to SARS-CoV-2 infection from different populations under the similar conditions. In particular, we found that the eQTL variant rs35074065 is associated with high expression of TMPRSS2 but with a low expression of the interferon (IFN)-α/β-inducible gene, MX1, splicing isoform. Thus, these subjects could account for a more susceptibility either to viral infection or to a decrease in cellular antiviral response. https://www.biorxiv.org/content/10.1101/2020.04.23.057190v1

NEWLY REGISTERED CLINICAL TRIALS

- Here is a trial looking at whether an estrogen patch can reduce symptoms of SARS-CoV-2 infection. The purpose of this study is to find out if estrogen, a female sex hormone, given as a patch placed on skin of COVID19 positive or presumptive positive patients for 7 days can reduce the severity of COVID19 symptoms compared to regular care. As the COVID19 pandemic has spread, it has been observed that adult men of all ages and older women are at higher risk of developing serious complications from infection with the virus. Animal model studies of SARS suggest that the age and sex difference in COVID19 symptom severity may be due to protective and acute actions of the female sex hormone estrogen. Animal and human studies support immune modulating effects of estrogen that are acute acting in viral infections and wound repair processes that may reduce the damaging effects of the virus on the lung and symptom severity. NCT04359329

CLINICAL TRIAL RESULTS

- We are still waiting definitive data on remdesivir and hydroxychloroquine.

DRUG DEVELOPMENT

- Dear readers, I'm sure you all remember the wonderful Chiffon Margarine commercials, 'you can't fool Mother Nature.' The more I read about the coronavirus virus family, the more I'm amazed at how this pest evolved. Here a paper outlining the proofreading ability. Coronaviruses (CoVs) emerge as zoonoses and cause severe disease in humans, demonstrated by the SARS-CoV-2 (COVID-19) pandemic. RNA recombination is required during normal CoV replication for subgenomic mRNA (sgmRNA) synthesis and generates defective viral genomes (DVGs) of unknown function. However, the determinants and patterns of CoV recombination are unknown. Here, we show that divergent β-CoVs SARS-CoV-2, MERS-CoV, and murine hepatitis virus (MHV) perform extensive RNA recombination in culture, generating similar patterns of recombination junctions and diverse populations of DVGs and sgmRNAs. We demonstrate that the CoV proofreading nonstructural protein (nsp14) 3-to-5 exoribonuclease (nsp14-ExoN) is required for normal CoV recombination and that its genetic inactivation causes significantly decreased frequency and altered patterns of recombination in both infected cells and released virions. Thus, nsp14-ExoN is a key determinant of both high fidelity CoV replication and recombination, and thereby represents a highly-conserved and vulnerable target for virus inhibition and attenuation. https://www.biorxiv.org/content/10.1101/2020.04.23.057786v1
- These Louisiana researchers suggest looking at nelfinavir, an HIV protease inhibitor approved by the FDA, for early intervention in SARS-CoV-2 infection. Previous results with SARS and MERS CoV have shown that the Spike (S) glycoprotein is a major determinant of virus infectivity and immunogenicity. Herein, we show that expression of SARS CoV-2 S (S-n) glycoprotein after transient transfection of African green monkey kidney (Vero) cells caused extensive cell fusion in comparison to limited cell fusion caused by the SARS S (S-o) glycoprotein. S-n expression was detected intracellularly and on transfected Vero cell surfaces and caused the formation of very large multinucleated cells (syncytia) by 48 hours post transfection. These results are in agreement with published pathology observations of extensive syncytial formation in lung tissues of COVID-19 patients. This differential S-n versus S-o-mediated cell fusion suggests that SARS-CoV-2 is able to spread from cell-to-cell much more efficiently than SARS effectively avoiding extracellular spaces and neutralizing antibodies. A systematic screening of several drugs for ability to inhibit S-n and S-o cell fusion revealed that the FDA approved HIV-protease inhibitor, nelfinavir mesylate (Viracept) drastically inhibited S-n and S-o-mediated cell fusion in a dose-dependent manner. Complete inhibition of cell fusion was observed at a 10 micromolar concentration. Computational modeling and in silico docking experiments suggested the possibility that nelfinavir may bind inside the S trimer structure, proximal to the S2 amino terminus directly inhibiting S-n and S-o-mediated membrane fusion. Also, it is possible that nelfinavir mesylate acts on cellular processes to inhibit S proteolytic processing. These results warrant further investigations of the potential of nelfinavir mesylate as an antiviral drug, especially at early times after SARS-CoV-2 symptoms appear. [**note: this is an older HIV drug and I don't think it's in general use these days. I just checked to see if there is any trial for**

