2020-07-13

Welcome to Week 17 of the Pandemic Newsletter!

I have one more opera duet to begin the week as we will be shifting music genres. Here are Nicolai Gedda and Lisa Della Casa in the prison scene from Gounod's Faust: https://www.youtube.com/watch?v=19R-Kk0ThGA Both were exquisite singers. Gedda sang roles in almost every single language and Della Casa was a renowned Strauss interpreter.

US COVID-19 STATISTICS - **Infection Rate: 1.0%; CRF: 4.0%** (CRF down 0.1%)

I was alerted by my voice teacher that the National Association of Teachers of Singing was working with scientists on a study of aerosol production and what can be done to minimize passing SARS-CoV-2. There was a big outbreak in Washington state choir early in the pandemic which pretty much shut down all choruses and other performing arts. A number of groups are commissioning a study by University of Colorado scientists to look at this. I will keep my readers apprised of this work. It will be interesting to see what they can add as there already has been some work done by a number of scientists.

A loyal reader writes with three pertinent questions that will answer based on my readings.

1. *Is there a good summary/review of the different tests for detecting the virus, whether by nasal swab or serology, including how long it takes to obtain the results? Is the test done locally or must it be sent to an outside lab?* For **PCR testing of SARS-CoV-2 virus**, nasal swab collection is still the principal mode of sample collection. Most of the testing is done by centralized laboratories by the big diagnostic companies. Although the tests themselves can be run in a couple of hours there is a major delay in getting results. The Washington Post documented problems in an article today. Multiple problems continue to plague the testing system. There has been a lot of research to shorten testing times at the instrumentation level but those do not solve supply shortages. The Abbott ID Now system is portable and can provide results in under 15 minutes. **Serology testing** is routinely done on automated machines that usually require a full blood draw. There is ongoing research to see if collected dried blood spots from finger pricks can be amplified for these tests. These also would be done at centralized labs. There are lateral flow immunoassay sticks that provide a readout from a finger prick drop of blood. Some have good specificity and sensitivity, but most do not. Results are available in 10-20 minutes and can be done locally.
2. *How soon after a person is exposed to the virus will a test detect a positive reaction? E.g., if you are known to be exposed and do acquire the virus, will the test show up within 1 hour, 1 day, etc. of initial transmission? Does it depend on the initial viral load?* Laboratory PCR tests can be very sensitive. I don't know about the commercial lab tests. I do not know of any studies that specifically look testing times post exposure in humans. One of the problems in answering this question is access to testing which was covered by the article referenced in the answer to the previous question.
3. *While we know how important it is to cover the nose and mouth and keep our distance, what is known about transmission/acquisition through the eye? A face mask does not help. A face shield might.* The Lancet published a review on physical distancing, face masks and eye protection. A popular press story on ocular transmission is HERE. It remains unclear to me how common ocular transmission is in the real world. It is difficult to do laboratory studies on this mode of transmission. At my dentist office, staff wore masks and face shields. I was given glasses to wear during the visit as there was close contact and possibility of aerosol generation.

I do not wear eye protection when going to the store. However, a variety of inexpensive goggles and shields are available if one wants to add this layer of protection.

The Washington Post asked readers to create some pandemic themed artworks. The results are interesting and inspiring. There is also a nice story on a how a cheeseburger made it to a customer's table (of course there were a lot of intermediate steps involved as there is no such thing as a cheeseburger tree!).

Seriously, reliance on FAX machines is still something our healthcare system is battling with! Governors have been thrust into dealing with the pandemic. Finally, in an effort to bring all my readers the most important news of the day, here is the New York Times on how to shoot a sex scene in a pandemic as television and movie production begins to start up.

The Guardian has a story on a documentary on 1990 Biosphere 2 experiment (remember that one?). that was the ultimate 'lockdown' where eight people would be in the sealed dome for two years. This sounds like an interesting film to view.

For the CDC alums on the newsletter mail list, STAT has a good article on the current state of the agency.

MODELING

- After a recent spate of models, nothing new today.

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow

CLINICAL TRIAL RESULTS

- Nothing new.

DRUG DEVELOPMENT

- A series of epidemiological explorations has suggested a negative association between national bacillus Calmette–Guérin (BCG) vaccination policy and the prevalence and mortality of coronavirus disease 2019 (COVID-19). However, these comparisons are difficult to validate due to broad differences between countries such as socioeconomic status, demographic structure, rural vs. urban set-tings, time of arrival of the pandemic, number of diagnostic tests and criteria for testing, and national control strategies to limit the spread of COVID-19. We review evidence for a potential biological basis of BCG cross-protection from severe COVID-19, and refine the epidemiological analysis to mitigate effects of potentially con-founding factors (e.g., stage of the COVID-19 epidemic, development, rurality, population density, and age structure). A strong correlation between the BCG index, an estimation of the degree of universal BCG vaccination deployment in a country, and COVID-19 mortality in different socially similar European countries was observed (r_2=0.88;P=8×10$_{-7}$), indicating that every 10% in-crease in the BCG index was associated with a 10.4% reduction inCOVID-19 mortality. Results fail to confirm the null hypothesis of no association between BCG vaccination and COVID-19 mortality and suggest that BCG could have a protective effect. Nevertheless, the analyses are restricted to coarse-scale signals and should be considered with caution. BCG vaccination clinical trials are required to

corroborate the patterns detected here, and to establish causal-ity between BCG vaccination and protection from severe COVID-19. Public health implications of a plausible BCG cross-protection from severe COVID-19 are discussed. [**note: I saw this paper yesterday. It's not better or worse than any of the other BCG papers that have come across my computer of the past several months. I only note that there are a variety of clinical trials going on to demonstrate whether this vaccine offers any protection. I could make the argument that allergen desensitization offers COVID-19 protection as it also stimulates the immune system to a degree. Observational trials need to be carefully done in order to get useful information.**] https://www.pnas.org/content/pnas/early/2020/07/07/2008410117.full.pdf

- The COVID-19 pandemic is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and has spread world-wide with millions of cases and hundreds of thousands of deaths to date. The gravity of the situation mandates accelerated efforts to identify safe and effective vaccines. Here, we generated measles virus (MeV)-based vaccine candidates expressing the SARS-CoV-2 spike glycoprotein (S). Insertion of the full-length S protein gene in two different MeV genomic positions resulted in modulated S protein expression. The variant with lower S protein expression levels was genetically stable and induced high levels of effective Th1-biased antibody and T cell responses in mice after two immunizations. In addition to neutralizing IgG antibody responses in a protective range, multifunctional $CD8^+$ and $CD4^+$ T cell responses with S protein-specific killing activity were detected. These results are highly encouraging and support further development of MeV-based COVID-19 vaccines. Significance: The COVID-19 pandemic has caused hundreds of thousands of deaths, yet. Therefore, effective vaccine concepts are urgently needed. In search for such a concept, we have analysed a measles virus-based vaccine candidate targeting SARS-CoV-2. Using this well known, safe vaccine backbone, we demonstrate here induction of functional immune responses in both arms of adaptive immunity with the desired immune bias. Therefore, occurrence of immunopathologies such as antibody-dependent enhancement or enhanced respiratory disease is rather unlikely. Moreover, the candidate still induces immunity against the measles, recognized as a looming second menace, when countries are entrapped to stop routine vaccination campaigns in the face of COVID-19. Thus, a bivalent measles-based COVID-19 vaccine could be the solution for two significant public health threats. [**note: way cool!!! These German researchers genetically engineer measles vaccine to also produce the SARS-CoV-2 Spike protein. It elicited antibodies in mice. Get two immunizations in one shot. Outstanding thinking outside the box!!**]
  https://www.biorxiv.org/content/10.1101/2020.07.11.198291v1
- While vaccine development will hopefully quell the global pandemic of COVID-19 caused by SARS-CoV-2, small molecule drugs that can effectively control SARS-CoV-2 infection are urgently needed. Here inhibitors of two coronavirus spike proteins (S) were identified by screening a library of approved drugs with SARS-S and MERS-S pseudotyped particle entry assays. Using high-throughput screening technology, we discovered three compounds (cepharanthine, abemaciclib and trimipramine) to be broad spectrum inhibitors for spike-mediated entry. This work should contribute to the development of effective treatments against the initial stage of viral infection, thus reducing viral burden in COVID-19 patients. [**note: another decent drug discovery paper. Two oncology drugs and a tricyclic antidepressant. Drugs from these two classes have come up in other papers.**]
  https://www.biorxiv.org/content/10.1101/2020.07.10.197988v1

- Tafenoquine [TQ] exhibited EC50/90s of approximately 2.6/5.1 microM against SARS-CoV-2 in VERO E6 cells and was 4-fold more potent than hydroxychloroquine [HCQ]. Time-of-addition experiments were consistent with a different mechanism for TQ v HCQ. Physiologically based pharmacokinetic (PBPK) modeling suggested that lung unbound concentrations of TQ in COVID-19 patients may exceed the EC90 for at least 8 weeks after administration. The therapeutic potential for TQ in management of COVID-19 should be further evaluated. [**note: this is an approved antimalarial drug with a very long half life. This is not even a new drug as this article notes. Fascinating history.**]
  https://www.biorxiv.org/content/10.1101/2020.07.12.199059v1

VIRUS BIOCHEMISTRY

- Angiotensin-converting enzyme 2 (ACE2) is a key receptor mediating the entry of SARS-CoV-2 into the host cell. Through a systematic analysis of publicly available mouse brain sc/snRNA-seq data, we found that ACE2 is specifically expressed in small sub-populations of endothelial cells and mural cells, namely pericytes and vascular smooth muscle cells. Further, functional changes in viral mRNA transcription and replication, and impaired blood-brain barrier regulation were most prominently implicated in the aged, ACE2-expressing endothelial cells, when compared to the young adult mouse brains. Concordant EC transcriptomic changes were further found in normal aged human brains. Overall, this work reveals an outline of ACE2 distribution in the mouse brain and identify putative brain host cells that may underlie the selective susceptibility of the aging brain to viral infection. [**note: this indicates how the virus might get into the brain in older individuals.**] https://www.biorxiv.org/content/10.1101/2020.07.11.198770v1

DIAGNOSTIC DEVELOPMENT

- With the COVID-19 pandemic surpassing 12M confirmed cases and 550K deaths worldwide, defining the key components of the immune response to SARS-CoV-2 infection is critical. Of particular importance is the identification of immune correlates of infection that would support public health decision-making on treatment approaches, vaccination strategies, and convalescent plasma therapy. While ELISA-based assays to detect and quantitate antibodies to SARS-CoV-2 in patient samples have been developed, the detection of neutralizing antibodies typically requires more demanding cell-based viral assays. Here, we present and validate a safe and efficient protein-based assay for the detection of serum and plasma antibodies that block the interaction of the SARS-CoV-2 spike (S) protein receptor binding domain (RBD) with its receptor, angiotensin converting-enzyme 2 (ACE2). This test is performed on the same platform and in parallel with an enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against the RBD and serves as a surrogate neutralization assay. [**note: here is a clever approach to surrogate assay for neutralizing antibodies that can be run in parallel with an ELISA**] https://www.biorxiv.org/content/10.1101/2020.07.10.197913v1

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2020-07-14

Let us have a listen to another pandemic recital from Wigmore Hall. Canadian pianist Angela Hewitt is an excellent pianist who has specialized in Bach both in recordings and recitals. One could do worse than listen to Bach though it sometimes brings back nightmarish memories of my music theory battles analyzing Bach chorales. I think my loyal listeners will enjoy this solo piano recital as much as I have: https://www.youtube.com/watch?v=tfFXCiUHOgU

US COVID-19 STATISTICS - **Infection Rate: 1.0%; CRF: 4.0%** (CRF unchanged)

There was an email exchange among loyal readers late yesterday regarding a post from this website that as far as I can see is run by a group of lawyers. The relevant quote –

> *Is there any reason why this cycle will not continue into 2021, 2022 and 2023? At the current pace, herd immunity will not be achieved for some years. I suppose those who love to control our lives, like Gavin Newsom, purport to be hoping for a vaccine. A safe and effective vaccine is possible, but not at all inevitable; certainly not in the next year. I think we need to seriously consider whether the best policy would be to stop slowing the spread of COVID so that we will suffer damage only from the disease, and not from the disease plus, in addition, from the arbitrary dictates of our governors.*

overlooks what is in the public health interest, something governors are charged with protecting. This was addressed in part by a piece in STAT this morning on 'how to fix the COVID-19 dumpster fire.' Ideally responses to this pandemic would be free of political invective but we do not live in an ideal world. All that I/we can do is continue focusing on the science and try to reduce morbidity and mortality to the best of our abilities. Anyone desiring to delve into the politics of COVID-19 can do so. For me, it is an endless sinkhole of know nothingism and I have better things to do with my time.

STAT also has a good article on how patient-reported data can help monitor the safety of fast track vaccines. This is useful but more needs to be done in terms of data capture that might inform future vaccine development work.

Here is another article on the need for vaccine glass vials from the Washington Post Staying with the Washington Post, here is a neat article on pandemic architecture. The is a push for getting new therapeutic for COVID-19 approved quickly by the FDA. Janet Woodcock also noted that plasma from convalescent patients are needed. For the former PHS readers, some former CDC directors have a good op-ed on the current state of affairs. And finally from the Post and article comparing the current COVID-19 vaccine effort to the search for an HIV vaccine. Let us hope this effort is successful.

New York Times health columnist, Jane Brody, discusses smart COVID-19 testing. Here is a first person account of battle against the virus.

For the former Pfizer folks on the email list, Derek Lowe has a column on the company.

MODELING

- This pandemic overlaps with the ongoing epidemics of cigarette smoking and electronic cigarette (e-cig) vaping, with over 1 billion smokers and vapers worldwide. However, there is

scarce data relating COVID-19 risks and outcome with cigarette or e-cig use. In this study, we mined 3 independent RNA expression datasets from smokers and vapers to understand the potential relationship between vaping/smoking and the dysregulation of key genes and pathways related to COVID-19. We found that smoking, but not vaping, upregulates ACE2, the cellular receptor that SARS-CoV-2 requires for infection. Both smoking and use of nicotine and flavor-containing e-cig led to upregulations of pro-inflammatory cytokine production and expression of genes related to inflammasomes. Vaping flavor-less and nicotine-less e-cig, however, did not lead to significant cytokine dysregulation and inflammasome activation. Release of inflammasome products, such as IL-1B, and cytokine storms are hallmarks of COVID-19 infection, especially in severe cases. Therefore, our findings demonstrated that smoking or vaping, specifically use of flavored or nicotine-containing e-cigs, may critically exacerbate COVID-19-related inflammation or increase susceptibility to the disease. Further scientific and public health investigations should be undertaken to address these concerning links between COVID-19 and e-cig/smoking. [**note; Save money; don't smoke or vape**]
https://www.biorxiv.org/content/10.1101/2020.07.13.198630v1

NEWLY REGISTERED CLINICAL TRIALS

- The overall objective of this project is to develop an emergent treatment protocol using adoptive T-cell therapy for the treatment of severe COVID-19. The central hypothesis is that SARS-CoV-2 specific T cells from convalescent donors who have recovered from COVID-19 can be manufactured expeditiously and these cells are safe and effective for the treatment of severe SARS-CoV-2 infections. [**note: this is a Singapore trial that refines the use of convalescent blood looking at SARS-CoV-2 specific T cells.**] NCT04457726
- Coronavirus Disease 19 (COVID-19) is a global pandemic caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Severe disease occurs in 15% of the cases with COVID-19 and may progress to critical disease in only 5% of the cases with a high risk of mortality. Critical disease may present as acute respiratory failure secondary to Acute Respiratory Distress Syndrome (ARDS) and is caused by the body's hyper-immune response to the virus in the form of a cytokine storm syndrome (CSS). There is currently no effective anti-viral treatment against SARS-CoV-2 and the mainstay of treatment is supportive. Co-trimoxazole (combination of trimethoprim and sulphamethoxazole in a 1:5) ratio is a Sulphur containing anti-folate bactericidal antibiotic indicated for the treatment of respiratory tract infections. It has been around for over 60 years and is inexpensive and readily available with a good safety profile. It has a rapid onset of action with excellent bioavailability and lung penetration. In addition to having antimicrobial properties co-trimoxazole have immunomodulatory and anti-inflammatory properties and may be a potential treatment option for cytokine storm syndrome mediated severe COVID-19. [**note: this is a Bangladesh trial of two old antibiotics. I was unaware of the immunomodulatory properties of the drug pair.**] NCT04470531
- Patients will be randomized to receive Enzalutamide with standard of care (SOC) or SOC alone. Enzalutamide will be administered daily p.o. from Day 1 to Day 28 or until confirmed negativization of Nasopharyngeal swap (NPS) Polymerase chain reaction (PCR) (2 consecutive negative samples), whichever occurs first. High risk outpatient adult males with a confirmed SARS-CoV-2 infection will be included in the study. [**note: this is another approach to treat**

males who have more severe outcomes than females. There are ongoing trials with other sex related hormone therapy.] NCT04456049

