2020-08-31

Welcome to Week 24 of the Pandemic Newsletter

Let's stay with Dvorak for another day. Here is the fine Serenade for Strings with the Netherlands Chamber Orchestra. It is a live performance from the Concertgebouw, filmed in 2016: https://www.youtube.com/watch?v=CRcbDMg56yg

US COVID-19 STATISTICS - **Infection Rate: 1.8%; CFR: 3.0%** (IR up O.1%; CFR down 0.1%; **note:** the CFR for this current outbreak continues to hover at 2%)

The Washington Post has more on the politics behind the FDA decision on convalescent plasma. The new medical advisor to President Trump is pushing a herd immunity strategy. Should we be more like Sweden? Certainly there are parts of the US that have inadvertently (or ignorantly, I'm unsure about this) adopted this strategy. Here is the WaPo story on the Maine wedding that turned out to be a super spreader event. We can learn from Japan how to protect the elderly among us from the ravages of COVID-19.

According to the New York Times, with wealth there come privilege at least as far as getting quick tests for COVID-19. COVID-19 cases among children are rising fast along with hospitalizations and mortality.

The Guardian weighs in on a potential US regulatory decision on a COVID-19 vaccine. This flight from Greece to the UK did not end well for the passengers.

My Yahoo newsfeed gives me an intriguing story about potential weaknesses of the Russian and Chinese adenovirus COVID-19 vaccines. Other companies have selected viral vectors that are less rare to human immune systems.

STAT have a story on the Vir-GSK monoclonal antibody that is about to enter clinical trials. Execs from the two companies think they may have a better mAb. One wonders what impact the FDA EUA on convalescent plasma will have on this study. Maybe that should be used as the 'placebo' arm as patients may be reluctant to accept standard of care.

MODELING

- We document four facts about the COVID-19 pandemic worldwide relevant for those studying the impact of non-pharmaceutical interventions (NPIs) on COVID-19 transmission. First: across all countries and U.S. states that we study, the growth rates of daily deaths from COVID-19 fell from a wide range of initially high levels to levels close to zero within 20-30 days after each region experienced 25 cumulative deaths. Second: after this initial period, growth rates of daily deaths have hovered around zero or below everywhere in the world. Third: the cross section standard deviation of growth rates of daily deaths across locations fell very rapidly in the first 10 days of the epidemic and has remained at a relatively low level since then. Fourth: when interpreted through a range of epidemiological models, these first three facts about the growth rate of COVID deaths imply that both the effective reproduction numbers and transmission rates of COVID-19 fell from widely dispersed initial levels and the effective reproduction number has hovered around one after the first 30 days of the epidemic virtually everywhere in the world. We argue that failing to account for these four stylized facts may result in overstating the importance of policy mandated NPIs for shaping the progression of this deadly pandemic.

[**note: this is from the National Bureau of Economic Research and I picked up from the Marginal Revolution blog. The authors are economists and while it is an interesting read they, as have many others, try to identify elusive factors governing transmission and making arguments that various interventions are good, bad or indifferent. Most papers of this ilk tend to focus on mortality which is a pretty definite end point. Few if any look at the real reason NPIs were taken, to avoid crushing the health care system. Of course we expect (certainly I did) that mortality rates would drop from the scary levels seen in Lombardy this past March but this does not mean that hospitalizations would show a corresponding decrease. We have seen in many US communities high levels of hospitalization. As we still don't do a good job of testing, it is not clear at all what the true case fatality rate is. I can only go by published numbers and not conjecture. Since the summer outbreak began, it continues to hover at the 2% mark and we are still over 3% since the pandemic began in the US. One can make arguments based on stylized models, but if these three are so convinced that NPIs are not so important then I want to know whether they practice social distancing or wear masks. Just looking at a model does not necessarily predict what is happening in real time. I think there are other flaws with this paper but I don't want to spend the rest of what looks to be a nice Sunday gong over them. I'll let others read the paper and draw their own conclusions.**]
https://www.nber.org/papers/w27719.pdf

- We report the stability of SARS-CoV-2 on various surfaces under indoor, summer and spring/fall conditions. The virus was more stable under the spring/fall condition with virus half-lives ranging from 17.11 to 31.82 hours, whereas under indoor and summer conditions the virus half-lives were 3.5-11.33 and 2.54-5.58 hours, respectively. [**note: another study of surface persistence of SARS-CoV-2. While interesting, it is just a lab study and won't force me to change any of the precautions I am currently taking to keep my house COVID-19 free.**]
https://www.biorxiv.org/content/10.1101/2020.08.30.274241v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check sometime this week as the number of newly registered trials is way down.