SARS-CoV-2 and there are not. Maybe it doesn't work, but nothing tested to date seems to be a game changer. Someone ought to take a look at this one.]
https://www.biorxiv.org/content/10.1101/2020.04.24.060376v1

DIAGNOSTIC DEVELOPMENT

- Your fearless curator of COVID-19 preprints is always enamored of outside the box DIY project. This clever approach from an Indian team deserves an award!!! Here, we report an in-house built biosensor device (eCovSens) and compare it with a commercial potentiostat machine for the detection of nCovid-19 spike protein antigen (nCovid-19 Ag) in spiked saliva samples. A potentiostat based sensor was fabricated using fluorine doped tin oxide electrode (FTO) drop casted with gold nanoparticle (AuNPs) and immobilized with nCovid-19 monoclonal antibody (nCovid-19 Ab) to measure change in the electrical conductivity. Similarly, eCovSens was used to measure change in electrical conductivity by immobilizing nCovid-19 Ab on screen printed carbon electrode (SPCE). The performances of both sensors were recorded upon interaction of nCovid-19 Ab with its specific nCovid-19 Ag. Under optimum conditions, the FTO based immunosensor and proposed SPCE-based biosensor device displayed high sensitivity for early detection of nCovid-19 Ag, ranging from 1 fM to 1 uM. Our in-house developed eCovSens device can successfully detect nCovid-19 Ag at 10 fM concentration in standard buffer that is in close agreement with FTO/AuNPs sensor where AuNPs were used for the amplification of the electrical signal. The limit of detection (LOD) was found to be 90 fM with eCovSens and 120 fM with potentiostst in case of spiked saliva samples. The proposed portable point of care (PoC) eCovSens device can be used as an alternative diagnostic tool for the rapid (within 10-30 s) detection of nCovid-19 Ag traces directly in patient saliva samples that displayed high sensitivity, stability, and specificity. [**note: obviously there will need to be validation work to see if this can be deployed in the field.**] https://www.biorxiv.org/content/10.1101/2020.04.24.059204v1
- YES!!! The CDC weighs in on validation of a SARS-CoV-2 spike ELISA for use in contact investigations. Since emergence of SARS-CoV-2 in late 2019, there has been a critical need to understand transmission patterns, to calculate the burden of disease and case fatality rates. Molecular diagnostics, the gold standard for identifying viremic cases, are not ideal for determining true case counts and rates of asymptomatic infection. Serological detection of SARS-CoV-2 specific antibodies can contribute to filling these knowledge gaps. In this study, we describe optimization and validation of a SARS-CoV-2-specific-enzyme linked immunosorbent assay (ELISA) using the prefusion-stabilized form of the spike protein. We performed receiver operator characteristic (ROC) analyses to define the specificities and sensitivities of the optimized assay and examined cross reactivity with immune sera from persons confirmed to have had infections with other coronaviruses. These assays will be used to study chains of transmission and to conduct large-scale, cross sectional surveillance to define disease burden in the population. https://www.biorxiv.org/content/10.1101/2020.04.24.057323v1
- I have long felt that a lot of laboratory expertise is going unused. Here is a nice paper from Britain that discusses repurposing academic labs into testing centers. The emergence of the novel coronavirus SARS-CoV-2 has led to a pandemic infecting more than two million people worldwide in less than four months, posing a major threat to healthcare systems. This is compounded by the shortage of available tests causing numerous healthcare workers to

unnecessarily self-isolate. We provide a roadmap instructing how a research institute can be repurposed in the midst of this crisis, in collaboration with partner hospitals and an established diagnostic laboratory, harnessing existing expertise in virus handling, robotics, PCR, and data science to derive a rapid, high throughput diagnostic testing pipeline for detecting SARS-CoV-2 in patients with suspected COVID-19. The pipeline is used to detect SARS-CoV-2 from combined nose-throat swabs and endotracheal secretions/ bronchoalveolar lavage fluid. Notably, it relies on a series of in-house buffers for virus inactivation and the extraction of viral RNA, thereby reducing the dependency on commercial suppliers at times of global shortage. We use a commercial RT-PCR assay, from BGI, and results are reported with a bespoke online web application that integrates with the healthcare digital system. This strategy facilitates the remote reporting of thousands of samples a day with a turnaround time of under 24 hours, universally applicable to laboratories worldwide.
https://www.medrxiv.org/content/10.1101/2020.04.19.20071373v1