- To assess the safety and efficacy of CK0802 in treatment of patients with COVID-19 induced moderate-to-severe PNA-ARDS. **[note: this is a selective T cell therapy sponsored by Cellenkos, Inc.**] NCT04468971
- Inflammatory diseases favour the onset of venous thromboembolic events in hospitalized patients. Thromboprophylaxis with a fixed dose of heparin/low molecular weight heparin (LMWH) is recommended if concomitant inflammatory disease. In severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) pneumonia an inflammation-dependent thrombotic process occurs and platelet activation may promote thrombosis and amplify inflammation, as indicated by previous experimental evidence , and the similarities with atherothrombosis and thrombotic microangiopathies. Antiplatelet agents represent the cornerstone in the prevention and treatment of atherosclerotic arterial thromboembolism, with limited efficacy in the context of venous thromboembolism. The use of purinergic receptor P2Y12 inhibitors in pneumococcal pneumonia may improve inflammation and respiratory function in humans. There are no validated protocols for thrombosis prevention in Covid-19. There is scientific rationale to consider a P2Y12 inhibitor for the prevention of thrombosis in the pulmonary circulation and attenuation of inflammation. This is supported by numerous demonstrations of the anti-inflammatory activity of P2Y12 inhibitors and the evidence of improvement in respiratory function both in human and experimental pathology. Prasugrel could be considered as an ideal candidate drug for Covid-19 patients because of higher efficacy and limited Interactions with drugs used in the treatment of Sars-CoV2. The hypothesis underlying the present study project is that in Covid-19 platelet activation occurs through an inflammation-dependent mechanism and that early antithrombotic prophylaxis in non-critical patients could reduce the incidence of pulmonary thrombosis and respiratory and multi-organ failure improving clinical outcome in patients with SARS-CoV2 pneumonia. The prevention of thrombogenic platelet activity with a P2Y12 inhibitor could be superior to fixed dose enoxaparin alone. The proposed treatment is feasible in all coronavirus disease 2019 (COVID-19) patients, regardless of the treatment regimen (antivirals, anti-inflammatory drugs, antibiotics), except for specific contraindications. [**note: another blood thinning drug to combat blood clots in sever COVID-19.**] NCT04445623
- This is a randomized, randomized controlled trial to investigate the efficacy and safety of Neurokinin-1 Receptor (NK-1R) 80 mg orally given daily to treat cytokine storm causing inflammatory lung injury and respiratory failure associated with severe or critical COVID-19 infection. NK-1R is the receptor of Substance P (SP) and responsible for its functionality. Here, we propose that SP via its tachykinin receptor, NK-1R may cause inflammation in Covid-19 infection. It may initiate the cytokine storming via binding to its receptor NK-1 and many inflammatory mediators are released. If SP release is reduced by NK-1R antagonist, it may control the cytokine storming and hence the hyper-responsiveness of the respiratory tract through reduction in cytokine storming It may serve as the treatment strategy for Covid-19 infected patients. [**note: this is a Pakastani trial!**] NCT04468646

CLINICAL TRIAL RESULTS

- For yet unknown reasons, severely ill COVID-19 patients often become critically ill around the time of activation of adaptive immunity. Here, we show that anti-Spike IgG from serum of

severely ill COVID-19 patients induces a hyper-inflammatory response by human macrophages, which subsequently breaks pulmonary endothelial barrier integrity and induces microvascular thrombosis. The excessive inflammatory capacity of this anti-Spike IgG is related to glycosylation changes in the IgG Fc tail. Moreover, the hyper-inflammatory response induced by anti-Spike IgG can be specifically counteracted in vitro by use of the active component of fostamatinib, an FDA- and EMA-approved therapeutic small molecule inhibitor of Syk. [**note: this is an interesting immunological finding. It's also the first mention of this drug though there have been other tyrosine kinase inhibitors come up in possible therapies.**] https://www.biorxiv.org/content/10.1101/2020.07.13.190140v1

DRUG DEVELOPMENT

- In an effort to interfere with the biology of SARS-CoV-2, the virus responsible for the COVID-19 pandemic, we focused on restoring the transcriptional response induced by infection. Utilizing expression patterns of SARS-CoV-2-infected cells, we identified a region in gene expression space that was unique to virus infection and inversely proportional to the transcriptional footprint of known compounds characterized in the Library of Integrated Network-based Cellular Signatures. Here we demonstrate the successful identification of compounds that display efficacy in blocking SARS-CoV-2 replication based on their ability to counteract the virus-induced transcriptional landscape. These compounds were found to potently reduce viral load despite having no impact on viral entry or modulation of the host antiviral response in the absence of virus. RNA-Seq profiling implicated the induction of the cholesterol biosynthesis pathway as the underlying mechanism of inhibition and suggested that targeting this aspect of host biology may significantly reduce SARS-CoV-2 viral load. [**note: another area to target drug therapy.**] https://www.biorxiv.org/content/10.1101/2020.07.12.199687v1
- PT150 is a clinical stage molecule, taken orally, with a strong safety profile having completed Phase 1 and Phase 2 clinical trials for its original use as an anti-depressant. It has an active IND for COVID-19. Antiviral activities have been found for PT150 and other members of its class in a variety of virus families; thus, it was now tested against SARS-CoV-2 in human bronchial epithelial lining cells and showed effective 90% inhibitory antiviral concentration (EC90) of 5.55 µM. PT150 is a member of an extended platform of novel glucocorticoid receptor (GR) and androgen receptor (AR) binding molecules. In vivo, their predominant net effect is one of systemic glucocorticoid antagonism, but they also show direct downregulation of AR and minor GR agonism at the cellular level. We hypothesize that anti-SARS-CoV-2 activity depends in part on this AR downregulation through diminished TMPRSS2 expression and modulation of ACE2 activity. Given that hypercortisolemia is now suggested to be a significant co-factor for COVID-19 progression, we also postulate an additive role for its potent immunomodulatory effects through systemic antagonism of cortisol. [**note: I don't know what the structure of this compound is. It is not shown in the paper and the link to the company product page does not exist.**] https://www.biorxiv.org/content/10.1101/2020.07.12.199505v1

VIRUS BIOCHEMISTRY

- Nothing new

DIAGNOSTIC DEVELOPMENT

- Nothing new

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2020-07-15

Let's get back to country!! Tennessee Waltz is just plain wonderful. Most people think it was written for Patti Paige but they would be wrong. Lots of singers have covered this and this rendition by Bonnie Raitt and Norah Jones is fine despite the screwed up video: https://www.youtube.com/watch?v=zzDUi_L6MzA Paige's original 1950 version is here: https://www.youtube.com/watch?v=5c-rVoBdZDE

US COVID-19 STATISTICS - **Infection Rate: 1.0%; CFR: 3.9%** (CFR down 0.1%)

Merck CEO Ken Frazier weighs in on COVID-19 vaccine development (disclosure: I am a Merck shareholder). Do listen to the sound clips.

The Atlantic has an excellent story on herd immunity . Everyone ought to read James Hamblin's piece which also has links to some interesting papers. There have been a number of modelers who have been looking at the pandemic using chaos theory principles and examining it as a heterogenous event. Herd immunity for SARS-CoV-2 infections might range from 20 to 60% though I have not seen many papers arguing for the lower number. For my own calculations I have been using the higher number. Having a lower number for herd immunity does not imply that geographical regions should just suck it up and let the virus do its think. This approach would lead to infections far above the 'true' herd immunity number as chances increase that everyone becomes infected. Alas, the herd immunity concepts are not easily boiled down into sound bites. I think it is good to have experts from diverse fields coming up with new models for the spread of infectious diseases. We will learn something from this effort.

The New York Times is the first to report on a move to shift hospital reporting of COVID-19 hospital information away from the CDC to a new central database in Washington DC. The COVID-19 Task Force announcement is HERE. Are you Doomscrolling again? This article may help. Until I read this article, I had no clue that this was even a word much less a psychological disturbance. The Times also asks whether a nasal vaccine will perform better than the traditional shot (I'm still partial to the Canadian 'yogurt-like' oral vaccine though it has not started clinical trials.). Science writer Donald McNeil has a good article on pandemic history; recommended reading.

Kaiser Health News dispels the conspiracy theories (I hope!!) about what contact tracers really do. I still do not see a big effort in this area which is too bad. The site also has a good article on how you can visit friends and relatives safely.

JAMA has a short viewpoint article on airborne transmissions of SARS-CoV-2. Perhaps more importantly is this viewpoint on communicating science in the time of a pandemic.

STAT notes the US may be the accidental Sweden in it's response to SARS-CoV-2.

Here is a link to an interesting preprint on dust cleaning materials for use in face masks. They use the material in Swiffer dusters!! (disclosure: I am a shareholder in Procter & Gamble and encourage everyone of my readers to use a Swiffer for everyday cleaning.)

Wrapping up the news for today is a Washington Post story on why bats have so many viruses.

MODELING

- Forecasting models have provided timely and critical information about the course of the COVID-19 pandemic, predicting both the timing of peak mortality, and the total magnitude of mortality, which can guide health system response and resource allocation. Out-of-sample predictive validation--checking how well past versions of forecasting models predict subsequently observed trends--provides insight into future model performance. As data and models are updated regularly, a publicly available, transparent, and reproducible framework is needed to evaluate them in an ongoing manner. We reviewed 384 published and unpublished COVID-19 forecasting models, and evaluated seven models for which publicly available, multi-country, and date-versioned mortality estimates could be downloaded. These included those modeled by: DELPHI-MIT (Delphi), Youyang Gu (YYG), the Los Alamos National Laboratory (LANL), Imperial College London (Imperial), and three models produced by the Institute for Health Metrics and Evaluation (IHME), a curve fit model (IHME-CF), a hybrid curve fit and epidemiological compartment model (IHME-CF SEIR), and a hybrid mortality spline and epidemiological compartment model (IHME - MS SEIR). Collectively models covered 171 countries, as well as the 50 states of the United States, and Washington, D.C., and accounted for >99% of all reported COVID-19 deaths on July 11th, 2020. As expected, errors in mortality predictions increased with a larger number of weeks of extrapolation. For the most recent models, released in June, at four weeks of forecasting the best performing model was the IHME-MS SEIR model, with a cumulative median absolute percent error of 6.4%, followed by YYG (6.5%) and LANL (8.0%). Looking across models, errors in cumulative mortality predictions were highest in sub-Saharan Africa and lowest in high-income countries, reflecting differences in data availability and prediction difficulty in earlier vs. later stages of the epidemic. For peak timing prediction, among models released in April, median absolute error values at six weeks ranged from 23 days for the IHME-CF model to 36 days for the YYG model. In sum, we provide a publicly available dataset and evaluation framework for assessing the predictive validity of COVID-19 mortality forecasts. We find substantial variation in predictive performance between models, and note large differences in average predictive validity between regions, highlighting priority areas for further study in sub-Saharan Africa and other emerging-epidemic contexts. [**note: this comes from the group at University of Washington and is a useful comparison**]
  https://www.medrxiv.org/content/10.1101/2020.07.13.20151233v1
- As of 1st June 2020, the US Centers for Disease Control and Prevention reported 104,232 confirmed or probable COVID-19-related deaths in the US. This was more than twice the number of deaths reported in the next most severely impacted country. We jointly modelled the US epidemic at the state-level, using publicly available death data within a Bayesian hierarchical semi-mechanistic framework. For each state, we estimate the number of individuals that have been infected, the number of individuals that are currently infectious and the time-varying reproduction number (the average number of secondary infections caused by an infected person). We used changes in mobility to capture the impact that non-pharmaceutical interventions and other behaviour changes have on the rate of transmission of SARS-CoV-2. Nationally, we estimated 3.7% [3.4%-4.0%] of the population had been infected by 1st June 2020, with wide variation between states, and approximately 0.01% of the population was

infectious. We also demonstrated that good model forecasts of deaths for the next 3 weeks with low error and good coverage of our credible intervals. [**note: this is a model from the Imperial College group. They estimate that the infection level as of June 1 was 3.7%. they number I give every day is based on reported infections and there is no extrapolation using undiagnosed cases.**] https://www.medrxiv.org/content/10.1101/2020.07.13.20152355v1

- To determine the effectiveness of non-medical grade washable masks or face coverings in controlling airborne dispersion from exhalation (both droplet and aerosol), and to aid in establishing public health strategies on the wearing of masks to reduce COVID-19 transmission. Design: This comparative effectiveness study using an exhalation simulator to conduct 94 experiment runs with combinations of 8 different fabrics, 5 mask designs, and airflows for both talking and coughing. Setting: Non-airtight fume hood and multiple laser scattering particle sensors. Participants: No human participants. Exposure: 10% NaCl nebulized solution delivered by an exhalation simulator through various masks and fabrics with exhalation airflows representative of "coughing" and "talking or singing." Main Outcomes and Measures: The primary outcome was reduction in aerosol dispersion velocity, quantity of particles, and change in dispersion direction. Measurements used in this study included peak expiratory flow (PEF), aerosol velocity, concentration area under curve (AUC), and two novel metrics of expiratory flow dispersion factor (EDF) and filtration efficiency indicator (FEI). Results: Three-way multivariate analysis of variance establishes that factors of fabric, mask design, and exhalation breath level have a statistically significant effect on changing direction, reducing velocity or concentration (Fabric: P = < .001, Wilks' Λ = .000; Mask design: P = < .001, Wilks' Λ = .000; Breath level: P = < .001, Wilks' Λ = .004). There were also statistically significant interaction effects between combinations of all primary factors. Conclusions and Relevance: The application of facial coverings or masks can significantly reduce the airborne dispersion of aerosolized particles from exhalation. The results show that wearing of non-medical grade washable masks or face coverings can help increase the effectiveness of non-pharmaceutical interventions (NPI) especially where infectious contaminants may exist in shared air spaces. However, the effectiveness varies greatly between the specific fabrics and mask designs used. [**note: this is a sound study of various types of non-medical grade masks.**
https://www.medrxiv.org/content/10.1101/2020.07.12.20152157v1
- Comparisons of the utility and accuracy of methods for measuring social interactions relevant to disease transmission are rare. To increase the evidence base supporting specific methods to measure social interaction, we compared data from self-reported contact surveys and wearable proximity sensors from a cohort of schoolchildren in the Pittsburgh metropolitan area. Although the number and type of contacts recorded by each participant differed between the two methods, we found good correspondence between the two methods in aggregate measures of age-specific interactions. Fewer, but longer, contacts were reported in surveys, relative to the generally short proximal interactions captured by wearable sensors. When adjusted for expectations of proportionate mixing, though, the two methods produced highly similar, assortative age-mixing matrices. These aggregate mixing matrices, when used in simulation, resulted in similar estimates of risk of infection by age. While proximity sensors and survey methods may not be interchangeable for capturing individual contacts, they can generate highly correlated data on age-specific mixing patterns relevant to the dynamics of respiratory virus transmission. [**note: the study compares self reported interaction with data from wearable**

proximity sensors in a cohort of Pittsburgh school children.]
https://www.medrxiv.org/content/10.1101/2020.07.12.20151696v1

- Most respiratory viruses show pronounced seasonality, but for SARS-CoV-2 this still needs to be documented. Methods We examined the disease progression of COVID-19 in 6,911 patients admitted to hospitals in Europe and China. In addition, we evaluated progress of disease symptoms in 37,187 individuals reporting symptoms into the COVID Symptom Study application. Findings Meta-analysis of the mortality risk in eight European hospitals estimated odds ratios per one day increase in the admission date to be 0.981 (0.973-0.988, p<0.001) and per increase in ambient temperature of one degree Celsius to be 0.854 (0.773-0.944, p=0.007). Statistically significant decreases of comparable magnitude in median hospital stay, probability of transfer to Intensive Care Unit and need for mechanical ventilation were also observed in most, but not all hospitals. The analysis of individually reported symptoms of 37,187 individuals in the UK also showed the decrease in symptom duration and disease severity with time. Interpretation *Severity of COVID-19 in Europe decreased significantly between March and May and the seasonality of COVID-19 is the most likely explanation. Mucosal barrier and mucociliary clearance can significantly decrease viral load and disease progression, and their inactivation by low relative humidity of indoor air might significantly contribute to severity of the disease*. [**note: data comes from a large number of hospitals in Europe and China looking for an environmental association with morbidity and mortality.**]
https://www.medrxiv.org/content/10.1101/2020.07.11.20147157v1

NEWLY REGISTERED CLINICAL TRIALS

- Did not check today.