CLINICAL TRIAL RESULTS

- Sadly, nothing new

DRUG DEVELOPMENT

- With the rapid rate of Covid-19 infections and deaths, treatments and cures besides hand washing, social distancing, masks, isolation, and quarantines are urgently needed. The treatments and vaccines rely on the basic biophysics of the complex viral apparatus. While proteins are serving as main drug and vaccine targets, therapeutic approaches targeting the 30,000 nucleotide RNA viral genome form important complementary approaches. Indeed, the high conservation of the viral genome, its close evolutionary relationship to other viruses, and the rise of gene editing and RNA-based vaccines all argue for a focus on the RNA agent itself. One of the key steps in the viral replication cycle inside host cells is the ribosomal frameshifting required for translation of overlapping open reading frames. The frameshifting element (FSE), one of three highly conserved regions of coronaviruses, includes an RNA pseudoknot considered essential for this ribosomal switching. In this work, we apply our graph-theory-based framework

for representing RNA secondary structures, "RAG" (RNA-As Graphs), to alter key structural features of the FSE of the SARS-CoV-2 virus. Specifically, using RAG machinery of genetic algorithms for inverse folding adapted for RNA structures with pseudoknots, we computationally predict minimal mutations that destroy a structurally-important stem and/or the pseudoknot of the FSE, potentially dismantling the virus against translation of the polyproteins. Additionally, our microsecond molecular dynamics simulations of mutant structures indicate relatively stable secondary structures. These findings not only advance our computational design of RNAs containing pseudoknots; they pinpoint to key residues of the SARS-CoV-2 virus as targets for anti-viral drugs and gene editing approaches. [**note: this is an interesting paper from NYU on the possibility of targeting the viral RNA as a therapeutic target.**] https://www.biorxiv.org/content/10.1101/2020.08.28.271965v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Global health has been threatened by the COVID-19 pandemic, caused by the novel severe acute respiratory syndrome coronavirus (SARS-CoV-2). Although considered primarily a respiratory infection, many COVID-19 patients also suffer severe cardiovascular disease. Improving patient care critically relies on understanding if cardiovascular pathology is caused directly by viral infection of cardiac cells or indirectly via systemic inflammation and/or coagulation abnormalities. Here we examine the cardiac tropism of SARS-CoV-2 using human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) and three-dimensional engineered heart tissues (3D-EHTs). We observe that hPSC-CMs express the viral receptor ACE2 and other viral processing factors, and that SARS-CoV-2 readily infects and replicates within hPSC-CMs, resulting in rapid cell death. Moreover, infected hPSC-CMs show a progressive impairment in both electrophysiological and contractile properties. Thus, COVID-19-related cardiac symptoms likely result from a direct cardiotoxic effect of SARS-CoV-2. Long-term cardiac complications might be possible sequelae in patients who recover from this illness. [**note: this might be a model for cardiac effects of SARS-CoV-2**]
https://www.biorxiv.org/content/10.1101/2020.08.30.274464v1
- The COVID-19 pandemic presents an urgent health crisis. Human neutralizing antibodies (hNAbs) that target the host ACE2 receptor-binding domain (RBD) of the SARS-CoV-2 spike[1-5] show therapeutic promise and are being evaluated clincally[6-8]. To determine structural correlates of SARS-CoV-2 neutralization, we solved 8 new structures of distinct COVID-19 hNAbs[5] in complex with SARS-CoV-2 spike trimer or RBD. Structural comparisons allowed classification into categories: (1) VH3-53 hNAbs with short CDRH3s that block ACE2 and bind only to up RBDs, (2) ACE2-blocking hNAbs that bind both up and down RBDs and can contact adjacent RBDs, (3) hNAbs that bind outside the ACE2 site and recognize up and down RBDs, and (4) Previously-described antibodies that do not block ACE2 and bind only up RBDs[9]. Class 2 comprised four hNAbs whose epitopes bridged RBDs, including a VH3-53 hNAb that used a long CDRH3 with a hydrophobic tip to bridge between adjacent down RBDs, thereby locking spike into a closed conformation. Epitope/paratope mapping revealed few interactions with host-derived N-glycans and minor contributions of antibody somatic hypermutations to epitope contacts. Affinity measurements and mapping of naturally-occurring and in vitro-selected spike mutants in 3D provided insight into the potential for SARS-CoV-2 escape from antibodies elicited during infection or delivered therapeutically. These classifications and structural analyses