- More good information on how to design a solid antibody test. Quantitative measurements of plasma or serum antibodies by luciferase immunoprecipitation assay systems (LIPS) to the nucleocapsid and spike proteins were analyzed in 100 cross-sectional or longitudinal samples from SARS-CoV-2-infected patients. A subset of samples was tested with and without heat inactivation. Results: Fifteen or more days after symptom onset, antibodies against SARS-CoV-2 nucleocapsid protein showed 100% sensitivity and 100% specificity, while antibodies to spike protein were detected with 91% sensitivity and 100% specificity. Neither antibody levels nor the rate of seropositivity were significantly reduced by heat inactivation of samples. *Analysis of daily samples from six patients with COVID-19 showed anti-nucleocapsid and spike antibodies appearing between day 8 to day 14 after initial symptoms.* Immunocompromised patients generally had a delayed antibody response to SARS-CoV-2 compared to immunocompetent patients. Conclusions: Antibody to the nucleocapsid protein of SARS-CoV-2 is more sensitive than spike protein antibody for detecting early infection. Analyzing heat-inactivated samples by LIPS is a safe and sensitive method for detecting SARS-CoV-2 antibodies. [**note: antibody appearance fall in the range I've seen in previous papers.**]
https://www.medrxiv.org/content/10.1101/2020.04.20.20071423v1
- Good to know information!!! RT-PCR test kits from multiple manufacturers behave similarly; someone needed to do this research and these Dutch researchers did it. Many commercial kits have recently become available, but their performance has not yet been independently assessed. The aim of this study was to compare basic analytical and clinical performance of selected RT-PCR kits from seven different manufacturers (Altona Diagnostics, BGI, CerTest Biotec, KH Medical, PrimerDesign, R-Biopharm AG, and Seegene). >We used serial dilutions of viral RNA to establish PCR efficiency and estimate the 95% limit of detection (LOD95%). Furthermore, we ran a panel of SARS-CoV-2-positive clinical samples (n=16) for a preliminary evaluation of clinical sensitivity. Finally, we used clinical samples positive for non-coronavirus respiratory viral infections (n=6) and a panel of RNA from related human coronaviruses to evaluate assay specificity. PCR efficiency was ≥96% for all assays and the estimated LOD95% varied within a 6-fold range. Using clinical samples, we observed some variations in detection rate between kits. Importantly, none of the assays showed cross-reactivity with other respiratory (corona)viruses, except as expected for the SARS-CoV-1 E-gene. We conclude that all RT-PCR kits assessed in this study may be used for routine diagnostics of COVID-19 in patients by

experienced molecular diagnostic laboratories.
https://www.biorxiv.org/content/10.1101/2020.04.22.056747v1

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2020-04-26

The BIG NEWS of the day is I finally finished my working paper on how to optimize clinical trials during a pandemic. **CLINICAL TRIALS DURING A PANDEMIC is up on my COVID-19 website** and it's a FREE download (I thought about putting a PayPal link to it but didn't think there would be too many takers). This is absolutely a work in progress and *input from all of you is welcome*!!! We need to have a flexible clinical trial program that tests as many drugs as possible and reports out results quickly to inform medical practice. This is no time for business as usual. Feel free to share the link with all your friends and neighbors (at least they will get some good cookie recipes!!).

We absolutely have to stay with pop music for today's selection. I have been a fan of Aretha Franklin since she hit the charts big time in the 1960s. There are so many good selections but I return to 'Think' which for me made The Blues Brothers an iconic film:
https://www.youtube.com/watch?v=WY66elCQkYk

I suspect it will be some time before the cruise ship industry regains public confidence. This Washington Post story covers the pandemic at sea.

More good stuff from Derek Lowe, it's not all that encouraging.