CLINICAL TRIAL RESULTS

- Cardiac sequelae of past SARS-CoV-2 infection are still poorly documented. We conducted a cross-sectional study in health-care workers to report evidence of pericarditis and myocarditis after SARS-CoV-2 infection. Methods We studied 139 health-care workers with confirmed past SARS-CoV-2 infection (103 diagnosed by RT-PCR and 36 by serology). Participants underwent clinical assessment, electrocardiography, laboratory tests including immune cell profiling and cardiac magnetic resonance (CMR) imaging. Pericarditis was diagnosed when classical criteria were present, and the diagnosis of myocarditis was based on the updated CMR Lake-Louise-Criteria. Results: Median age was 52 years (IQR 41-57), 100 (72%) were women, and 23 (16%) were previously hospitalized for Covid-19 pneumonia. At examination (10.4 [9.3-11.0] weeks after infection-like symptoms), all participants presented hemodynamic stability. Chest pain, dyspnoea or palpitations were observed in 58 (42%) participants; electrocardiographic abnormalities in 69 (50%); NT-pro-BNP was elevated in 11 (8%); troponin in 1 (1%); and CMR abnormalities in 104 (75%). Isolated pericarditis was diagnosed in 4 (3%) participants, myopericarditis in 15 (11%) and isolated myocarditis in 36 (26%). Participants diagnosed by RT-PCR were more likely to still present symptoms than participants diagnosed by serology (73 [71%] vs 18 [50%]; p=0.027); nonetheless, the prevalence of pericarditis or myocarditis was high in both groups (44 [43%] vs 11 [31%]; p=0.238). Most participants (101 [73%]) showed altered immune cell counts in blood, particularly decreased eosinophil (37 [27%]; p<0.001) and increased CD4-CD8-/loT alpha beta-cell numbers (24 [17%]; p<0.001). Pericarditis was

associated with elevated CD4-CD8-/loT alpha beta-cell numbers (p=0.011), while participants diagnosed with myopericarditis or myocarditis had lower (p<0.05) plasmacytoid dendritic cell, NK-cell and plasma cell counts and lower anti-SARS-CoV-2-IgG antibody levels (p=0.027). Conclusions: Pericarditis and myocarditis with clinical stability are frequent long after SARS-CoV-2 infection, even in presently asymptomatic subjects. These observations will probably apply to the general population infected and may indicate that cardiac sequelae might occur late in association with an altered (delayed) innate and adaptative immune response. [**note: this highlights some health issues that may emerge after ingection diagnosis. Don't assume that silent infections are necessarily always going to be symptom free!**] https://www.medrxiv.org/content/10.1101/2020.07.12.20151316v1

- Clinical manifestations of COVID-19 caused by the novel coronavirus SARS-CoV-2 are associated with age. While children are largely spared from severe respiratory disease, they can present with a SARS-CoV-2-associated multisystem inflammatory syndrome (MIS-C) similar to Kawasaki's disease. Here, we show distinct antibody (Ab) responses in children with MIS-C compared to adults with severe COVID-19 causing acute respiratory distress syndrome (ARDS), and those who recovered from mild disease. There was a reduced breadth and specificity of anti-SARS-CoV-2-specific antibodies in MIS-C patients compared to the COVID patient groups; MIS-C predominantly generated IgG Abs specific for the Spike (S) protein but not for the nucleocapsid (N) protein, while both COVID-19 cohorts had anti-S IgG, IgM and IgA Abs, as well as anti-N IgG Abs. Moreover, MIS-C patients had reduced neutralizing activity compared to COVID-19 cohorts, indicating a reduced protective serological response. These results suggest a distinct infection course and immune response in children and adults who develop severe disease, with implications for optimizing treatments based on symptom and age. [**note: this is from Columbia University and shows the differential antibody responses in children compared with adults having COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.07.12.20151068v1
- The retrospective analysis of clinical data of patients suffering from COVID-19 has indicated that statin therapy, used to lower plasma cholesterol levels, is associated with a better clinical outcome. We therefore investigated the effect of statins on SARS-CoV-2 infection and found that selective statins reduced SARS-CoV-2 cell entry and inhibited high and low pathogenic coronavirus infection in human cells. A retrospective study on hospitalized patients with COVID-19 implies that reduced high density lipoprotein levels, which are typically counteracted by statin therapy, are associated with aggravated disease outcome. These results suggest that statin therapy poses no additional risk to individuals exposed to SARS-CoV-2 and that some statins may have a mild beneficial effect on COVID-19 outcome. [**note: this is a combination of a very small observational study and *in vitro* data. I discount the second part of this as lots of things have displayed inhibitory activity in such studies. I know that OHDSI has a large observational study planned on the whole class of drugs but I've not seen anything here.**] https://www.medrxiv.org/content/10.1101/2020.07.13.20152272v1
- There is a concern that proton pump inhibitors (PPI) induced hypochlorhydria could potentially predispose to severe COVID-19. Methods We studied the association between prehospitalization PPI use and clinical outcomes among hospitalized COVID-19 patients. Results In our study, 15.6% of hospitalized COVID-19 patients were on PPI. Mortality among PPI-users was 2.3 times higher than non-users, along with 2.5 times higher risk of mechanical ventilation. This relationship existed even after adjusting for confounding variables. Discussion These results

warrant further investigation to evaluate if PPI-induced hypochlorhydria is associated with a higher risk of GI symptoms and worse outcomes because of the omnipresence of ACE-2 in the gastrointestinal tract. [**note: if you are taking PPIs don't get COVID-19! The patient size is small as it is only a single center study. Switch everyone to famotidine!!!**] https://www.medrxiv.org/content/10.1101/2020.07.12.20151084v1

- The novel coronavirus disease 2019 (COVID-19) has rapidly spread across the globe, overwhelming healthcare systems and depleting resources. The infection has a wide spectrum of presentations, and pre-existing comorbidities have been found to have a dramatic effect on the disease course and prognosis. We sought to analyze the effect of asthma on the disease progression and outcomes of COVID-19 patients. Methods We conducted a multi-center retrospective study of positively confirmed COVID-19 patients from multiple hospitals in Louisiana. Demographics, medical history, comorbidities, clinical presentation, daily laboratory values, complications, and outcomes data were collected and analyzed. The primary outcome of interest was in-hospital mortality. Secondary outcomes were Intensive Care Unit (ICU) admission, risk of intubation, duration of mechanical ventilation, and length of hospital stay. Results A total of 502 COVID-19 patients (72 asthma and 430 non-asthma cohorts) were included in the study. The frequency of asthma in hospitalized cohorts was 14.3%, higher than the national prevalence of asthma (7.7%). Univariate analysis revealed that asthma patients were more likely to be obese (75% vs 54.2%, p=0.001), with higher frequency of intubation (40.3% vs 27.8%, p = 0.036), and required longer duration of hospitalization (15.1±12.5 vs 11.5±10.6, p=0.015). After adjustment, multivariable analysis showed that asthmatic patients were not associated with higher risk of ICU admission (OR=1.81, 95%CI=0.98-3.09, p=0.06), endotracheal intubation (OR=1.77, 95%CI=0.99-3.04, p=0.06) or complications (OR=1.37, 95%CI=0.82-2.31, p=0.23). Asthmatic patients were not associated with higher odds of prolonged hospital length of stay (OR=1.48, 95%CI=0.82-2.66, p=0.20) or with the duration of ICU stay (OR=0.76, 95%CI=0.28-2.02, p=0.58). Kaplan-Meier curve showed no significant difference in overall survival of the two groups (p=0.65). Conclusion Despite the increased prevalence of hospitalization in asthmatic COVID-19 patients compared to the general population, after adjustment for other variables, it was neither associated with increased severity nor worse outcomes. [**note: this is good news for asthmatics. These Tulane researchers did not see increased severity or outcomes in asthmatic patients that contracted COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.07.13.20153130v1

DRUG DEVELOPMENT

- We investigated the ability of Luminore CopperTouch™ copper and copper–nickel surfaces to inactivate filoviruses and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). For this purpose, we compared viral titers in Vero cells from viral droplets exposed to copper surfaces for 30 min. The copper and copper–nickel surfaces inactivated 99.9% of the viral titer of both Ebola and Marburg viruses. The copper surfaces also inactivated 99% of SARS-CoV-2 titers in 2 hours to close to the limit of detection. These data add Ebolavirus, Marburgvirus, and SARS-CoV-2 (COVID-19) to the list of pathogens that can be inactivated by exposure to copper ions, validating Luminore CopperTouch™ technology (currently the only Environmental Protection Agency [EPA]-registered cold spray antimicrobial surface technology) as an efficacious, cost-friendly tool to improve infection control in hospitals, long-term care facilities, schools, hotels,

buses, trains, airports, and other highly trafficked areas. [**note: this is really an environmental control agent (see the link). Loyal readers can chip in and purchase a nice copper/nickel mouse for me as I'm spending so much more time at the computer these days doing COVID-19 curator work!]** https://www.medrxiv.org/content/10.1101/2020.07.05.20146043v1

- Guess what? Moderna publishes the results of their Phase 1 mRNA vaccine - https://www.nejm.org/doi/full/10.1056/NEJMoa2022483 There is still no word as far as I know from FDA about a Phase 2/3 study. Here is an accompanying editorial.
- Neutralization antibodies and vaccines for treating COVID-19 are desperately needed. For precise development of antibodies and vaccines, the key is to understand which part of SARS-CoV-2 Spike protein is highly immunogenic on a systematic way. We generate a linear epitope landscape of Spike protein by analyzing serum IgG response of 1,051 COVID-19 patients with a peptide microarray. We reveal two regions that rich of linear epitopes, i.e., CTD and a region close to the S2' cleavage site and fusion peptide. Unexpectedly, we find RBD is lack of linear epitope. Besides 3 moderate immunogenic peptides from RBD, 16 highly immunogenic peptides from other regions of Spike protein are determined. These peptides could serve as the base for precise development of antibodies and vaccines for COVID-19 on a systematic level. [**note: interesting research from China about specific domains that can serve as antibody and vaccine development targets.]** https://www.medrxiv.org/content/10.1101/2020.07.13.20152587v1
- Neutralizing antibodies targeting the receptor-binding domain (RBD) of the SARS-CoV-2 spike (S) block severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) entry into cells using surface-expressed angiotensin-converting enzyme 2 (ACE2). We developed a surrogate neutralization test (sVNT) to assess at what degree serum antibodies interfere with the binding of SARS-CoV-2-S-RBD to ACE2. The sVNT revealed neutralizing anti-SARS-CoV-2-S antibodies in the sera of 90% of mildly and 100% of severely affected coronavirus-disease-2019 (COVID-19) convalescent patients. Importantly, sVNT results correlated strongly to the results from pseudotyped-vesicular stomatitis virus-vector-based neutralization assay and to levels of anti-SARS-CoV-2-S1 IgG and IgA antibodies. Moreover, levels of neutralizing antibodies also correlated to duration and severity of clinical symptoms, but not patient age or gender. These findings together with the sVNT will not only be important for evaluating the prevalence of neutralizing antibodies in a population but also for identifying promising plasma donors for successful passive antibody therapy. [**note: this German paper outlines an approach for a new test for neutralizing antibodies in the convalescent sera. It might be used to screen donors plasma.**] https://www.medrxiv.org/content/10.1101/2020.07.12.20151407v1

VIRUS BIOCHEMISTRY

- Nothing today

DIAGNOSTIC DEVELOPMENT

- The Lancet has a commentary on rapid genome sequencing of COVID-19 clinical samples and for those interested in pool testing of SARS-CoV-2 samples there are several correspondences: HERE, HERE, & HERE.

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2020-07-16

I am going to stay with country for a day or two more. Country music is evocative of many moods, having its roots in a number of sectors within the United States. I mentioned Ken Burns fine PBS documentary about this genre a number weeks ago and it is worth watching if you haven't. Here is a song of remembrance by Vince Gill, "Go Rest High on That Mountain" accompanied by Alison Krauss and Ricky Skaggs: https://www.youtube.com/watch?v=NwFiWCUkk4M

US COVID-19 STATISTICS - **Infection Rate: 1.1%; CFR: 3.9%** (IR up 0.1%; CFR unchanged)

NOTE TO READERS: I am going to shorten the news discussion portion of the newsletter while posting links to articles. I assume most of you read the above fold highlights and click on the links for more information. This will include the newsfeeds and medical journal general articles. This helps me focus more time on preprints.

For the school teachers and parents of school teachers who are loyal readers – The New York Times has a story on a National Academy of Sciences report on school reopening urging younger children and those with special needs should attend school in person. The full report is HERE.

Ed Yong on what happens if there is a 2nd pandemic. I surely hope not! Staying with The Atlantic, an interview with Tony Fauci.

I mentioned the other day an effort by various performing arts groups to look at aerosol formation via vocal production and wind instruments. Here is the first draft paper on the topic. Lots of good diagrams that will help inform us of risks. Good reading for musicians and singers!

I don't want to besieged my readers with lots of regional news on COVID-19 outbreaks, but this cautionary tale about what is happening in Washington state is worthwhile as it was the first epicenter of the disease in the US. Some New York Times writers are able to get away for vacations!

The Washington Post covers recent announcements by big retailers who will begin requiring the wearing of masks when shopping. From the 'Know-Nothing' side of the aisle, Georgia Governor, Brian Kemp, signs an executive order banning cities in the state from enacting their own mask mandates.

Derek Lowe on the Moderna vaccine data paper and on the Nature paper regarding T cells that I link to below.

The British Medical Journal (BMJ) as a cautionary opinion piece on vaccines and antibody therapies for COVID-19.

JAMA have several interesting papers. The current data on droplet and aerosol transmission is discussed HERE; the authors note that *currently available evidence suggests that long-range aerosol-based transmission is not the dominant mode of SARS-CoV-2 transmission*. Universal masking of healthcare workers at the Massachusetts General Brigham hospital system (>75,000 employees) suggest a decrease in SARS-CoV-2 positivity rate. An editorial urging universal masking from CDC is also on line (it has not escaped my attention that CDC is located in Georgia whose governor does not want to take this step). An original paper on factors associated with death in critically ill US patients with COVID-19 is available. It looks at 2215 patients from 65 sites. A larger cohort study from the Lombardy region in Italy is a useful comparison read.

The Lancet offers a good panel checklist to assess national performance in response to COVID-19. It won't surprise any of my readers that "…communities must have the capacity to detect cases early and interrupt transmission chains. This capacity requires a strong community-based public health system that adjusts its functioning according to locally disaggregated data about the whereabouts of the virus and the effectiveness of the response…" This is mandatory reading for next week's monthly test 😉

Wrapping of the news of the day, STAT has a story on the use of low-dose radiation as a treatment for COVID-19. I have not focused on this but have seen several registered clinical trials for this approach. The US needs to invest $75 billion in order to fix the flawed system of diagnostic testing for COVID-19. The full Rockefeller Foundation report is HERE; this is another paper worth reading!

Lots of reading today including an observational study showing the lack of usefulness of chlorpromazine as a therapeutic and some biochemical studies on the mode of action of famotidine (they couldn't identify and direct drug target).

MODELING

- COVID-19 containment efforts in the United States so far have largely focused on physical distancing, including school and workplace closures. However, these interventions have come at an enormous societal and economic cost. Here, we use an agent-based model, calibrated to detailed demographic, mobility, and epidemiological data for the Seattle region, to investigate the feasibility of alternative control strategies, focusing on "test-trace-quarantine": a combination of (a) routine testing of primarily symptomatic individuals, (b) tracing and testing their known contacts, and (c) placing their contacts in quarantine. We assess the requirements for implementing this strategy, including its robustness to low compliance, delays, and other factors such as variability in overall transmission rates. *We find that for the Seattle setting, if mask compliance remains high and schools remain closed, realistic levels of testing and tracing are sufficient to maintain epidemic control under a return to full workplace and community mobility*. [**note: mask wearing coupled with testing and tracing can bring this virus under control. Well worth looking at this paper.**]
  https://www.medrxiv.org/content/10.1101/2020.07.15.20154765v1
- Covid-19 pandemic is the most critical challenge nowadays for the manhood, and the infection and death cases are still speedily increasing. Since there are no available vaccine and specifically effective treatment, to break the infectious way of the pandemic remains the unique measure to efficiently combat Covid-19 infection. Understanding factors that affect the Covid-19 infection can help make better balance between activity restriction and infection dynamics. This study sought to investigate association between Covid-19 infection and blood type distribution. Methods: The big data provided by World Health Organization and Johns Hopkins University were taken to assess epidemic dynamics of Covid-19 infection. Growth rate and doubling time of infection and death cases, reproductive number, infection and death cases in the mid-exponential phase were analyzed in relation to blood type distribution. Results: Growth rate of infection and death cases correlated significantly to blood type A proportion of the population positively while to blood type B proportion negatively. In comparison with lower blood type A population (< 30%) people with higher blood type A (≥ 30%) had more infection and death cases

in the early exponential phase, higher growth rates, and shorter case doubling time for infection and death. Discussion: Covid-19 infection is significantly associated with blood type distribution and people with blood type A are more susceptible to Covid-19 infection and have higher epidemic dynamics and higher case fatality rate. The results of this study provide important and useful information for fighting Covid-19 pandemic. [**note: more on the linkage between COVID-19 and blood type.**] https://www.medrxiv.org/content/10.1101/2020.07.12.20152074v1

- The province of British Columbia (BC) has been recognized for successful SARS-CoV-2 control, with surveillance data showing amongst the lowest case and death rates in Canada. We estimate sero-prevalence for two periods flanking the start (March) and end (May) of first-wave mitigation measures in BC. Methods: Serial cross-sectional sampling was conducted using anonymized residual sera obtained from an outpatient laboratory network, including children and adults in the Greater Vancouver Area (population ~3 million) where community attack rates were expected to be highest. Screening used two chemiluminescent immuno-assays for spike (S1) and nucleocapsid antibodies. Samples sero-positive on either screening assay were assessed by a third assay targeting the S1 receptor binding domain plus a neutralization assay. Age-standardized sero-prevalence estimates were based on dual-assay positivity. The May sero-prevalence estimate was extrapolated to the source population to assess surveillance under-ascertainment, quantified as the ratio of estimated infections versus reported cases. Results: Serum collection dates spanned March 5-13 and May 15-27, 2020. In March, two of 869 specimens were dual-assay positive, with age-standardized sero-prevalence of 0.28% (95%CI=0.03-0.95). Neither specimen had detectable neutralizing antibodies. In May, four of 885 specimens were dual-assay positive, with age-standardized sero-prevalence of 0.55% (95%CI=0.15-1.37%). All four specimens had detectable neutralizing antibodies. We estimate ~8 times more infections than reported cases. Conclusions: Less than 1% of British Columbians had been infected with SARS-CoV-2 when first-wave mitigation measures were relaxed in May 2020. Our findings indicate successful suppression of community transmission in BC, but also substantial residual susceptibility. Further sero-survey snapshots are planned as the pandemic unfolds. [**note: here is a serology study from British Columbia which has had good luck in minimizing SARS-CoV-2 infections. They note that probably less than 1% of the province has been infected leaving residual susceptibility.**]
https://www.medrxiv.org/content/10.1101/2020.07.13.20153148v1
- Uncertainty in the immune response to SARS-CoV-2 may have implications for future outbreaks. We use simple epidemiological models to explore estimates for the magnitude and timing of future Covid-19 cases given different impacts of the adaptive immune response to SARS-CoV-2 as well as its interaction with vaccines and nonpharmaceutical interventions. We find that variations in the immune response to primary SARS-CoV-2 infections and a potential vaccine can lead to dramatically different immunity landscapes and burdens of critically severe cases, ranging from sustained epidemics to near elimination. Our findings illustrate likely complexities in future Covid-19 dynamics, and highlight the importance of immunological characterization beyond the measurement of active infections for adequately characterizing the immune landscape generated by SARS-CoV-2 infections. [**note: this 'life-history and dynamics of SARS-CoV-2 over the next five years' comes from a Princeton University group. I'm not fond of five year projections particularly in the absence of full information which we do not have at present.**] https://www.medrxiv.org/content/10.1101/2020.07.15.20154401v1

NEWLY REGISTERED CLINICAL TRIALS

- Did not look today.