MODELING

- This one is interesting only in that it discusses a model for closed environments and uses the Diamond Princess cruise ship as an example. We take the case of the Diamond Princess (DP) cruise ship as an experimental model for studying the transmission potential of COVID-19 in a closed environment. We investigate the changes in R0 for COVID-19 on the DP from January 21 to February 19, 2020 with a chain-binomial model at different times under two scenarios: no quarantine assuming a random mixing condition, and quarantine of passengers in cabins -- passengers may get infected either by an infectious case in a shared cabin or by asymptomatic crew who continued to work. Our modeling approach takes account of the asymptomatic ratio of crew members during the quarantine of passengers, which has not been explored in the literature. Assuming an asymptomatic ratio 0.505 and the proportion of infections in cabins 0.2, R0 at the beginning of the epidemic was 3.27 (95% CI (3.02, 3.54)) and 3.78 (95% CI (3.49, 4.09)) respectively for serial intervals of 5 and 6 days, and increased for passengers in contact with asymptomatic crew during quarantine. We find evidence to support a CDC report that "a high proportion of asymptomatic infections could partially explain the high attack rate among cruise ship passengers and crew." This emphasizes the importance of improved reporting and quarantine of asymptomatic cases, and raises questions on quarantine procedures in closed

environments such as military vessels, cruise ships, dormitories, prisons, and other enclosed living complexes with high population densities. https://www.medrxiv.org/content/10.1101/2020.04.22.20074286v1

- Anyone supporting mass testing gets a nod in my newsletter!! The ratios offer simple ways to account for variations in testing and reporting. Tracking the ratios in addition to cases offer a more precise view of the pandemic. Our observations underscore the need to scale mass testing with accurate and reliable tests, to implement testing systematically and report results consistently. https://www.medrxiv.org/content/10.1101/2020.04.21.20074070v1

NEWLY REGISTERED CLINICAL TRIALS

- Because the NIH Clinical Trials database is too cumbersome to search, I've decided to only do this every other day. Hence, nothing new to report.

CLINICAL TRIAL RESULTS

- ACE2 levels in normal tissues of cancer patients appears to be elevated raising an increased risk. The rapidly developing COVID-19 pandemic has raised a concern that cancer patients may have increased susceptibility to SARS-CoV-2 infection. This discussion has mostly focused on therapy-induced immune suppression. Here, we examined the expression patterns of ACE2, the receptor through which SARX-CoV2 enters human cells, and found that ACE2 mRNA levels are elevated in tumor-adjacent normal tissues of cancer patients, including in normal-adjacent lung tissues of lung cancer patients. These observations raise the possibility that the elevated COVID-19 risk of cancer patients may not be limited to those undergoing immune-suppressing treatment. https://www.biorxiv.org/content/10.1101/2020.04.25.061200v1
- Now this stuff is really interesting!! Here we describe constructing a proteomic risk score based on 20 blood proteomic biomarkers which predict the progression to severe COVID-19. We demonstrate that in our own cohort of 990 individuals without infection, this proteomic risk score is positively associated with proinflammatory cytokines mainly among older, but not younger, individuals. We further discovered that a core set of gut microbiota could accurately predict the above proteomic biomarkers among 301 individuals using a machine learning model, and that these gut microbiota features are highly correlated with proinflammatory cytokines in another set of 366 individuals. Fecal metabolomic analysis suggested potential amino acid-related pathways linking gut microbiota to inflammation. This study suggests that gut microbiota may underlie the predisposition of normal individuals to severe COVID-19. [**note: there was no mention in the paper as to the utility of taking probiotic supplements. I may head out to CVS later today to stock up.**] https://www.medrxiv.org/content/10.1101/2020.04.22.20076091v1
- Absolutely no surprise here!! Hydroxychloroquine and azithromycin need to be administered with CAUTION. Despite a paucity of clinical evidence, hydroxychloroquine and azithromycin are being administered widely to patients with verified or suspected COVID-19. Both drugs may increase risk of lethal arrhythmias associated with QT interval prolongation. Methods: We performed a case series of COVID-19 positive/suspected patients admitted between 2/1/2020

and 4/4/2020 who were treated with azithromycin, hydroxychloroquine or a combination. We evaluated baseline and post-medication QT interval (QTc, Bazett) using 12-lead ECGs. Critical QTc prolongation was defined as: a) maximum QTc ≥500 ms (if QRS <120 ms) or QTc ≥550 (if QRS ≥120 ms) and b) increased QTc of ≥60 ms. Tisdale score and Elixhauser comorbidity index were calculated. Results: Of 490 COVID-19 positive/suspected patients, 314 (64%) received either/both drugs, and 98 (73 COVID-19 positive, 25 suspected) met study criteria (age 62±17 yrs, 61% male). Azithromycin was prescribed in 28%, hydroxychloroquine in 10%, and both in 62%. Baseline mean QTc was 448±29 ms and increased to 459±36ms (p=0.005) with medications. Significant prolongation was observed only in men (18±43 ms vs -0.2±28 ms in women, p=0.02). 12% of patients reached critical QTc prolongation. In a multivariable logistic regression, age, sex, Tisdale score, Elixhauser score, and baseline QTc were not associated with critical QTc prolongation (p>0.14). Changes in QTc were highest with the combination compared to either drug, with many-fold greater prolongation with the combination vs. azithromycin alone (17±39 vs. 0.5±40 ms, p=0.07). No patients manifested torsades de pointes. Conclusions: *Overall, 12% of patients manifested critical QTc interval prolongation, and traditional risk indices failed to flag these patients. With the drug combination, QTc prolongation was several-fold higher compared to azithromycin alone.* The balance between uncertain benefit and potential risk when treating COVID-19 patients with these drugs should be carefully assessed prior to use. [**note: I hope community physicians realize the risk in prescribing to patients and furthermore pharmacies MUST flag co-prescriptions of these drugs and flag them.**]
https://www.medrxiv.org/content/10.1101/2020.04.22.20075671v1

- Here is a large cohort study from the OHDSI group on characterization of patients hospitalized with SARS-CoV-2 and those previously hospitalized with influenza. Background To better understand the profile of individuals with severe coronavirus disease 2019 (COVID-19), we characterised individuals hospitalised with COVID-19 and compared them to individuals previously hospitalised with influenza. Methods We report the characteristics (demographics, prior conditions and medication use) of patients hospitalised with COVID-19 between December 2019 and April 2020 in the US (Columbia University Irving Medical Center [CUIMC], STAnford Medicine Research data Repository [STARR-OMOP], and the Department of Veterans Affairs [VA OMOP]) and Health Insurance Review & Assessment [HIRA] of South Korea. Patients hospitalised with COVID-19 were compared with patients previously hospitalised with influenza in 2014-19. Results 6,806 (US: 1,634, South Korea: 5,172) individuals hospitalised with COVID-19 were included. Patients in the US were majority male (VA OMOP: 94%, STARR-OMOP: 57%, CUIMC: 52%), but were majority female in HIRA (56%). Age profiles varied across data sources. Prevalence of asthma ranged from 7% to 14%, diabetes from 18% to 43%, and hypertensive disorder from 22% to 70% across data sources, while between 9% and 39% were taking drugs acting on the renin-angiotensin system in the 30 days prior to their hospitalisation. Compared to 52,422 individuals hospitalised with influenza, patients admitted with COVID-19 were more likely male, younger, and, in the US, had fewer comorbidities and lower medication use. Conclusions Rates of comorbidities and medication use are high among individuals hospitalised with COVID-19. However, *COVID-19 patients are more likely to be male and appear to be younger and, in the US, generally healthier than those typically admitted with influenza*.
https://www.medrxiv.org/content/10.1101/2020.04.22.20074336v1