CLINICAL TRIAL RESULTS

- Hydroxychloroquine and chloroquine have been proposed as treatments for coronavirus disease 2019 (COVID-19) on the basis of in vitro activity, uncontrolled data, and small randomized studies. Methods: The Randomised Evaluation of COVID-19 therapy (RECOVERY) trial is a randomized, controlled, open-label, platform trial comparing a range of possible treatments with usual care in patients hospitalized with COVID-19. We report the preliminary results for the comparison of hydroxychloroquine vs. usual care alone. The primary outcome was 28-day mortality. Results: 1561 patients randomly allocated to receive hydroxychloroquine were compared with 3155 patients concurrently allocated to usual care. Overall, 418 (26.8%) patients allocated hydroxychloroquine and 788 (25.0%) patients allocated usual care died within 28 days (rate ratio 1.09; 95% confidence interval [CI] 0.96 to 1.23; P=0.18). Consistent results were seen in all pre-specified subgroups of patients. Patients allocated to hydroxychloroquine were less likely to be discharged from hospital alive within 28 days (60.3% vs. 62.8%; rate ratio 0.92; 95% CI 0.85-0.99) and those not on invasive mechanical ventilation at baseline were more likely to reach the composite endpoint of invasive mechanical ventilation or death (29.8% vs. 26.5%; risk ratio 1.12; 95% CI 1.01-1.25). There was no excess of new major cardiac arrhythmia. Conclusions: *In patients hospitalized with COVID-19, hydroxychloroquine was not associated with reductions in 28-day mortality but was associated with an increased length of hospital stay and increased risk of progressing to invasive mechanical ventilation or death*. [**note: this is the manuscript from the UK RECOVERY HCQ trial. Researchers issued a press statement several weeks ago on the results but had finished writing the paper. Are Zombies killed with a wooden stake or silver cross? I cannot remember but I hope this Zombie drug is now sent home!**] https://www.medrxiv.org/content/10.1101/2020.07.15.20151852v1
- The major clinical feature of severe COVID-19 requiring ventilation is acute Respiratory Distress Syndrome (ARDS) with multi-functional failure as a result of a cytokine storm with increased serum levels of cytokines such as TNF-α and IL-6 being reported. TNF-α levels are increased during the cytokine storm in very ill patients and soluble receptors for IL-6 and IL-2 are present in the blood of COVID-19 patients, Objectives: To elucidate the involvement of serum levels of soluble TNF-Receptor of severe and mild COVID-19 patients to determine for severity of disease. Method: We recruited 16 severe COVID-19 patients in the ICU on ventilator support and 26 milder COVID-19 patients who were hospitalised but not within the intensive care unit (ICU) between March-May 2020 at the Masih Daneshvari Hospital Tehran, Iran. After harvesting of whole blood the serum was isolated and soluble TNF-Receptor levels measured by ELISA. Results: *Serum levels of the usually inhibitory soluble TNFα receptor 1 (sTNFaR1) were significantly elevated in severe COVID-19 patients at admission to ICU. High serum levels of sTNFaR1 were associated with mortality of severe COVID-19 patients treated within ICU.* Conclusions: This pilot study demonstrates for role of STNF-aR1 receptor in severity of disease. Future studies should examine whether lower levels of systemic sTNFaR1 at admission may indicate a better disease outcome. [**note: this is from Iran and points to another cytokine marker for severe COVID-19**
https://www.medrxiv.org/content/10.1101/2020.07.12.20152066v1

- Acute respiratory distress syndrome (ARDS) may be the main cause of death in patients with coronavirus disease 2019 (COVID-19). Herein, we retrospect clinical features, outcomes and ARDS characteristics of 75 intensive care unit (ICU) patients with COVID-19 in Chongqing, China. We found a 5.3% case fatality rate of the ICU patients in Chongqing. 93% patients developed ARDS during the intensive care, and more than half were moderate. However, most of the patients (55%) supported with high flow nasal cannula (HFNC) oxygen therapy, but not mechanical ventilation. Nearly one third of patients with ARDS got an early improvement (eiARDS), and the rate is much higher than the other causes of ARDS in a previous study. Patients with eiARDS had a higher survival rate and lower length of ICU stay. The age (< 55 years) is an independent predictor for the eiARDS, and stratification of COVID-19 patients by age is recommended. [**note: this is useful information from China on ICU patients with ARDS. I think their CFR is on the low side relative to some other data I've seen. Age is an important independent predictor for early improvement.**]
  https://www.medrxiv.org/content/10.1101/2020.07.15.20154047v1
- RT-PCR to detect SARS-CoV-2 RNA in clinical specimens was key to manage the COVID-19 pandemic. We monitored SARS-CoV-2 viral loads over time and across different patient populations. We analyzed RT-PCR results according to samples types, gender, age, and health units and compared SARS-CoV-2 viral load to other respiratory viruses, representing a total of 28,373 RT-PCR results including 22,323 SARS-CoV-2 RT-PCR. The importance of viral load to predict contagiousness and clinical prognosis is discussed. [**note: the abstract does not do this paper justice! There are a lot of good histograms looking at viral load across age groups and they see little difference. They also compare viral load of SARS-CoV-2 to a number of other infectious viruses. For those interested in this topic, this is a good paper to read.**]
  https://www.medrxiv.org/content/10.1101/2020.07.15.20154518v1
- On the grounds of its anti-inflammatory and potential antiviral effects, chlorpromazine has been suggested to be effective treatment for Covid-19. We examined the association between chlorpromazine use and respiratory failure among all hospitalized adults with Covid-19 at the 39 Greater Paris University hospitals since the beginning of the epidemic. Study baseline was defined as the date of hospital admission. The primary endpoint was a composite of intubation or death in a time-to-event analysis adjusting for numerous potential confounders. We used a multivariable Cox model with inverse probability weighting according to the propensity score. Of the 12,217 adult inpatients with a positive Covid-19 RT-PCR test included in the analyses, 57 (0.47%) received chlorpromazine. Over a mean follow-up of 20.8 days, the primary endpoint occurred in 29 patients (50.9%) exposed to chlorpromazine and 1,899 patients (15.6%) who were not. In the main analysis, there was a positive significant association between chlorpromazine use and the outcome (HR, 1.67; 95% CI, 1.09 to 2.56, p=0.019), while a Cox regression in a matched analytic sample yielded non-significant association (1.38; 95% CI, 0.91 to 2.09, p=0.123). These findings suggest that chlorpromazine is unlikely to have a clinical efficacy for Covid-19. [**note: chlorpromazine was identified early on as a potential therapeutic and there is at least on registered clinical trial. This observational study from France suggests it may not be useful though the number of patients is small. I think it is useful for researchers to put forth these findings but remember, TIWWDCT!!!!**]
  https://www.medrxiv.org/content/10.1101/2020.07.15.20154310v1

DRUG DEVELOPMENT

- Molecular understanding of neutralizing antibody responses to SARS-CoV-2 could accelerate vaccine design and drug discovery. We analyzed 294 anti-SARS-CoV-2 antibodies and found that IGHV3-53 is the most frequently used IGHV gene for targeting the receptor-binding domain (RBD) of the spike protein. Co-crystal structures of two IGHV3-53 neutralizing antibodies with RBD, with or without Fab CR3022, at 2.33 to 3.20 Å resolution revealed that the germline-encoded residues dominate recognition of the ACE2 binding site. This binding mode limits the IGHV3-53 antibodies to short CDR H3 loops, but accommodates light-chain diversity. These IGHV3-53 antibodies show minimal affinity maturation and high potency, which is promising for vaccine design. Knowledge of these structural motifs and binding mode should facilitate design of antigens that elicit this type of neutralizing response. [**note: more work on antibody characterization** https://science.sciencemag.org/content/early/2020/07/10/science.abd2321
- In vitro antibody selection against pathogens from naive combinatorial libraries can yield various classes of antigen-specific binders that are distinct from those evolved from natural infection. Also, rapid neutralizing antibody discovery can be made possible by a strategy that selects for those interfering with pathogen and host interaction. Here we report the discovery of antibodies that neutralize SARS-CoV-2, the virus responsible for the COVID-19 pandemic, from a highly diverse naive human Fab library. Lead antibody 5A6 blocks the receptor binding domain (RBD) of the viral spike from binding to the host receptor angiotensin converting enzyme 2 (ACE2), neutralizes SARS-CoV-2 infection of Vero E6 cells, and reduces viral replication in reconstituted human nasal and bronchial epithelium models. 5A6 has a high occupancy on the viral surface and exerts its neutralization activity via a bivalent binding mode to the tip of two neighbouring RBDs at the ACE2 interaction interface, one in the "up" and the other in the "down" position, explaining its superior neutralization capacity. Furthermore, 5A6 is insensitive to several spike mutations identified in clinical isolates, including the D614G mutant that has become dominant worldwide. Our results suggest that 5A6 could be an effective prophylactic and therapeutic treatment of COVID-19. [**note: a mainly Singapore group identify a potent neutralizing mAb.**] https://www.biorxiv.org/content/10.1101/2020.07.14.203414v1
- Typically, the fastest route to identifying and licensing a safe and effective antiviral drug is to test those already shown safe in early clinical trials for other infections or diseases. Here, we tested in vitro oleandrin, derived from the Nerium oleander plant and shown previously to have inhibitory activity against several viruses. Using Vero cells, we found that prophylactic oleandrin administration at concentrations down to 0.05 µg/ml exhibited potent antiviral activity against SARS-CoV-2, with an 800-fold reduction in virus production, and a 0.1 µg/ml dose resulted in a greater than 3,000-fold reduction in infectious virus production. The EC50 values were 11.98 ng/ml when virus output was measured at 24 hours post-infection, and 7.07 ng/ml measured at 48 hours post-infection. Therapeutic (post-infection) treatment up to 24 hours after infection of Vero cells also reduced viral titers, with the 0.1 µg/ml dose causing greater than 100-fold reductions as measured at 48 hours, and the 0.05 µg/ml dose resulting in a 78-fold reduction. The potent prophylactic and therapeutic antiviral activities demonstrated here strongly support the further development of oleandrin to reduce the severity of COVID-19 and potentially also to reduce spread by persons diagnosed early after infection. [**note: oleanders were frequently used for public space gardens in San Diego. I remember my mother cautioning us about the flowers and leaves of the plant being quite poisonous. This noted compound is a cardiac**

glycoside and quite poisonous.]
https://www.biorxiv.org/content/10.1101/2020.07.15.203489v1

- The lack of coronavirus-specific antiviral drugs has instigated multiple drug repurposing studies to redirect previously approved medicines for the treatment of SARS-CoV-2, the coronavirus behind the ongoing COVID-19 pandemic. A recent, large-scale, retrospective clinical study showed that famotidine, when administered at a high dose to hospitalized COVID-19 patients, reduced the rates of intubation and mortality. A separate, patient-reported study associated famotidine use with improvements in mild to moderate symptoms such as cough and shortness of breath. While a prospective, multi-center clinical study is ongoing, two parallel *in silico* studies have proposed one of the two SARS-CoV-2 proteases, $3CL^{pro}$ or PL^{pro}, as potential molecular targets of famotidine activity; however, this remains to be experimentally validated. In this report, we systematically analyzed the effect of famotidine on viral proteases and virus replication. Leveraging a series of biophysical and enzymatic assays, we show that famotidine neither binds with nor inhibits the functions of $3CL^{pro}$ and PL^{pro}. Similarly, no direct antiviral activity of famotidine was observed at concentrations of up to 200 µM, when tested against SARS-CoV-2 in two different cell lines, including a human cell line originating from lungs, a primary target of COVID-19. These results rule out famotidine as a direct-acting inhibitor of SARS-CoV-2 replication and warrant further investigation of its molecular mechanism of action in the context of COVID-19. [**note: If you cannot identify the molecular action of a drug, does it really work? Here is a systematic look at famotidine which has been touted as a useful drug.**]
https://www.biorxiv.org/content/10.1101/2020.07.15.203059v1
- The COVID-19 pandemic has taken a significant toll on people worldwide, and there are currently no specific antivirus drugs or vaccines. We report herein a therapeutic based on catalase, an antioxidant enzyme that can effectively breakdown hydrogen peroxide and minimize the downstream reactive oxygen species, which are excessively produced resulting from the infection and inflammatory process. Catalase assists to regulate production of cytokines, protect oxidative injury, and repress replication of SARS-CoV-2, as demonstrated in human leukocytes and alveolar epithelial cells, and rhesus macaques, without noticeable toxicity. Such a therapeutic can be readily manufactured at low cost as a potential treatment for COVID-19. [**note: maybe this is in the 'throw it against the wall and see if it sticks' category.**]
https://www.biorxiv.org/content/10.1101/2020.07.15.205211v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- **NOTE: Going forward I will put all the immunology posts in this section so there is some general place for the emerging work.**
- The ongoing evolution of SARS-CoV-2 is expected to be at least partially driven by the selective pressure imposed by the human immune system. We exploited the availability of a large number of high-quality SARS-CoV-2 genomes, as well as of validated epitope predictions, to show that B cell epitopes in the spike glycoprotein (S) and in the nucleocapsid protein (N) have higher diversity than non-epitope positions. Similar results were obtained for other human coronaviruses. Conversely, in the SARS-CoV-2 population, epitopes for CD4+ and CD8+ T cells were not more variable than non-epitope positions. A significant reduction in epitope variability was instead observed for some of the most immunogenic proteins (S, N, ORF8, and ORF3a). Analysis over longer evolutionary time-frames indicated that this effect is not due to differential

constraints. These data indicate that SARS-CoV-2 is evolving to elude the host humoral immune response, whereas recognition by T cells might benefit the virus. [**note: more confounding and potentially troublesome information on the immunogenicity of the Spike and Nucleocapsid proteins.**] https://www.biorxiv.org/content/10.1101/2020.07.15.204610v1

- COVID-19 is currently a global pandemic, but human immune responses to the virus remain poorly understood. We analyzed 125 COVID-19 patients, and compared recovered to healthy individuals using high dimensional cytometry. Integrated analysis of ~200 immune and ~50 clinical features revealed activation of T cell and B cell subsets in a proportion of patients. A subgroup of patients had T cell activation characteristic of acute viral infection and plasmablast responses reaching >30% of circulating B cells. However, another subgroup had lymphocyte activation comparable to uninfected subjects. Stable versus dynamic immunological signatures were identified and linked to trajectories of disease severity change. These analyses identified three "immunotypes" associated with poor clinical trajectories versus improving health. These immunotypes may have implications for the design of therapeutics and vaccines for COVID-19. [**note: This is a good analysis of the variability of the immune response in COVID-19 patients.**] https://science.sciencemag.org/content/early/2020/07/15/science.abc8511
- Memory T cells induced by previous pathogens can shape the susceptibility to, and clinical severity of, subsequent infections[1]. Little is known about the presence of pre-existing memory T cells in humans with the potential to recognize SARS-CoV-2. Here, we first studied T cell responses to structural (nucleocapsid protein, NP) and non-structural (NSP-7 and NSP13 of ORF1) regions of SARS-CoV-2 in COVID-19 convalescents (n=36). In all of them we demonstrated the presence of CD4 and CD8 T cells recognizing multiple regions of the NP protein. We then showed that SARS-recovered patients (n=23) still possess long-lasting memory T cells reactive to SARS-NP 17 years after the 2003 outbreak, which displayed robust cross-reactivity to SARS-CoV-2 NP. Surprisingly, we also frequently detected SARS-CoV-2 specific T cells in individuals with no history of SARS, COVID-19 or contact with SARS/COVID-19 patients (n=37). SARS-CoV-2 T cells in uninfected donors exhibited a different pattern of immunodominance, frequently targeting the ORF-1-coded proteins NSP7 and 13 as well as the NP structural protein. Epitope characterization of NSP7-specific T cells showed recognition of protein fragments with low homology to "common cold" human coronaviruses but conserved amongst animal betacoranaviruses. Thus, infection with betacoronaviruses induces multispecific and long-lasting T cell immunity to the structural protein NP. Understanding how pre-existing NP- and ORF-1-specific T cells present in the general population impact susceptibility and pathogenesis of SARS-CoV-2 infection is of paramount importance for the management of the current COVID-19 pandemic. [**note: this is a Nature article and I believe I linked to the preprints several weeks ago. It provides a better understanding of T cell immunity. It also indicates that there is some prior immunity based on previous other coronavirus exposure.**] https://www.nature.com/articles/s41586-020-2550-z_reference.pdf
- It is widely believed that the herd immunity threshold (HIT) required to prevent a resurgence of SARS-CoV-2 is in excess of 50% for any epidemiological setting. Here, we demonstrate that HIT may be greatly reduced if a fraction of the population is unable to transmit the virus due to innate resistance or cross-protection from exposure to seasonal coronaviruses. The drop in HIT

is proportional to the fraction of the population resistant only when that fraction is effectively segregated from the general population; however, when mixing is random, the drop in HIT is more precipitous. Significant reductions in expected mortality can also be observed in settings where a fraction of the population is resistant to infection. These results help to explain the large degree of regional variation observed in seroprevalence and cumulative deaths and suggest that sufficient herd-immunity may already be in place to substantially mitigate a potential second wave. [**note: a useful paper from an Oxford group on herd immunity. This fits in with the current discussion of what % constitutes herd immunity. I took a quick look at the paper and the rationale looks sound and there is a good graphical presentation.**]
https://www.medrxiv.org/content/10.1101/2020.07.15.20154294v1