- Dutch research into phenotypic characterization is revealing some interesting findings. Large differences in outcome have also been observed between males and females. The causes for this variability are likely to be multifactorial, and to include genetics. The SARS-CoV-2 virus responsible for the infection uses the human receptor angiotensin converting enzyme 2 (ACE2) for cell invasion, and the serine protease TMPRSS2 for S protein priming. Genetic variation in these two genes may thus modulate an individual's genetic predisposition to infection and virus clearance. While genetic data on COVID-19 patients is being gathered, we carried out a phenome-wide association scan (PheWAS) to investigate the role of these genes in other human phenotypes in the general population. We examined 178 quantitative phenotypes including cytokines and cardio-metabolic biomarkers, as well as 58 medications in 36,339 volunteers from the Lifelines population biobank, in relation to 1,273 genetic variants located in or near *ACE2* and *TMPRSS2*. While none reached our threshold for significance, we observed a suggestive association of polymorphisms within the ACE2 gene with (1) the use of ARBs combination therapies (p=5.7x10-4), an association that is significantly stronger in females (p_{diff}=0.01), and (2) with the use of non-steroid anti-inflammatory and antirheumatic products (p=5.5x10-4). While these associations need to be confirmed in larger sample sizes, they suggest that these variants play a role in diseases such as hypertension and chronic inflammation that are often observed in the more severe COVID-19 cases. Further investigation of these genetic variants in the context of COVID-19 is thus promising for better understanding of disease variability. Full results are available at https://covid19research.nl.
https://www.medrxiv.org/content/10.1101/2020.04.22.20074963v1
- Linkage data from Iran on outcomes. Background − The Covid−19 pandemic imposed the most devastating challenge on healthcare systems worldwide. Iran was among the first countries that had to confront serious shortages in RT−PCR testing for SARS−CoV−2 and ventilators availabilities throughout the COVID−19 outbreak. This study aimed to investigate the clinical course of hospitalized COVID−19 patients with different rRT−PCR test results during the first 3 weeks of the outbreak in Qazvin province, Iran. Methods −For this retrospective cohort study, data of hospitalized patients primarily diagnosed as having COVID−19 in all 12 centers across the whole Qazvin province during Feb 20−Mar 11, 2020 was analyzed. A multivariate logistic regression model was applied to assess the independent associates of death among COVID−19 patients. Results − 998 patients (57% male, median age 54 years) with positive chest CT−scan changes were included in this study. Among them, 558 patients were examined with rRT−PCR test and 73.8% tested positive. Case fatality rate was 20.68% and 7.53% among test−positive and test negative hospitalized patients, respectively. While only 5.2% of patients were ICU admitted, case fatality rates outside ICU were 17.70% and 4.65% in test-positive and test-negative non−ICU admitted patients, correspondingly. The independent associates of death were age ≥ 70 years, testing positive with rRT−PCR test, having immunodeficiency disorders and ICU admission. Conclusions − Hospitalized COVID−19 patients with mild symptoms despite positive chest CT changes and major comorbidities were more probable to have negative rRT−PCR test result, hence lower case fatality rate and a more favorable outcome. [**note: I try not to make political statements in this newsletter and will only state that this is a global fight. Researchers and clinicians in all countries should be saluted for the hard work they are putting in during this pandemic!**]
https://www.medrxiv.org/content/10.1101/2020.04.21.20074641v1

DRUG DEVELOPMENT

- OH NO!!!! YIKES!! The labeled ivermectin dose may not be effective!! Introduction: Caly, Druce (1) reported that ivermectin inhibited SARS-CoV-2 in vitro for up to 48 h using ivermectin at 5 uM. The concentration resulting in 50% inhibition (IC50, 2 uM) was >35x higher than the maximum plasma concentration (Cmax) after oral administration of the approved dose of ivermectin when given fasted. Method: Simulations were conducted using an available population pharmacokinetic model to predict total (bound and unbound) and unbound plasma concentration-time profiles after a single and repeat fasted administration of the approved dose of ivermectin (200 ug/kg), 60 mg, and 120 mg. Plasma total Cmax was determined and then multiplied by the lung:plasma ratio reported in cattle to predict the lung Cmax after administration of each single dose. Results: Plasma ivermectin concentrations of total (bound and unbound) and unbound concentrations do not reach the IC50, even for a dose level 10x higher than the approved dose. Even with higher exposure in lungs than plasma, ivermectin is unlikely to reach the IC50 in lungs after single oral administration of the approved dose (predicted lung: 0.0857 uM) or at doses 10x higher that the approved dose administered orally (predicted lung: 0.817 uM). Conclusions: The likelihood of a successful clinical trial using the approved dose of ivermectin is low. Combination therapy should be evaluated in vitro. Re-purposing drugs for use in COVID-19 treatment is an ideal strategy but is only feasible when product safety has been established and experiments of re-purposed drugs are conducted at clinically relevant concentrations. [**note: this points out the value of clinical pharmacology input following *in vitro* studies. At least hydroxychloroquine has high plasma levels and it hangs around for a long time.**]
  https://www.medrxiv.org/content/10.1101/2020.04.21.20073262v1
- More work at looking at repurposed drugs. SARS-CoV-2, a member of the coronavirus family, is responsible for the current COVID-19 worldwide pandemic. We previously demonstrated that five nucleotide analogues inhibit the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp), including the active triphosphate forms of Sofosbuvir, Alovudine, Zidovudine, Tenofovir alafenamide and Emtricitabine. We report here the evaluation of a library of additional nucleoside triphosphate analogues with a variety of structural and chemical features as inhibitors of the RdRps of SARS-CoV and SARS-CoV-2. These features include modifications on the sugar (2′ or 3′ modifications, carbocyclic, acyclic, or dideoxynucleotides) or on the base. The goal is to identify nucleotide analogues that not only terminate RNA synthesis catalyzed by these coronavirus RdRps, but also have the potential to resist the viruses' exonuclease activity. We examined these nucleotide analogues with regard to their ability to be incorporated by the RdRps in the polymerase reaction and then prevent further incorporation. While all 11 molecules tested displayed incorporation, 6 exhibited immediate termination of the polymerase reaction (Carbovir triphosphate, Ganciclovir triphosphate, Stavudine triphosphate, Entecavir triphosphate, 3′-O-methyl UTP and Biotin-16-dUTP), 2 showed delayed termination (Cidofovir diphosphate and 2′-O-methyl UTP), and 3 did not terminate the polymerase reaction (2′-fluoro-dUTP, 2′-amino-dUTP and Desthiobiotin-16-UTP). The coronavirus genomes encode an exonuclease that apparently requires a 2′-OH group to excise mismatched bases at the 3′-terminus. In this study, all of the nucleoside triphosphate analogues we evaluated form Watson-