DIAGNOSTIC DEVELOPMENT

- We describe a cost-effective, scalable technology to produce SARS-COV-2 spike (S) protein based on stable expression in HEK293 cells, and its use to develop a highly specific and sensitive ELISA test. The assay allows early detection of anti-S IgG seroconversion and endpoint titers correlate with virus neutralization. The low-cost S-antigen production, together with sample collection by finger prick and dried blood spots, allowed the development of a half-dollar test that fits the urgent need for large-scale serological surveillance in low-income countries. [**note: from Brazil, a low cost serology test**]
https://www.medrxiv.org/content/10.1101/2020.07.13.20152884v2
- With the ongoing COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, there is need for sensitive, specific and affordable diagnostic tests to identify infected individuals, not all of whom are symptomatic. The most sensitive test involves the detection of viral RNA using RT-qPCR, with many commercial kits now available for this purpose. However, these are expensive and supply of such kits in sufficient numbers cannot always be guaranteed. We therefore developed a multiplex assay using well-established SARS-CoV-2 targets alongside internal controls that monitor sample quality and nucleic acid extraction efficiency. Here, we establish that this test performs as well as widely used commercial assays, but at substantially reduced cost. Furthermore, we demonstrate >1,000-fold variability in material routinely collected by nose-and-throat swabbing. The inclusion of a human control probe in our assay provides additional information that could help reduce false negative rates. [**note: some good work from Scotland on a multiplex PCR approach.**]
https://www.medrxiv.org/content/10.1101/2020.07.14.20154005v1
- Background The detection of SARS-CoV-2 by real-time polymerase chain reaction (PCR) in respiratory samples collected from persons recovered from COVID-19 does not necessarily indicate shedding of infective virions. By contrast, the isolation of SARS-CoV-2 using cell-based culture likely indicates infectivity, but there are limited data on the correlation between SARS-CoV-2 culture and PCR. Here we review our experience using SARS-CoV-2 culture to determine infectivity and safe de-isolation of COVID-19 patients. Methods 195 patients with diverse severity of COVID-19 were tested (outpatients [n=178]), inpatients [n=12] and ICU [n=5]). SARS-CoV-2 PCR positive samples were cultured in Vero C1008 cells and inspected daily for cytopathic effect (CPE). SARS-CoV-2-induced CPE was confirmed by PCR of culture supernatant. Where no CPE was documented, PCR was performed on day four to confirm absence of virus replication. Cycle threshold (Ct) values of the day four PCR (Ctculture) and the PCR of the original clinical

sample (Ctsample) were compared, and positive cultures were defined as a Ctsample - Ctculture value of greater than or equal to 3. Findings Of 234 samples collected, 228 (97%) were from the upper respiratory tract. SARS-CoV-2 was only successfully isolated from samples with Ctsample values <32, including in 28/181 (15%), 19/42 (45%) and 9/11 samples (82%) collected from outpatients, inpatients and ICU patients, respectively. The mean duration from symptom onset to culture positivity was 4.5 days (range 0-18 days). SARS-CoV-2 was significantly more likely to be isolated from samples collected from inpatients ($p<0.001$) and ICU patients ($p<0001$) compared with outpatients, and in samples with lower Ctsample values. Conclusion SARS-CoV-2 culture may be used as a surrogate marker for infectivity and inform de-isolation protocols. [**note: this is a pretty cool study from Australia that compares PCR data with cell culture data on patient samples. I don't think I have seen such data before. I am unsure how viable viral culture testing can be in terms of large scale testing.**]
https://www.medrxiv.org/content/10.1101/2020.07.14.20153981v1

- Mitigating transmission of SARS-CoV-2 has been complicated by the inaccessibility and, in some cases, inadequacy of testing options to detect present or past infection. Immunochromatographic lateral flow assays (LFAs) are a cheap and scalable modality for tracking viral transmission by testing for serological immunity, though systematic evaluations have revealed the low performance of some SARS-CoV-2 LFAs. Here, we re-analyzed existing data to present a proof-of-principle machine learning framework that may be used to inform the pairing of LFAs to achieve superior classification performance while enabling tunable False Positive Rates optimized for the estimated seroprevalence of the population being tested. [**note: this may be one approach to using lateral flow assays which tend to be less accurate than ELISA tests. It does add another layer of complexity to the testing system.**]
https://www.medrxiv.org/content/10.1101/2020.07.15.20154773v1

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2020-07-17

This is another one of my country faves!!! Pancho and Lefty was composed by Townes Van Zandt who battled various addictions throughout his life but manged to have great influence on a lot of performers. Here is the video of the song performed by Willie Nelson and Merle Haggard: https://www.youtube.com/watch?v=UoKvUYbGu7A just good music telling a good story, it doesn't get much better than this!

US COVID-19 STATISTICS - **Infection Rate: 1.1%; CFR: 3.8%** (IR unchanged; CFR down 0.1%)

This Australian story came across my news feed this AM. Researchers have developed an agglutination blood test that can tell is someone is currently infected with SARS-CoV-2 or if they have been infected in the past. If this can readily be automated, it will be a big deal. I look forward to reading the paper on this research (see diagnostics section below). Here is the University's press release.

The Atlantic on a second coronavirus death surge. Magical thinking doesn't really work.

The New York Times has a dashboard that rates various therapies as good, bad or indifferent. Vaccine development in China is indeed different from other major countries. Russia seems to think that spying on vaccine companies can help their internal vaccine efforts. A small Maryland biotechnology company

gets big bucks to develop a COVID-19 vaccine despite never having brought a product to the market before.

What is wrong with this New York Times picture taken this week in St. Petersburg, Florida? On the topic of mask donning, the Times has a nice survey and accompanying map of the US on this.

The Guardian covers some UK research that provides a nice grouping of COVID-19 symptoms from mild to serious.

The BMJ has a research paper on physical distancing interventions and incidence of coronavirus disease in 149 countries! Physical distancing interventions were associated with reductions in the incidence of covid-19 globally. No evidence was found of an additional effect of public transport closure when the other four physical distancing measures were in place. Earlier implementation of lockdown was associated with a larger reduction in the incidence of covid-19. These findings might support policy decisions as countries prepare to impose or lift physical distancing measures in current or future epidemic waves. There is an accompanying editorial on the topic that looks at need for supporting evidence

The Lancet is full of good reading today. Can digital contact tracing make up for lost time? Towards pandemic preparedness beyond COVID-19 (it will happen again). Richard Horton, the editor of the journal, offers an editorial on the dangers of Sinophobia and there is an unsigned editorial entitled 'No more normal.' Finally, for the docs in the audience, histopathology and ultrastructural findings of fatal COVID infections in Washington state, the first US epicenter.

The results of the Minnesota HCQ trial in symptomatic, non-hospitalized adults with laboratory-confirmed COVID-19 are in. Guess what? Paging Peter Navarro, put on your reading glasses: it doesn't work. An editorial on this cautionary tale is also in the Annals of Internal Medicine. Bring me a wooden stake to kill this Zombie drug.

Some interesting stuff in the diagnostics section and a nasal SARS-CoV-2 vaccine project showing the viability of that approach.

MODELING

- Background: School closures are part of the SARS-CoV-2 pandemic control measures in many countries, based on the assumption that children play a similar role in transmitting SARS-CoV-2 as they do in transmitting influenza. We therefore performed a SARS-CoV-2 seropraevalence-study in students and teachers to assess their role in the SARS-CoV-2 transmission. Methods: Students grade 8-11 and their teachers in 13 secondary schools in eastern Saxony, Germany, were invited to participate in the SchoolCoviDD19 study. Blood samples were collected between May 25th and June 30th, 2020. Anti-SARS-CoV-2 IgG were assed using chemiluminescence immunoassay technology and all samples with a positive or equivocal test result were re-tested with two additional serological tests. Findings: 1538 students and 507 teachers participated in this study. The seropraevalence for SARS-CoV-2 was 0.6%. Even in schools with reported Covid-19 cases before the Lockdown of March 13th no clusters could be identified. 23/24 participants with a household history of COVID-91 were seronegative. By using a combination of three different immunoassays we could exclude 16 participants with a positive or equivocal results after initial testing. Interpretation: *Students and teachers do not play a crucial role in driving the SARS-CoV-2 pandemic in a low prevalence setting. Transmission in families occurs very infrequently, and the number of unreported cases is low in this age group, making school closures not appear appropriate as a strategy in this low prevalence settings*. Funding: This study was supported by a grant from the state of Saxony [**note: it would be good to have similar data from other locales and countries. It would provide better information as decisions to open schools are made. Germany may be a special case with few hot spots of infections. Certainly there are some regions of the US that cannot be thought of as 'low prevalence.**] https://www.medrxiv.org/content/10.1101/2020.07.16.20155143v1
- Superspreading events shape the COVID-19 pandemic. Here we provide a national-scale analysis of SARS-CoV-2 outbreaks in Austria, a country that played a major role for virus transmission across Europe and beyond. Capitalizing on a national epidemiological surveillance system, we performed deep whole-genome sequencing of virus isolates from 576 samples to cover major Austrian SARS-CoV-2 clusters. Our data chart a map of early viral spreading in Europe, including the path from low-frequency mutations to fixation. Detailed epidemiological surveys enabled us to calculate the effective SARS-CoV-2 population bottlenecks during transmission and unveil time-resolved intra-patient viral quasispecies dynamics. This study demonstrates the power of integrating deep viral genome sequencing and epidemiological data to better understand how SARS-CoV-2 spreads through populations. [**note: this is a good model for the spread of SARS-CoV-2 in Austria.**] https://www.biorxiv.org/content/10.1101/2020.07.15.204339v1

NEWLY REGISTERED CLINICAL TRIALS

- This is an First In Human (FIH), observer-blinded, randomized, placebo-controlled, parallel group study to evaluate the safety and immunogenicity of KBP-COVID-19 vaccine in healthy CoV-2seronegative adult subjects in 2 age groups, Part A (18-49 years) and Part B (50-70 years).

[**note: this is being manufactured in genetically engineered tobacco plants by Kentucky BioProcessing Inc. It's not a new technology.]** NCT04473690

CLINICAL TRIAL RESULTS

- The relationship between SARS-CoV-2 viral load and risk of disease progression remains largely undefined in coronavirus disease 2019 (COVID-19). We quantified SARS-CoV-2 viral load from participants with a diverse range of COVID-19 severity, including those requiring hospitalization, outpatients with mild disease, and individuals with resolved infection. SARS-CoV-2 plasma RNA was detected in 27% of hospitalized participants and 13% of outpatients diagnosed with COVID-19. Amongst the participants hospitalized with COVID-19, higher prevalence of detectable SARS-CoV-2 plasma viral load was associated with worse respiratory disease severity, lower absolute lymphocyte counts, and increased markers of inflammation, including C-reactive protein and IL-6. SARS-CoV-2 viral loads, especially plasma viremia, were associated with increased risk of mortality. SARS-CoV-2 viral load may aid in the risk stratification of patients with COVID-19 and its role in disease pathogenesis should be further explored. [**note: another study showing that increased viral load is not good!**]
  https://www.medrxiv.org/content/10.1101/2020.07.15.20131789v1
- The impact of COVID-19 on patients with Interstitial Lung Disease (ILD) has not been established. Objectives: To assess outcomes following COVID-19 in patients with ILD versus those without in a contemporaneous age, sex and comorbidity matched population. Methods: An international multicentre audit of patients with a prior diagnosis of ILD admitted to hospital with COVID-19 between 1 March and 1 May 2020 was undertaken and compared with patients, without ILD obtained from the ISARIC 4C cohort, admitted with COVID-19 over the same period. The primary outcome was survival. Secondary analysis distinguished IPF from non-IPF ILD and used lung function to determine the greatest risks of death. Measurements and Main Results: Data from 349 patients with ILD across Europe were included, of whom 161 were admitted to hospital with laboratory or clinical evidence of COVID-19 and eligible for propensity-score matching. Overall mortality was 49% (79/161) in patients with ILD with COVID-19. After matching ILD patients with COVID-19 had higher mortality (HR 1.60, Confidence Intervals 1.17-2.18 p=0.003) compared with age, sex and co-morbidity matched controls without ILD. Patients with a Forced Vital Capacity (FVC) of <80% had an increased risk of death versus patients with FVC ≥80% (HR 1.72, 1.05-2.83). Furthermore, obese patients with ILD had an elevated risk of death (HR 1.98, 1.13−3.46). Conclusions: Patients with ILD are at increased risk of death from COVID-19, particularly those with poor lung function and obesity. Stringent precautions should be taken to avoid COVID-19 in patients with ILD. [**note: this is a large multi-center study. It is not a surprise that patients with interstitial lung disease are at a greater risk for COVID-19 adverse outcome.**] https://www.medrxiv.org/content/10.1101/2020.07.15.20152967v1
- Objective To characterise the clinical features of children and young people admitted to hospital with laboratory-confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in the UK, and explore factors associated with admission to critical care, mortality, and development of multisystem inflammatory syndrome in children and adolescents temporarily related to covid-19 (MIS-C). Design Prospective observational cohort study with rapid data gathering and near real time analysis. Setting 260 acute care hospitals in England, Wales, and

Scotland between 17th January and 5th June 2020, with a minimal follow-up time of two weeks (to 19th June 2020). Participants 451 children and young people aged less than 19 years admitted to 116 hospitals and enrolled into the International Severe Acute Respiratory and emergency Infections Consortium (ISARIC) WHO Clinical Characterisation Protocol UK study with laboratory-confirmed SARS-CoV-2. Main Outcome Measures Admission to critical care (high dependency or intensive care), in-hospital mortality, or meeting the WHO preliminary case definition for MIS-C. Results Median age was 3.9 years [interquartile range (IQR) 0.3-12.9 years], 36% (162/451) were under 12 months old, and 57% (256/450) were male. 56% (224/401) were White, 12% (49/401) South Asian and 10% (40/401) Black. 43% (195/451) had at least one recorded comorbidity. A muco-enteric cluster of symptoms was identified, closely mirroring the WHO MIS-C criteria. 17% of children (72/431) were admitted to critical care. On multivariable analysis this was associated with age under one month odds ratio 5.05 (95% confidence interval 1.69 to 15.72, p=0.004), age 10 to 14 years OR 3.11 (1.21 to 8.55, p=0.022) and Black ethnicity OR 3.02 (1.30 to 6.84, p=0.008). Three young people died (0.7 %, 3/451) aged 16 to 19 years, all of whom had profound comorbidity. Twelve percent of children (36/303) met the WHO MIS-C criteria, with the first patient developing symptoms in mid-March. Those meeting MIS-C criteria were older, (median age 10.8 years ([IQR 8.4-14.1] vs 2.0 [0.2-12.6]), p<0.001) and more likely to be of non-White ethnicity (70% (23/33) vs 43% (101/237), p=0.005). Children with MIS-C were four times more likely to be admitted to critical care (61% (22/36) vs 15% (40/267, p<0.001). In addition to the WHO criteria, children with MIS-C were more likely to present with headache (45% (13/29) vs 11% (19/171), p<0.001), myalgia (39% (11/28) vs 7% (12/170), p<0.001), sore throat (37% (10/27) vs (13% (24/183, p = 0.004) and fatigue (57% (17/30) vs 31% (60/192), p =0.012) than children who did not and to have a platelet count of less than 150 x109/L (30% (10/33) vs 10% (24/232), p=0.004). Conclusions Our data confirms less severe covid-19 in children and young people than in adults and we provide additional evidence for refining the MIS-C case definition. The identification of a muco-enteric symptom cluster also raises the suggestion that MIS-C is the severe end of a spectrum of disease. [**note: this is look at children from 260 acute care hospitals in the UK. Good analysis of the spectrum of symptoms.**] https://www.medrxiv.org/content/10.1101/2020.07.14.20153320v1

- Children are less susceptible to COVID-19 and manifests lower morbidity and mortality after infection, for which a multitude of mechanisms may be proposed. Whether the normal development of gut-airway microbiome is affected by COVID-19 has not been evaluated. We demonstrate that COVID-19 alters the respiratory and gut microbiome of children. Alteration of the microbiome was divergent between the respiratory tract and gut, albeit the dysbiosis was dominated by genus Pseudomonas and sustained for up to 25-58 days in different individuals. The respiratory microbiome distortion persisted in 7/8 children for at least 19-24 days after discharge from the hospital. The gut microbiota showed early dysbiosis towards later restoration in some children, but not others. Disturbed development of both gut and respiratory microbiomes, and prolonged respiratory dysbiosis in children imply possible long-term complications after clinical recovery from COVID-19, such as predisposition to an increased health risk in the post-COVID-19 era. [**note: the numbers in this study are quite small: 9 who had COVID-19 vs 14 age-matched controls. I have no doubt the findings are of interest but what is the percent of children who actually have this problem. More research is necessary**

but it does point to an issue that might not be widely appreciated by the broader medical community.] https://www.medrxiv.org/content/10.1101/2020.07.13.20152181v1

DRUG DEVELOPMENT

- To assess the overall effect of vitamin D supplementation on risk of acute respiratory infection (ARI), and to identify factors modifying this effect. Design: We conducted a systematic review and meta-analysis of data from randomised controlled trials (RCTs) of vitamin D for ARI prevention using a random effects model. Pre-specified sub-group analyses were done to determine whether effects of vitamin D on risk of ARI varied according to baseline 25-hydroxyvitamin D (25[OH]D) concentration or dosing regimen. Data Sources: MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science, ClinicalTrials.gov and the International Standard RCT Number (ISRCTN) registry from inception to May 2020. Eligibility Criteria for Selecting Studies: Double-blind RCTs of supplementation with vitamin D or calcidiol, of any duration, were eligible if they were approved by a Research Ethics Committee and if ARI incidence was collected prospectively and pre-specified as an efficacy outcome. Results: We identified 40 eligible RCTs (total 30,956 participants, aged 0 to 95 years). Data were obtained for 29,841 (96.5%) of 30,909 participants in 39 studies. For the primary comparison of vitamin D supplementation vs. placebo, the intervention reduced risk of ARI overall (Odds Ratio [OR] 0.89, 95% CI 0.81 to 0.98; P for heterogeneity 0.009). No statistically significant effect of vitamin D was seen for any of the sub-groups defined by baseline 25(OH)D concentration. However, protective effects were seen for trials in which vitamin D was given using a daily dosing regimen (OR 0.75, 95% CI 0.61 to 0.93); at daily dose equivalents of 400-1000 IU (OR 0.70, 95% CI 0.55 to 0.89); and for a duration of ≤12 months (OR 0.82, 95% CI 0.72 to 0.94). Vitamin D did not influence the proportion of participants experiencing at least one serious adverse event (OR 0.94, 95% CI 0.81 to 1.08). Risk of bias within individual studies was assessed as being low for all but two trials. A funnel plot showed asymmetry, suggesting that small trials showing non-protective effects of vitamin D may have been omitted from the meta-analysis. Conclusions*: Vitamin D supplementation was safe and reduced risk of ARI, despite evidence of significant heterogeneity across trials. The overall effect size may have been over-estimated due to publication bias. Protection was associated with administration of daily doses of 400-1000 IU vitamin D for up to 12 months. The relevance of these findings to COVID-19 is not known and requires investigation*. [**note: this was done by a large group of multi-national researchers. They were looking broadly at acute respiratory infection and not COVID-19. There are some clinical trials going on right now with Vitamin D. I think there ought to be enough patients that a well designed observational study would be informative. Unfortunately, dietary supplement intake is not always in medical records to the same degree Rx drugs are.**] https://www.medrxiv.org/content/10.1101/2020.07.14.20152728v1
- The Coronavirus Disease 2019 pandemic has made deployment of an effective vaccine a global health priority. We evaluated the protective activity of a chimpanzee adenovirus-vectored vaccine encoding a pre-fusion stabilized spike protein (ChAd-SARS-CoV-2-S) in challenge studies with Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and mice expressing the human angiotensin-converting enzyme 2 receptor. Intramuscular dosing of ChAd-SARS-CoV-2-S induces robust systemic humoral and cell-mediated immune responses and protects against lung infection, inflammation, and pathology but does not confer sterilizing immunity, as

evidenced by detection of viral RNA and induction of anti-nucleoprotein antibodies after SARS-CoV-2 challenge. In contrast, a single intranasal dose of ChAd-SARS-CoV-2-S induces high levels of systemic and mucosal IgA and T cell responses, completely prevents SARS-CoV-2 infection in the upper and lower respiratory tracts, and likely confers sterilizing immunity in most animals. Intranasal administration of ChAd-SARS-CoV-2-S is a candidate for preventing SARS-CoV-2 infection and transmission, and curtailing pandemic spread. [**note: nasal delivery of a SARS-CoV-2 vaccine may be a viable approach. The only caveat I have about this is the history of the nasal flu vaccine here in the US which did not have the same potency as the injectable version. This vaccine was developed by these investigators and is NOT in any current clinical trials.**] https://www.biorxiv.org/content/10.1101/2020.07.16.205088v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- SARS-CoV-2 has caused a global pandemic with millions infected and numerous fatalities. Questions regarding the robustness, functionality and longevity of the antibody response to the virus remain unanswered. Here we report that the vast majority of infected individuals with mild-to-moderate COVID-19 experience robust IgG antibody responses against the viral spike protein, based on a dataset of 19,860 individuals screened at Mount Sinai Health System in New York City. We also show that titers are stable for at least a period approximating three months, and that anti-spike binding titers significantly correlate with neutralization of authentic SARS-CoV-2. Our data suggests that more than 90% of seroconverters make detectible neutralizing antibody responses and that these titers are stable for at least the near-term future. [**note: here is some good news. Lots of robust antibody production in infected individuals appears to last for at least 3 months.**] https://www.medrxiv.org/content/10.1101/2020.07.14.20151126v1
- Héma-Québec, the blood supplier in the Province of Québec, Canada, collects and tests convalescent plasma used in a clinical trial to determine the clinical efficacy of this product for the treatment of hospitalized COVID-19 patients. So far, we have collected 1159 plasma units from 282 COVID-19 convalescent donors. The presence of antibodies to the receptor binding domain (RBD) of SARS-CoV-2 spike protein in convalescent donors was established at the first donation. Seropositive donors were asked to donate additional plasma units every six days. Until now, 15 donors have donated at least four times and, in some cases, up to nine times. This allowed us to perform a longitudinal analysis of the persistence of SARS-CoV-2 RBD-specific antibodies in these repeat donors, with the first donation occurring 33-77 days after symptoms onset and donations up to 71-114 days after symptoms onset thereafter. In all donors, the level of antibodies remained relatively stable up to about 76 days after symptoms onset but then started to decrease more rapidly to reach, in some convalescent donors, a seronegative status within 100-110 days after symptoms onset. The decline in anti-RBD antibodies was not related to the number of donations but strongly correlated with the numbers of days after symptoms onset ($r = 0.821$). This suggests that de novo secretion of SARS-CoV-2 RBD antibodies by short-lived plasma cells stopped about 2-3 months after disease onset, an observation that has important implications for convalescent plasma collection and seroprevalence studies undertaken several months after the peak of infection. [**note: this data from Quebec on antibody persistence runs counter to the Mt. Sinai report immediately above. As I always say, more data is better!**] https://www.biorxiv.org/content/10.1101/2020.07.16.206847v1

DIAGNOSTIC DEVELOPMENT

- High-throughput and rapid serology assays to detect the antibody response specific to severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) in human blood samples are urgently required to improve our understanding of the effects of COVID-19 across the world. Short-term applications include rapid case identification and contact tracing to limit viral spread, while population screening to determine the extent of viral infection across communities is a longer-term need. Assays developed to address these needs should match the ASSURED criteria. We have identified agglutination tests based on the commonly employed blood typing methods as a viable option. These blood typing tests are employed in hospitals worldwide, are high-throughput, fast (10–30 min), and automated in most cases. Herein, we describe the application of agglutination assays to SARS-CoV-2 serology testing by combining column agglutination testing with peptide–antibody bioconjugates, which facilitate red cell cross-linking only in the presence of plasma containing antibodies against SARS-CoV-2. This simple, rapid, and easily scalable approach has immediate application in SARS-CoV-2 serological testing and is a useful platform for assay development beyond the COVID-19 pandemic. [**note: I had a story on this one above the fold. This might be a real game changer once labs have had a chance to deploy and validate it.]** https://pubs.acs.org/doi/10.1021/acssensors.0c01050
- In the present study, we have determined SARS-CoV-2-specific antibody responses in a cohort of 96 individuals during the acute phase of infection and in a cohort of 578 individuals enrolled in a seroprevalence population study in Switzerland including three groups, i.e. subjects with previous RT-PCR confirmed SARS-CoV-2 infections (n=90), positive patient contact (n=177) and random selected subjects (n=311). Six serological assays detecting predominantly IgG antibodies targeting either the Spike (S) and/or the nucleocapsid (N) proteins were used including also a Luminex based assay using an S protein in its native trimeric form. Antibody responses against the S and/or the N proteins were equally sensitive in the acute infection phase although differences in sensitivity (range 83 to 97% 16-33 days post-initial symptoms) were observed between the different assays and the Luminex S protein trimer assay was the most sensitive. Interestingly, antibody responses against the N protein appear to wane in the post-infection phase of the infection while those against the S protein persist over time, as indicated by the drop in sensitivity of the assays targeting the N protein (sensitivity range 71-77%). Assays detecting anti-N IgG antibodies may substantially underestimate the proportion of SARS-CoV-2 infections in the groups of patient positive contacts, i.e. 10.9 to 32.2% reduction ($P<0.05$-<0.0001) and in the random selected general population, i.e. up to 45% reduction ($P<0.05$). The overall reduction in seroprevalence for the total cohort ranged from 9.4 to 31% ($P<0.0009$-<0.0001). Of note, the assay using the S protein in its native trimer form was more sensitive as compared to those using monomeric S proteins. These results indicate that assays targeting the S protein, ideally the trimeric form, should be implemented as reference test to estimate SARS-CoV-2 infections in seroprevalence population studies. [**note: this is a Swiss study that looks a different serology studies and notes that one focusing on the native trimer form of the S protein is more sensitive.**]
  https://www.medrxiv.org/content/10.1101/2020.07.14.20153536v1
- Up to 70% of SARS-CoV-2 infections in working- and school-age people are asymptomatic (Poletti et al., 2020), creating anxiety over reopening workplaces and schools around the world. In the absence of effective treatments or a vaccine, peace of mind will come only with community-based SARS-CoV-2 screening, where many people are tested on a regular basis.

However, recent models show that short sample-to-answer turnaround time will be a critical property of effective screening strategies (Larremore et al., 2020). Here, we describe an RT-LAMP test for SARS-CoV-2 in raw saliva that takes about 45 minutes from sample to answer and requires only simple equipment (pipettes and a heating source). The assay has a limit of detection of 100 virions per microliter, and targets two separate regions of the SARS-CoV-2 genome. By combining rapid sample-to-answer turnaround time with the use of saliva, our RT-LAMP assay provides a low-complexity, portable, and robust system for real-time community screening. [**note: more good work on the development of a saliva based diagnostic. Turnaround time for this one is 45 minutes. Let's get the validation done and get these tests out!**] https://www.medrxiv.org/content/10.1101/2020.07.16.20150250v1

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2020-07-18

Let's wrap the country selection with The Chicks who despite dropping the 'Dixie' from their name continue to stay true to their country rock roots. They have always been political going back to their protest of the Iraq war which pretty much ended their Nashville career. Here is a song from their newest album, March March: https://www.youtube.com/watch?v=xwBjF_VVFvE I'll let the music and video do the talking. Let us also take a moment to remember Congressman John Lewis who passed away yesterday. He was a stalwart of the Civil Rights movement. Also from the new album is the title track 'Gaslighter': https://www.youtube.com/watch?v=sbVPcPL30xc

US COVID-19 STATISTICS - **Infection Rate: 1.1%; CFR: 3.8%** (IR unchanged; CFR unchanged; *there was a spike in deaths yesterday and the CFR for the day was over 1%; while lower than the outbreak in the Northeast earlier this year, it is still high and no cause for rejoicing. As a comparison the UK mortality rate for the 1958-59 flu epidemic was 0.3%. I still think the CFR will continue to drop to below 1% but marginally worse than the UK number. I do not find a 1% CFR to be acceptable from a public health point of view even though it represents a decrease in mortality compared to the New York City epicenter.*)

The New York Times has a good story on the Johnson & Johnson vaccine effort. The collaboration is with Beth Israel Deaconess in Boston. Why is Thailand and other countries in Southeast Asia seeing so few cases and mortality? Is it an immune system difference or they are just good at observing proper public health practices? They mention high exposures to Dengue virus but that can't be it as there is equally high levels in Brazil.

The Washington Post covers the abrupt rise in COVID-19 cases. A Minnesota nurse comes out of retirement to help out the facility where she used to work. Responding to a Freedom of Information Act lawsuit, the State Department releases a cable that expressed concerns about the lack of trained personnel at the virology lab in Wuhan China.

The Guardian reports progress on a UK quick antibody test that appears to be 98% accurate. This is part of Rapid Test Consortium in the UK.

The New England Journal of Medicine publishes the UK RECOVERY dexamethasone trial along with an editorial comment by Cliff Lane and Tony Fauci. Note that there are some confounding papers from Spain and Italy today.

JAMA have a story on the use of Amabié, a Japanese symbol of the COVID-19 pandemic. It's an interesting read as the symbol goes back to the mid-1800s when '…according to legend, an unnamed officer went to investigate a strange light that had been appearing at sea. The officer encountered the strange creature who explained, "I live in the sea. My name is Amabié. Good harvest will continue for six years. At the same time disease will spread. Draw me and show me to the people as soon as possible," before submerging.'

Here is one for the endocrinologists in the crowd (I was one for a short period of time in my research career). The Lancet provide a review on why diabetics may suffer worse clinical outcomes from COVID-19. The journal also has a viewpoint on serology testing in the pandemic response.

This has to be one of the **most intriguing preprints** I've read all week. It discusses a DIY home method to decontaminate your non-medical face mask. You have to read the paper very carefully to understand how they are using household hygroscopic materials In this case they use short cut dried pasta, not specifying the type or brand. I'll need to try this out but wonder what the impact of the high heat will be on the elastic strap, but this is what experimentation is for!! Right now I use a hot water detergent treatment (I always use Dawn™ detergent another fine Procter & Gamble product – I am a P&G stockholder). Anyway, this is an ultra-cool method!!!!

Lots of reading today. I hope I have a quiet day tomorrow!

MODELING

- Due to the substantial proportion of asymptomatic and mild courses many SARS-CoV-2 infections remain unreported. Therefore, assessment of seroprevalence may detect the real burden of disease. We aimed at determining and characterizing the rate of SARS-CoV-2 infections and the resulting immunity in a defined population. Methods: CoNAN is a population-based cohort study in the previously quarantined community Neustadt-am-Rennsteig, Germany six weeks after a SARS-CoV-2 outbreak with 49 cases identified by PCR screening of all 883 inhabitants. The primary objective of the study was to assess SARS-CoV-2 antibody seroconversion rate using six different IgG detecting immunoassays. Secondary objectives of the study were: i.) to determine the rate of seroconversion in children; ii.) to determine potential risk factors for symptomatic vs. asymptomatic Covid19 courses; iii.) to investigate the rate of virus persistence. Findings: We enrolled 626 participants (71% of the community population). All actual SARS-CoV-2 PCR tests were negative; while a total of 8.4% (52 of 620 tested) had antibodies against SARS-CoV-2 in at least two independent tests. Twenty of the antibody positive participants had previously a positive SARS-CoV-2 PCR. On the contrary, of those 38 participants with SARS-CoV-2 infection, only 20 (52.6%) were antibody positive. Interpretation: Several antibody tests conducted six weeks after an outbreak of SARS-CoV-2 did not detect all previously PCR-positive tested individuals. Cautious evaluation of antibody testing strategies to assess immunity against the infection is warranted. [**note: any study named CoNAN (whether it is for the comedian or the barbarian, I don't know) deserves mention. Seriously though, this is an interesting study of small town that was quarantined. They were able to study 71% of the**

community population. Not all the confirmed cases by PCR showed antibody presentation using six different IgG assays. This same thing has been observed elsewhere and it still is unclear whether the antibody negative individuals are subject to reinfection.]
https://www.medrxiv.org/content/10.1101/2020.07.15.20154112v1

- Transmission of SARS-CoV-2 leading to COVID-19 occurs through exhaled respiratory droplets from infected humans. Currently, however, there is much controversy over whether respiratory aerosol microdroplets play an important role as a route of transmission. By measuring and modeling the dynamics of exhaled respiratory droplets we can assess the relative contribution of aerosols in the spreading of SARS-CoV-2. We measure size distribution, total numbers and volumes of respiratory droplets, including aerosols, by speaking and coughing from healthy subjects. Dynamic modelling of exhaled respiratory droplets allows to account for aerosol persistence times in confined public spaces. The probability of infection by inhalation of aerosols when breathing in the same space can then be estimated using current estimates of viral load and infectivity of SARS-CoV-2. In line with the current known reproduction numbers, our study of transmission of SARS-CoV-2 suggests that aerosol transmission is an inefficient route, in particular from non or mildly symptomatic individuals. [**note: more confounding information on aerosol transmission of SARS-CoV-2. I beginning to get totally confused (nothing new about this) regarding this. I continue to believe enclosed spaces with high population density are problematic.**] https://www.medrxiv.org/content/10.1101/2020.07.16.20155572v1
- Background Many foods have an antioxidant activity, and nutrition may mitigate COVID-19. To test the potential role of vegetables in COVID-19 mortality in Europe, we performed an ecological study. Methods The European Food Safety Authority (EFSA) Comprehensive European Food Consumption Database was used to study the country consumption of Brassica vegetables (broccoli, cauliflower, head cabbage (white, red and savoy cabbage), leafy brassica) and to compare them with spinach, cucumber, courgette, lettuce and tomato. The COVID-19 mortality per number of inhabitants was obtained from the Johns Hopkins Coronavirus Resource Center. EuroStat data were used for potential confounders at the country level including Gross Domestic Product (GDP) (2019), population density (2018), percentage of people over 64 years (2019), unemployment rate (2019) and percentage of obesity (2014, to avoid missing values). Mortality counts were analyzed with quasi-Poisson regression models to model the death rate while accounting for over-dispersion. Results Of all the variables considered, including confounders, only head cabbage and cucumber reached statistical significance with the COVID-19 death rate per country. For each g/day increase in the average national consumption of some of the vegetables (head cabbage and cucumber), the mortality risk for COVID-19 decreased by a factor of 11, down to 13.6 %. Lettuce consumption increased COVID-19 mortality. The adjustment did not change the point estimate and the results were still significant. Discussion The negative ecological association between COVID-19 mortality and the consumption of cabbage and cucumber supports the a priori hypothesis previously reported. The hypothesis needs to be tested in individual studies performed in countries where the consumption of vegetables is common. [**note: we had an associative study looking at pickled vegetables and COVID-19. Here is one looking at intake of fresh vegetables! Load up on cucumbers and cabbage!!!! Maybe decrease the lettuce consumption. What other newsletter is providing you with such good information?]** https://www.medrxiv.org/content/10.1101/2020.07.17.20155846v1

- Serological studies are critical for understanding pathogen−specific immune responses and informing public health measures (1,2). By developing highly sensitive and specific trimeric spike (S)−based antibody tests, we report IgM, IgG and IgA responses to SARS−CoV−2 in COVID−19 patients (n=105) representing different categories of disease severity. All patients surveyed were IgG positive against S. Elevated anti−SARS−CoV−2 antibody levels were associated with hospitalization, with IgA titers, increased circulating IL−6 and strong neutralizing responses indicative of intensive care status. Antibody−positive blood donors and pregnant women sampled during the pandemic in Stockholm, Sweden (weeks 14−25), displayed on average lower titers and weaker neutralizing responses compared to patients; however, inter−individual anti−viral IgG titers differed up to 1,000−fold. To provide more accurate estimates of seroprevalence, given the frequency of weak responders and the limitations associated with the dichotomization of a continuous variable (3,4), we used a Bayesian approach to assign likelihood of past infection without setting an assay cut−off. Analysis of blood donors (n=1,000) and pregnant women (n=900) sampled weekly demonstrated SARS-CoV-2-specific IgG in 7.2% (95% Bayesian CI [5.1−9.5]) of individuals two months after the peak of spring 2020 COVID−19 deaths. Seroprevalence in these otherwise healthy cohorts increased steeply before beginning to level−off, following the same trajectory as the Stockholm region deaths over this time period. [**note: this is a seroprevalence estimate in Sweden during the emergence of SARS-CoV-2. It is useful in that Sweden did not impose a strict lockdown.**]
  https://www.medrxiv.org/content/10.1101/2020.07.17.20155937v1

NEWLY REGISTERED CLINICAL TRIALS

- Did not check.