Crick-like base pairs. All the nucleotide analogues which demonstrated termination either lack a 2'-OH, have a blocked 2'-OH, or show delayed termination. These nucleotides may thus have the potential to resist exonuclease activity, a property that we will investigate in the future. Furthermore, prodrugs of five of these nucleotide analogues (Brincidofovir/Cidofovir, Abacavir, Valganciclovir/Ganciclovir, Stavudine and Entecavir) are FDA approved for other viral infections, and their safety profile is well known. *Thus, they can be evaluated rapidly as potential therapies for COVID-19.* [**note: my paradigm paper provides just the mechanism for getting things into trials! The old aphorism, 'throw a lot of things at the wall and see what sticks' is pretty apt today.**] https://www.biorxiv.org/content/10.1101/2020.04.23.058776v1

- This looks to be a different approach than the Moderna mRNA vaccine. The spread of the SARS-CoV-2 into a global pandemic within a few months of onset motivates the development of a rapidly scalable vaccine. Here, we present a self-amplifying RNA encoding the SARS-CoV-2 spike protein encapsulated within a lipid nanoparticle as a vaccine and demonstrate induction of robust neutralization of a pseudo-virus, proportional to quantity of specific IgG and of higher quantities than recovered COVID-19 patients. These data provide insight into the vaccine design and evaluation of immunogenicity to enable rapid translation to the clinic. [**note: there is some convincing data in the paper suggesting this is a viable vaccine candidate. I didn't see any mention about development. This company was involved in the research: https://acuitastx.com/**] https://www.biorxiv.org/content/10.1101/2020.04.22.055608v1
- Here is another target to go after. Coronaviruses (CoVs) emerge as zoonoses and cause severe disease in humans, demonstrated by the SARS-CoV-2 (COVID-19) pandemic. RNA recombination is required during normal CoV replication for subgenomic mRNA (sgmRNA) synthesis and generates defective viral genomes (DVGs) of unknown function. However, the determinants and patterns of CoV recombination are unknown. Here, we show that divergent β-CoVs SARS-CoV-2, MERS-CoV, and murine hepatitis virus (MHV) perform extensive RNA recombination in culture, generating similar patterns of recombination junctions and diverse populations of DVGs and sgmRNAs. We demonstrate that the CoV proofreading nonstructural protein (nsp14) 3-to-5 exoribonuclease (nsp14-ExoN) is required for normal CoV recombination and that its genetic inactivation causes significantly decreased frequency and altered patterns of recombination in both infected cells and released virions. Thus, nsp14-ExoN is a key determinant of both high fidelity CoV replication and recombination, and thereby represents a highly-conserved and vulnerable target for virus inhibition and attenuation.
https://www.biorxiv.org/content/10.1101/2020.04.23.057786v1

DIAGNOSTIC DEVELOPMENT

- Here is a new low cost diagnostic approach using whole genome sequencing. Here, we introduce a low-cost, high-throughput method for diagnosis of SARS-CoV-2 infection, dubbed Pathogen- Oriented Low-Cost Assembly & Re-Sequencing (POLAR), that enhances sensitivity by aiming to amplify the entire SARS-CoV-2 genome rather than targeting particular viral loci, as in typical RT- PCR assays. To achieve this goal, we combine a SARS-CoV-2 enrichment method developed by the ARTIC Network (https://artic.network/) with short-read DNA sequencing and de novo genome assembly. *We are able to reliably (>95% accuracy) detect SARS-CoV-2 at concentrations of 84 genome equivalents per milliliter, better than the reported limits of*