CLINICAL TRIAL RESULTS

- Anosmia has been listed as a key-symptom associated with the COVID-19 infection. Because it often occurs without any sign of rhinitis, lesions of the central olfactory system have been suspected. To date, however, there is no evidence that anosmia caused by SARS-CoV2 could be the result of brain damage. Methods: We conducted a case-series on 10 consecutive COVID-19 patients who reported anosmia. Each patient prospectively underwent a validated olfactory test (Sniffin Sticks test) and a brain MRI. Results: Hypersignal intensity lesions of the central olfactory system were found in 3 subjects on 3D T2 FLAIR and 2D T2 High Resolution images with a lesion involving the olfactory bulbs and/or the orbitofrontal cortex. These 3 subjects showed a severe and persistent loss of smell on the olfactory test. Mucosal hyperplasia of the upper nasal cavities was found in two other subjects with significant smell disorders. There was no MRI anomaly in two subjects with good smell restoration. Conclusions: Anomalies of the central olfactory system could be responsible for anosmia in patients with COVID-19 infection. Further studies are needed to assess the impact on long-term functional prognosis of these lesions. [**note: Oh No, I think my patent application for the COVID Scent Strip is dead in the water. This paper refers to *Sniffin' Sticks test* which from the reference was developed in 1997. Prior art nails me on this one.** 😒 **the interesting and disturbing finding is the presence of olfactory system brain lesions in a small number of patients. This needs further investigation to see if some people may see prolonged loss of smell.**]
  https://www.medrxiv.org/content/10.1101/2020.07.08.20148692v1

- Coronavirus Disease 2019 (COVID-19) causes severe acute respiratory failure. Antibody-dependent enhancement (ADE) is known as the mechanism for severe forms of other coronavirus diseases. The clinical progression of COVID-19 before and after IgG antibody seroconversion was investigated. Methods: Fifty-three patients with reverse transcriptase PCR (RT-PCT)-confirmed COVID-19 viral pneumonia with or without respiratory failure were retrospectively investigated. The timing of the first IgG antibody against SARS-CoV-2-positive date, as well as changes of C-reactive protein (CRP) as an inflammatory marker and blood lymphocyte numbers, was assessed using serial preserved blood samples. Findings: Ten patients recovered without oxygen therapy (mild/moderate group), 32 patients had hypoxemia and recovered with antiviral drugs (severe/non-ICU group), and 11 patients had severe respiratory failure and were treated in the ICU (6 of them died; critical/ICU group). The first IgG-positive date (day 0) was observed from 5 to 18 days from the onset of disease. At day 0, a CRP peak was observed in the severe and critical groups, whereas there was no synchronized CRP peak on day 0 in the mild/moderate group. In the severe/non-ICU group, the blood lymphocyte number increased (P=0.0007) and CRP decreased (P=0.0007) after day 0, whereas CRP did not decrease and the blood lymphocyte number further decreased (P=0.0370) in the critical/ICU group. Interpretation: *The respiratory failure due to COVID-19 viral pneumonia observed in week 2 may be related to an antibody-related mechanism rather than uncontrolled viral replication. In the critical form of COVID-19, inflammation was sustained after IgG seroconversion*. [**note: more information on the clinical progression of COVID-19 and antibody and other marker response. C-reactive protein spikes have been observed by other researchers. This may be why dexamethasone works in some patients.**]
  https://www.medrxiv.org/content/10.1101/2020.07.16.20154088v1
- COVID-19-associated respiratory failure offers the unprecedented opportunity to evaluate the differential host response to a uniform pathogenic insult. Prior studies of Acute Respiratory Distress Syndrome (ARDS) have identified subphenotypes with differential outcomes. Understanding whether there are distinct subphenotypes of severe COVID-19 may offer insight into its pathophysiology. Objectives. To identify and characterize distinct subphenotypes of COVID-19 critical illness defined by the post-intubation trajectory of Sequential Organ Failure Assessment (SOFA) score. Methods. Intubated COVID-19 patients at two hospitals in New York city were leveraged as development and validation cohorts. Patients were grouped into mild, intermediate, and severe strata by their baseline post-intubation SOFA. Hierarchical agglomerative clustering was performed within each stratum to detect subphenotypes based on similarities amongst SOFA score trajectories evaluated by Dynamic Time Warping. Statistical tests defined trajectory subphenotype predictive markers. Measurements and Main Results. Distinct worsening and recovering subphenotypes were identified within each stratum, which had distinct 7-day post-intubation SOFA progression trends. Patients in the worsening suphenotypes had a higher mortality than those in the recovering subphenotypes within each stratum (mild stratum, 29.7% vs. 10.3%, p=0.033; intermediate stratum, 29.3% vs. 8.0%, p=0.002; severe stratum, 53.7% vs. 22.2%, p<0.001). Worsening and recovering subphenotypes were replicated in the validation cohort. Routine laboratory tests, vital signs, and respiratory variables rather than demographics and comorbidities were predictive of the worsening and recovering subphenotypes. Conclusions. There are clear worsening and recovering subphenotypes of COVID-19 respiratory failure after intubation, which are more predictive of

outcomes than baseline severity of illness. Organ dysfunction trajectory may be well suited as a surrogate for research in COVID-19 respiratory failure. [**note: work from New York City to identify subphenotypes that experience poor clinical outcomes. Organ dysfunction trajectory may be key.**] https://www.medrxiv.org/content/10.1101/2020.07.16.20155382v1

- Purpose: The outcomes of patients requiring invasive mechanical ventilation for COVID-19 remain poorly defined. We sought to determine clinical characteristics and outcomes of patients with COVID-19 managed with invasive mechanical ventilation in an appropriately resourced US health care system. Methods: Outcomes of COVID-19 infected patients requiring mechanical ventilation treated within the Inova Health System between March 5, 2020 and April 26, 2020 were evaluated through an electronic medical record review. Results: 1023 COVID-19 positive patients were admitted to the Inova Health System during the study period. Of these, 165 (16.1%) were managed with invasive mechanical ventilation. At the time of data censoring, 63/165 patients (38.1%) had died and 102/165 (61.8%) were still alive. Of the surviving 102 patients, 17 (10.3%) remained on mechanical ventilation, 51 (30.9%) were extubated but remained hospitalized, and 34 (20.6%) had been discharged. Deceased patients were older (median age of 66 vs. 55, p <0.0001). 75.7% of patients over 70 years old had died at the time of data analysis. Conversely, 71.2% of patients age 70 or younger were still alive at the time of data analysis. Younger age, non-Caucasian race and treatment at a tertiary care center were all associated with survivor status. Conclusion: Mortality of patients with COVID-19 requiring invasive mechanical ventilation is high, with particularly daunting mortality seen in patients of advanced age, even in a well-resourced health care system. A substantial proportion of patients requiring invasive mechanical ventilation were not of advanced age, and this group had a reasonable chance for recovery. [**note: this is an outcome study from a Norther Virginia hospital system looking at patients on mechanical ventilation. Data is similar to other studies, older patients don't do well.**]
  https://www.medrxiv.org/content/10.1101/2020.07.16.20155580v1
- Since the start of the novel coronavirus 2019 (COVID-19) pandemic, corticosteroid use has been the subject of debate. The available evidence is uncertain, and knowledge on the subject is evolving. The aim of our cohort study was to evaluate the association between corticosteroid therapy and hospital mortality, in patients hospitalized with COVID-19 after balancing for possible confounders. Results: One thousand four hundred forty four patients were admitted to our hospital with a positive RT-PCR test for SARS-CoV-2, 559 patients (39%) were exposed to corticosteroids during hospital stay, 844 (61%) were not exposed to corticosteroids.In the cohort of patients exposed to corticosteroids, 171 (30.6%) died. In the cohort of patients not exposed to corticosteroids, 183 (21.7%) died (unadjusted p <0.001). Nonetheless, exposure to corticosteroids was not associated with in-hospital mortality after balancing with overlap weight propensity score (adjusted p = 0.25). Patients in the corticosteroids cohort had reduced risk of ICU admission (adjusted p <0.001). Conclusions: Treatment with corticosteroids did not affect hospital mortality in patients with COVID-19 after balancing for confounders. A possible advantage of corticosteroid therapy was to reduce Intensive Care Unit admission, which could be useful in reducing pressure on the Intensive Care Units in times of limited resources, as during the COVID-19 pandemic. [**note: here is an observational study from a single Italian hospital showing that dexamethasone may not affect mortality. This is opposite from the UK**

RECOVERY trial and the authors note this. Another confounding piece of evidence.]
https://www.medrxiv.org/content/10.1101/2020.07.17.20155994v1

- Despite the increasing evidence of the benefit of corticosteroids for the treatment of moderate-severe Coronavirus disease 2019 (COVID-19) patients, no data are available about the potential role of high doses of steroids for these patients. METHODS: All consecutive confirmed COVID-19 patients admitted to a single center were selected, including those treated with steroids and an acute respiratory distress syndrome (ARDS). Patients were allocated to the high doses (HD, 250mg/day or more of methylprednisolone) of corticosteroids or the standard doses (SD, 1.5mg/kg/day or more of methylprednisolone) at discretion of treating physician. The primary endpoint was the mortality between both cohorts and secondary endpoints were the risk of need for mechanical ventilation (MV) or death and the risk of developing a severe ARDS. RESULTS: 573 patients were included: 428 (74.7%) men, with a median (IQR) age of 64 (54-73) years. In HD cohort, a worse baseline respiratory situation was observed and male sex, older age and comorbidities were significantly more common. After adjusting by baseline characteristics, HD were associated with a higher mortality than SD (adjusted-OR 2.46, 95% CI 1.58-3.83, p<0.001) and with an increased risk of needing MV or death (adjusted-OR 2.50, p=0.001). Conversely, the risk of developing a severe ARDS was similar between groups. Interaction analysis showed that HD increased mortality exclusively in elderly patients. CONCLUSION: Our real-world experience advises against exceeding 1-1.5mg/kg/day of corticosteroids for severe COVID-19 with an ARDS, especially in older subjects. This reinforces the rationale of modulating rather than suppressing immune responses in these patients. [**note: this Spanish corticosteroid observational study suggests that high dose therapy should be avoided.**]
https://www.medrxiv.org/content/10.1101/2020.07.17.20156315v1

DRUG DEVELOPMENT

- Antiviral strategies to inhibit Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) and the pathogenic consequences of COVID-19 are urgently required. Here we demonstrate that the NRF2 anti-oxidant gene expression pathway is suppressed in biopsies obtained from COVID-19 patients. Further, we uncover that NRF2 agonists 4-octyl-itaconate (4-OI) and the clinically approved dimethyl fumarate (DMF) induce a cellular anti-viral program, which potently inhibits replication of SARS-CoV2 across cell lines. The anti-viral program extended to inhibit the replication of several other pathogenic viruses including Herpes Simplex Virus-1 and-2, Vaccinia virus, and Zika virus through a type I interferon (IFN)-independent mechanism. In addition, induction of NRF2 by 4-OI and DMF limited host inflammatory responses to SARS-CoV2 infection associated with airway COVID-19 pathology. In conclusion, NRF2 agonists 4-OI and DMF induce a distinct IFN-independent antiviral program that is broadly effective in limiting virus replication and suppressing the pro-inflammatory responses of human pathogenic viruses, including SARS-CoV2. [**note: another interesting finding about how SARS-CoV-2 works and a possible therapeutic approach. I suspect we will find more pathways that are affected by the virus. Too bad none of these approaches will never make it into the clinic.**]
https://www.biorxiv.org/content/10.1101/2020.07.16.206458v1
- Observational studies of the ongoing coronavirus disease 2019 (COVID-19) outbreak suggest that a cytokine storm is involved in the pathogenesis of severe illness. However, the molecular mechanisms underlying the altered pathological inflammation in COVID-19 are largely unknown.

We report here that toll-like receptor (TLR) 4-mediated inflammatory signaling molecules are upregulated in peripheral blood mononuclear cells (PBMCs) from COVID-19 patients, compared with healthy controls. Among the most highly increased inflammatory mediators in severe/critically ill patients, S100A9, an alarmin and TLR4 ligand, was found as a noteworthy biomarker, because it inversely correlated with the serum albumin levels. These data support a link between TLR4 signaling and pathological inflammation during COVID-19 and contribute to develop therapeutic approaches through targeting TLR4-mediated inflammation. [**note: and the very next preprint shows another signaling pathway that is impacted by SARS-CoV-2!**] https://www.biorxiv.org/content/10.1101/2020.07.17.207878v1

- Nucleotide analogs targeting viral RNA polymerase have been approved to be an effective strategy for antiviral treatment and are attracting antiviral drugs to combat the current SARS-CoV-2 pandemic. In this report, we develop a robust in vitro nonradioactive primer extension assay to evaluate the incorporation efficiency of nucleotide analog by SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) quantitively. Our results show that many nucleotide analogs can be incorporated into RNA by SARS-CoV-2 RdRp, and that the incorporation of some of them leads to chain termination. The discrimination values of nucleotide analog over those of natural nucleotide were measured to evaluate the incorporation efficiency of nucleotide analog by RdRp. We found that the incorporation efficiency of Remdesivir-TP is higher than ATP, and we did not observe chain termination or delayed chain termination caused by single Remdesivir-TP incorporation, while multiple incorporations of Remdesivir-TP caused chain termination in our assay condition. The incorporation efficiency of Ribavirin-TP and Favipiravir-TP is very low either as ATP or GTP analogs, which suggested that mutagenesis may not be the mechanism of action of those two drugs against SARS-CoV-2. Incorporation of Sofosbuvir-TP is also very low suggesting that sofosbuvir may not be very effective in treating SARS-CoV-2 infection. As a comparison, 2'-C-Methyl-GTP can be incorporated into RNA efficiently, and the derivative of 2'-C-Methyl-GTP may have therapeutic application in treating SARS-CoV-2 infection. This report provides a simple screening method that should be useful in evaluating nucleotide-based drugs targeting SARS-CoV-2 RdRp, and for studying the mechanism of action of selected nucleotide analog. [**note: drug discovery is hot today! These Chinese researchers come up with a simple screening method for evaluating nucleotide analogues that might inhibit the RNA polymerase enzyme.**] https://www.biorxiv.org/content/10.1101/2020.07.16.205799v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- A dysregulated immune response against the SARS-CoV-2 virus plays a critical role in severe COVID-19. However, the molecular and cellular mechanisms by which the virus causes lethal immunopathology are poorly understood. Here, we utilize multi-omics single-cell analysis to probe dynamic immune responses in patients with stable or progressive manifestations of COVID-19, and assess the effects of tocilizumab, an anti-IL-6 receptor monoclonal antibody. Coordinated profiling of gene expression and cell lineage protein markers reveals a prominent type-1 interferon response across all immune cells, especially in progressive patients. An anti-inflammatory innate immune response and a pre-exhaustion phenotype in activated T cells are hallmarks of progressive disease. Skewed T cell receptor repertoires in CD8 T cells and uniquely enriched V(D)J sequences are also identified in COVID-19 patients. B cell repertoire and somatic hypermutation analysis are consistent with a primary immune response, with possible

contribution from memory B cells. Our in-depth immune profiling reveals dyssynchrony of the innate and adaptive immune interaction in progressive COVID-19, which may contribute to delayed virus clearance and has implications for therapeutic intervention. **[note: more work on the immune system that may lead to delayed viral clearance. Someone is going to eventually figure all of this out.**] https://www.medrxiv.org/content/10.1101/2020.07.16.20153437v1