detection of almost all diagnostic methods currently approved by the US Food and Drug Administration. At higher concentrations, we are able to reliably assemble the SARS-CoV-2 genome in the sample, often with no gaps and perfect accuracy. Such genome assemblies enable the spread of the disease to be analyzed much more effectively than would be possible with an ordinary yes/no diagnostic, and can help identify vaccine and drug targets. Using POLAR, a single person can process 192 samples over the course of an 8-hour experiment, at a cost of ~$30/patient, enabling a 24-hour turnaround with sequencing and data analysis time included. Further testing and refinement will likely enable greater enhancements in the sensitivity of the above approach. [**note: it's good to see lots of thinking about how to approach testing in different ways.**] https://www.biorxiv.org/content/10.1101/2020.04.25.061499v1

- And yet another approach to enhanced testing. In this study, we describe an alternative RT-PCR approach for the detection of SARS-CoV-2 RNA that can be used for the probe-based detection of clinical isolates in the diagnostics as well as in research labs using a low cost SYBR green method. For the evaluation, we used samples from patients with confirmed SARS-CoV-2 infection and performed RT-PCR assays along with successive dilutions of RNA standards to determine the limit of detection. We identified an M-gene binding primer and probe pair highly suitable for quantitative detection of SARS-CoV-2 RNA for diagnostic and research purposes. https://www.biorxiv.org/content/10.1101/2020.04.20.052258v1
- Oh, oh; cautionary report about the quick Abbot ID Now test. The SARS-CoV-2 pandemic has created an urgent and unprecedented need for rapid large-scale diagnostic testing to inform timely patient management. This study compared two recently-authorized rapid tests, Cepheid Xpert Xpress SARS-CoV-2 and Abbott ID Now SARS-CoV-2 to the Roche cobas SARS-CoV-2 assay. A total of 113 nasopharyngeal swabs were tested, including 88 positives spanning the full range of observed Ct values on the cobas assay. Compared to cobas, the overall positive agreement was 73.9% with ID Now and 98.9% with Xpert. Negative agreement was 100% and 92.0% for ID Now and Xpert, respectively. Both ID Now and Xpert showed 100% positive agreement for medium and high viral concentrations (Ct value <30). However, for Ct values >30, positive agreement was 34.3% for ID Now and 97.1% for Xpert. These findings highlight an important limitation of ID Now for specimens collected in viral or universal transport media with low viral concentrations. Further studies are needed to evaluate the performance of ID Now for direct swabs. https://www.biorxiv.org/content/10.1101/2020.04.22.055327v1

Here is a meta-analysis from Greek researchers on serology testing. With the emergence of SARS-CoV-2 and the associated Coronavirus disease 2019 (COVID-19), there is an imperative need for diagnostic tests that can identify the infection. Although Nucleic Acid Test (NAT) is considered to be the gold standard, serological tests based on antibodies could be very helpful. However, individual studies measuring the accuracy of the various tests are usually underpowered and inconsistent, thus, a comparison of different tests is needed. We performed a systematic review and meta-analysis following the PRISMA guidelines. We conducted the literature search in PubMed, medRxiv and bioRxiv. For the statistical analysis we used the bivariate method for meta-analysis of diagnostic tests pooling sensitivities and specificities. We evaluated IgM and IgG tests based on Enzyme-linked immunosorbent assay (ELISA), Chemiluminescence Enzyme Immunoassays (CLIA), Fluorescence Immunoassays (FIA) and the point-of-care (POC) Lateral Flow Immunoassays (LFIA) that are based on immunochromatography. In total, we identified 38 eligible studies that include data from 7,848 individuals. The analyses showed that tests using the S antigen are more sensitive than N antigen-based tests. IgG tests perform better

compared to IgM ones, and show better sensitivity when the samples were taken longer after the onset of symptoms. Moreover, irrespective of the method, a combined IgG/IgM test seems to be a better choice in terms of sensitivity than measuring either antibody type alone. All methods yielded high specificity with some of them (ELISA and LFIA) reaching levels around 99%. ELISA- and CLIA-based methods performed better in terms of sensitivity (90-94%) followed by LFIA and FIA with sensitivities ranging from 80% to 86%. ELISA tests could be a safer choice at this stage of the pandemic. POC tests (LFIA), that are more attractive for large seroprevalence studies show high specificity but lower sensitivity and this should be taken into account when designing and performing seroprevalence studies. https://www.medrxiv.org/content/10.1101/2020.04.22.20074914v1