- SARS-CoV-2 is the causative agent of the current COVID-19 pandemic. A major virulence factor of SARS-CoVs is the nonstructural protein 1 (Nsp1) which suppresses host gene expression by ribosome association. Here, we show that Nsp1 from SARS-CoV-2 binds to the 40S ribosomal subunit, resulting in shutdown of mRNA translation both in vitro and in cells. Structural analysis by cryo-electron microscopy (cryo-EM) of in vitro reconstituted Nsp1-40S and various native Nsp1-40S and -80S complexes revealed that the Nsp1 C terminus binds to and obstructs the mRNA entry tunnel. Thereby, Nsp1 effectively blocks RIG-I-dependent innate immune responses that would otherwise facilitate clearance of the infection. Thus, the structural characterization of the inhibitory mechanism of Nsp1 may aid structure-based drug design against SARS-CoV-2. [**note: here is another way that SARS-CoV-2 can gum up the cells it infects. It can interfere with the immune response via this inhibition of protein synthesis. Maybe this is a potential drug target.**] https://science.sciencemag.org/content/early/2020/07/16/science.abc8665
- Currently, there is a need for reliable tests that allow identification of individuals that have been infected with SARS-CoV-2 even if the infection was asymptomatic. To date, the vast majority of the serological tests for SARS-CoV-2 specific antibodies are based on serum detection of antibodies to either the viral spike glycoprotein (the major target for neutralising antibodies) or the viral nucleocapsid protein that are known to be highly immunogenic in other coronaviruses. Conceivably, exposure of antigens released from infected cells could stimulate antibody responses that might correlate with tissue damage and, hence, they may have some value as a prognostic indicator. We addressed whether other non-structural viral proteins, not incorporated into the infectious viral particle, specifically the viral cysteine-like protease, might also be potent immunogens. Using ELISA tests, coating several SARS-CoV-2 proteins produced in vitro, we describe that COVID-19 patients make high titre IgG, IgM and IgA antibody responses to the Cys-like protease from SARS-CoV-2, also known as 3CLpro or Mpro, and it can be used to identify individuals with positive serology against the coronavirus. Higher antibody titres in these assays associated with more severe disease and no cross-reactive antibodies against prior betacoronavirus were found. Remarkably, IgG antibodies specific for Mpro and other SARS-CoV-2 antigens can also be detected in saliva. In conclusion, Mpro is a potent antigen in infected patients that can be used in serological tests and its detection in saliva could be the basis for a rapid, non-invasive test for COVID-19 seropositivity. [**note: wow, is this an interesting finding! Most of the time we focus on infectious agent coat proteins and here is a key enzyme of the virus that elicits an antibody response. Maybe this turns out to be a good diagnostic test target.**] https://www.medrxiv.org/content/10.1101/2020.07.16.20155853v1

DIAGNOSTIC DEVELOPMENT

- PCR methods are presently the standard for the diagnosis of Coronavirus disease 2019 (COVID-19), but additional methodologies are needed to complement PCR methods, which have some limitations. Here, we validated and investigated the usefulness of measuring serum antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using the iFlash3000 CLIA

analyzer. We measured IgM and IgG titers against SARS-CoV-2 in sera collected from 26 PCR-positive COVID-19 patients, 53 COVID-19-suspected but PCR-negative patients, and 20 and 100 randomly selected non-COVID-19 patients who visited our hospital in 2020 and 2017, respectively. The within-day and between-day precisions were regarded as good, since the coefficient variations were below 5%. Linearity was also considered good between 0.6 AU/mL and 112.7 AU/mL for SARS-CoV-2 IgM and between 3.2 AU/mL and 55.3 AU/mL for SARS-CoV-2 IgG, while the linearity curves plateaued above the upper measurement range. We also confirmed that the seroconversion and no-antibody titers were over the cutoff values in all 100 serum samples collected in 2017. These results indicate that this measurement system successfully detects SARS-CoV-2 IgM/IgG. We observed four false-positive cases in the IgM assay and no false-positive cases in the IgG assay when 111 serum samples known to contain autoantibodies were evaluated. The concordance rates of the antibody test with the PCR test were 98.1% for SARS-CoV-2 IgM and 100% for IgG among PCR-negative cases and 30.8% for SARS-CoV-2 IgM and 73.1% for SARS-CoV-2 IgG among PCR-positive cases. In conclusion, the performance of this measurement system is sufficient for use in laboratory testing. [**note: this is a Japanese validation of a new chemiluminescent antibody assay system looking at both N and S proteins.**] https://www.medrxiv.org/content/10.1101/2020.07.16.20155796v1

- Given the importance of the humoral immune response to SARS-CoV-2 as a global benchmark for immunity, a detailed analysis is needed to (i) monitor seroconversion in the general population, (ii) understand manifestation and progression of the disease, and (iii) predict the outcome of vaccine development. Currently available serological assays utilize single analyte technologies such as ELISA to measure antibodies against SARS-CoV-2 antigens including spike (S) or nucleocapsid (N) protein. To measure individual antibody (IgG and IgA) responses against SARS-CoV-2 and the endemic human coronaviruses (hCoVs) NL63, 229E, OC43, and HKU1, we developed a multiplexed immunoassay (CoVi-plex), for which we included S and N proteins of these coronaviruses in an expanded antigen panel. Compared to commercial in vitro diagnostic (IVD) tests our CoVi-plex achieved the highest sensitivity and specificity when analyzing 310 SARS-CoV-2 infected and 866 uninfected individuals. Simultaneously we see high IgG responses against hCoVs throughout all samples, whereas no consistent cross reactive IgG response patterns can be defined. In summary, our CoVi-plex is highly suited to monitor vaccination studies and will facilitate epidemiologic screenings for the humoral immunity toward pandemic as well as endemic coronaviruses. [**note: this is from Germany and shows how a multi-plex immunoassay using six antigenic determinnents can improve sensitivity and specificity.**] https://www.medrxiv.org/content/10.1101/2020.07.17.20156000v1

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2020-07-19

For reflection Sunday let's go to a pandemic performance of the Dvorak 8th Symphony. The venue is an empty Concertgebouw in Amsterdam. Listen to the introduction on how they set up the stage. It's a

wonderful performance directed by Gustavo Gimeno:
https://www.youtube.com/watch?v=EQTgC1KeIcI

The researchers who rung the COVID Symptom Study, a patient reported program, put out a release saying there are six distinct 'types' of COVID-19 that have major implications for treatment and monitoring. I'm not sure the viral infection can be observed in such a simplistic manner and a lot of us are familiar with flaws in patient reported outcomes studies. Most of these have also been reported in the many preprints I've read over the past four months. Still, they are to be applauded for launching a project that has over 4 million users.

The Washington Post discusses the specter of superspreading events. The Trump Administration is seeking to block new funding for track and trace efforts (I kid you not). Black people in Maine are 25% of the state's COVID-19 cases. No Mask, No Entry; one person's experience enforcing the rule. Many large nationwide retailers have mask only rules for shoppers. Will schools lose teachers if they reopen this fall? A Massachusetts researcher is conducting some interesting research into a preventative yearly Lyme vaccine using a well characterized antibody against the bacterium. In an earlier newsletter I conjectured that a similar approach could be done with SARS-CoV-2 antibodies and I believe Regeneron have a clinical trial designed to explore this.

The New York Times has a lengthy story on the Trump Administration's response to the pandemic. This small Washington state town suffers as the border between the US and Canada is closed.

Here is an article from CDC on how South Korea handled track and trace during the first quarter of this year. Highest rate of virus was found for household contacts of school age children (ages 10-19) which has implications for school reopenings. It is a lot of work to get this right, but the reward is diminished infection nationwide. This Swiss study shows no difference in viral load between children and adults at a single tertiary care hospital (53 children & 352 adults).

MODELING

- Successful public health regimes for COVID-19 push below unity long-term global R_t -- the average number of secondary cases caused by an infectious individual. Most assessments use local information. Populations differ in R_t, amongst themselves and over time. We use a SIR model for two populations to make the conceptual point that even if each locality averages $R_t < 1$, the overall epidemic can still grow, provided these populations have asynchronous variation in transmission, and are coupled by movement of infectious individuals. This emergent effect in pandemic dynamics instantiates "Parrondo's Paradox," -- an entity comprised of distinct but interacting units can behave qualitatively differently than each part on its own. For effective COVID-19 disease mitigation strategies, it is critical that infectious individuals moving among locations be identified and quarantined. This does not warrant indiscriminate prevention of movement, but rather rational, targeted testing and national coordination. [**note: this is from the University of Florida and I wonder if Gov. DeSanis is asking them about modeling the pandemic in this state. Any paper that refers to Parrondo's Paradox is a must read for me! I'm always interested in game theory, having cut my teeth on the Monte Hall problem.]**
  https://www.medrxiv.org/content/10.1101/2020.07.17.20155762v1

- We use an individual based model and national level epidemic simulations to estimate the medical costs of keeping the US economy open during COVID-19 pandemic under different counterfactual scenarios. We model an unmitigated scenario and 12 mitigation scenarios which differ in compliance behavior to social distancing strategies and to the duration of the stay-home order. Under each scenario we estimate the number of people who are likely to get infected and require medical attention, hospitalization, and ventilators. Given the per capita medical cost for each of these health states, we compute the total medical costs for each scenario and show the tradeoffs between deaths, costs, infections, compliance and the duration of stay-home order. We also consider the hospital bed capacity of each Hospital Referral Region (HRR) in the US to estimate the deficit in beds each HRR will likely encounter given the demand for hospital beds. We consider a case where HRRs share hospital beds among the neighboring HRRs during a surge in demand beyond the available beds and the impact it has in controlling additional deaths. [**note: this is from the Univ of Virginia and models medical costs across the US for different scenarios. The abstract doesn't say much about the costs and you need to look at the paper for this. The data is well presented and it is one of the better approaches to looking at this problem. Well worth reading!**]
  https://www.medrxiv.org/content/10.1101/2020.07.17.20156232v1

NEWLY REGISTERED CLINICAL TRIALS

- Did not look today

CLINICAL TRIAL RESULTS

- Nothing today

DRUG DEVELOPMENT

- SARS-CoV-2 is the pathogen responsible for the COVID-19 pandemic. The SARS-CoV-2 papain-like cysteine protease has been implicated in virus maturation, dysregulation of host inflammation and antiviral immune responses. We showed that PLpro preferably cleaves the K48-ubiquitin linkage while also being capable of cleaving ISG15 modification. The multiple functions of PLpro render it a promising drug target. Therefore, we screened an FDA-approved drug library and also examined available inhibitors against PLpro. Inhibitor GRL0617 showed a promising IC50 of 2.1 μM. The co-crystal structure of SARS-CoV-2 PLpro-C111S in complex with GRL0617 suggests that GRL0617 is a non-covalent inhibitor. NMR data indicate that GRL0617 blocks the binding of ISG15 to PLpro. The antiviral activity of GRL0617 reveal that PLpro is a promising drug target for therapeutically treating COVID-19. [**note: from China, the papain like protease is one of two the virus produces. The experimental compound appears to have been developed at the Univ of Illinois back in 2008 and inhibits both the original SARS coronavirus as well as this one.**] https://www.biorxiv.org/content/10.1101/2020.07.17.208959v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- In efforts to synthesize a clear understanding of SARS-CoV-2 protective immunity, antibody analysis has been paralleled by T cell studies across asymptomatic, mild and severe COVID-19. Defining CD4 and CD8 effector functions in protection is important considering that antibody responses appear short-lived and T cell memory is potentially more durable. To fully understand

population level immunity, screening for both antibody and T cell immunity using standardized testing methods would be beneficial. [**note: this is a good perspective on T cell immunity to SARS-CoV-2. I don't know if it is behind a paywall. If you are a member of AAAS it is free to read.**] https://immunology.sciencemag.org/content/5/49/eabd6160

- Although critical illness has been associated with SARS-CoV-2-induced hyperinflammation, the immune correlates of severe COVID-19 remain unclear. Here, we comprehensively analyzed peripheral blood immune perturbations in 42 SARS-CoV-2 infected and recovered individuals. We identified extensive induction and activation of multiple immune lineages, including T cell activation, oligoclonal plasmablast expansion, and Fc and trafficking receptor modulation on innate lymphocytes and granulocytes, that distinguished severe COVID-19 cases from healthy donors or SARS-CoV-2-recovered or moderate severity patients. We found the neutrophil to lymphocyte ratio to be a prognostic biomarker of disease severity and organ failure. Our findings demonstrate broad innate and adaptive leukocyte perturbations that distinguish dysregulated host responses in severe SARS-CoV-2 infection and warrant therapeutic investigation. [**note: this is from UPenn and has a comprehensive map of immune perturbations associated with severe COVID-19.**] https://immunology.sciencemag.org/content/5/49/eabd7114
- Global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues unabated. Binding of SARS-CoV-2's Spike protein to host angiotensin converting enzyme 2 triggers viral entry, but other proteins may participate, including neuropilin-1 receptor (NRP-1). As both Spike protein and vascular endothelial growth factor-A (VEGF-A) - a pro-nociceptive and angiogenic factor, bind NRP-1, we tested if Spike could block VEGF-A/NRP-1 signaling. VEGF-A-triggered sensory neuronal firing was blocked by Spike protein and NRP-1 inhibitor EG00229. Pro-nociceptive behaviors of VEGF-A were similarly blocked via suppression of spontaneous spinal synaptic activity and reduction of electrogenic currents in sensory neurons. Remarkably, preventing VEGF-A/NRP-1 signaling was antiallodynic in a neuropathic pain model. A 'silencing' of pain via subversion of VEGF-A/NRP-1 signaling may underlie increased disease transmission in asymptomatic individuals. [**note: it seems that every place one looks, this virus has some effect. This is an interesting finding but how many people experience these symptoms?**] https://www.biorxiv.org/content/10.1101/2020.07.17.209288v1
- The responses of specific immune cell populations to the disease remain poorly defined, which hinders improvements in treatment and care management. Here, we utilized mass cytometry (CyTOF) to thoroughly phenotype peripheral myeloid cells and T lymphocytes from 30 convalescent patients with mild, moderate, and severe cases of COVID-19. We identified 10 clusters of monocytes and dendritic cells and 17 clusters of T cells. Examination of these clusters revealed that both CD14+CD16+ intermediate and CD14dimCD16+ nonclassical monocytes, as well as CD4+ stem cell memory T (TSCM) cells, correlated with COVID-19 severity, coagulation factor levels, and/or inflammatory indicators. We also identified two nonclassical monocyte subsets distinguished by expression of the sugar residue 6-Sulfo LacNac (Slan). One of these subsets (Slanlo, nMo1) was depleted in moderately and severely ill patients, while the other (Slanhi, nMo2) increased with disease severity and was linked to CD4+ T effector memory (TEM) cell frequencies, coagulation factors, and inflammatory indicators. Intermediate monocytes tightly correlated with loss of naive T cells as well as an increased abundance of effector memory T cells expressing the exhaustion marker PD-1. Our data suggest that both intermediate and non-classical monocyte subsets shape the adaptive immune response to SARS-CoV-2. In

summary, our study provides both broad and in-depth characterization of immune cell phenotypes in response to COVID-19 and suggests functional interactions between distinct cell types during the disease. [**note: more on the immune response. This paper from La Jolla looks at monocytes and T cells.**] https://www.biorxiv.org/content/10.1101/2020.07.17.209304v1

DIAGNOSTIC DEVELOPMENT

- We prospectively compared healthcare worker-collected nasopharyngeal swabs (NPS) to self-collected anterior nasal swabs (ANS) and straight saliva for the diagnosis of COVID-19 in 354 patients. The positive percent agreement between NPS and ANS or saliva was 86.3% (95% CI: 76.7-92.9) and 93.8% (95% CI: 86.0-97.9), respectively. Negative percent agreement was 99.6% (95% CI: 98-100) for NPS vs. ANS and 97.8% (95% CI: 95.3 – 99.2) for NPS vs. saliva. NPS (n=80) and saliva (n=81) detected more cases than ANS (n=70), but no single specimen type detected all SARS-CoV2 infections. [**note: from Utah a comparison of sample collection methods in healthcare workers. Saliva collection looks to be reasonable and easy to do even if it is somewhat less accurate.**] https://www.medrxiv.org/content/10.1101/2020.07.17.20155754v1
- Serological testing to evaluate antigen-specific antibodies in plasma is generally performed by rapid lateral flow test strips that lack quantitative results or by high complexity immunoassays that are time- and labor-intensive but provide quantitative results. Here, we describe a novel application of biolayer interferometry for the rapid detection of antigen-specific antibody levels in plasma samples, and demonstrate its utility for quantification of SARS-CoV-2 antibodies. Our biolayer interferometry immunosorbent assay (BLI-ISA) utilizes single-use biosensors in an automated "dip-and-read" format, providing real-time optical measurements of antigen loading, plasma antibody binding, and antibody isotype detection. Complete quantitative results are obtained in less than 20 minutes. BLI-ISA meets or exceeds the performance of high complexity methods such as Enzyme-Linked Immunosorbent Assay (ELISA) and Chemiluminescent Immunoassay. Importantly, our method can be immediately implemented on existing BLI platforms for urgent COVID-19 studies, such as serosurveillance and the evaluation of vaccine candidates. In a broader sense, BLI-ISA can be developed as a novel diagnostic platform to evaluate antibodies and other biomolecules in clinical specimens. [**note: another novel way of testing serology samples. I don't know how widely deployed the instrumentation for this approach is but 20 minutes to results is quite appealing.**] https://www.medrxiv.org/content/10.1101/2020.07.17.20156281v1
- Antibody neutralization is an important prognostic factor in many viral diseases. To easily and rapidly measure titers of neutralizing antibodies in serum or plasma, we developed pseudovirion particles composed of the spike glycoprotein of SARS-CoV-2 incorporated onto murine leukemia virus capsids and a modified minimal MLV genome encoding firefly luciferase. These pseudovirions provide a practical means of assessing immune responses under laboratory conditions consistent with biocontainment level 2. [**note: a novel antibody neutralization assay from these Utah scientists.**] https://www.biorxiv.org/content/10.1101/2020.07.17.207563v1