provide rules for assigning current and future human RBD-targeting antibodies into classes, evaluating avidity effects, suggesting combinations for clinical use, and providing insight into immune responses against SARS-CoV-2. [**note: from a Cal Tech and Rockfeller Univ team, structural classification of neutralizing antibodies.**]
https://www.biorxiv.org/content/10.1101/2020.08.30.273920v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

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2020-09-01

How have gone this long without ever once posting a clip of the wonderful Silk Road Ensemble? (Better question is why my readers have not written to me about this omission? 😉) Let us correct this right now! Here is the ensemble performing 'Arabian Waltz': https://www.youtube.com/watch?v=fZpEjrdf56k You can read more about the ensemble that was formed out of a project started by cellist Yo-Yo Ma. My wife and I saw them live as part of the Inauguration Week festivities back in 2008 which was a special treat. We had front row tickets right next to WaPo columnist E.J. Dionne. There is a wonderful documentary of the group that HBO did several years ago, 'The Music of Strangers: Yo-Yo Ma and The Silk Road Ensemble' that can be found on a number of streaming sites (Google is your friend) and here is a nice clip of a concert following the Berlin Film Festival screening: https://www.youtube.com/watch?v=BWYo388T1XU

US COVID-19 STATISTICS - **Infection Rate: 1.8%; CFR: 3.0%** (IR no change; CFR no change; **note:** the CFR for this current outbreak continues to hover at 2%)

The Washington Post has a very good summary of research into using Interferon 1β as a way to boost the immune system in COVID-19 patients. I've linked to a number of preprints in the past and NIH has a large trial going on right now. The Cuban biotech group developed a synthetic Interferon 2α that they found to be useful in-patient treatment there. I have not seen any results of this interferon version other than from Cuba. Here is an explanation of 'herd immunity' and a cute interactive that shows how to achieve it. This is why vaccine conspiracy theories are so dangerous (no editorial comment, just read the story). Maybe these folks don't read many firsthand accounts of young people with post COVID-19 complications.

Two worthwhile op-ed pieces in The New York Times: one on the need for greater testing despite what CDC may be saying and the second on the need for stepping up clinical research (one of the coauthors of the 2nd is my good friend Zeke Emanuel; I am glad that others are taking up this cause which outlined in my April white paper). There is an article on the DIY vaccine efforts and how one of these is running afoul of the FDA. Point Roberts WA looks like a safe place to live (no COVID-19 here!) but you can't get out right now because of the need to cross over to Canada before getting back into the US. Here is a documentary clip on how to stop the next pandemic. Some good news! The US is reviving the global virus hunting effort that was foolishly terminated last year. Finally, exercise may boost your immune response!! Get up and work out!

The Atlantic weighs in on contact tracing.

Nature have an article on how to measure COVID-19 mortality.

Medscape offer a harsh open letter from Eric Topol to FDA Commissioner Hahn. Almost one quarter of children hospitalized in China develop ocular symptoms. Fortunately, the symptoms resolved.

The British Medical Journal publishes a large retrospective study on COVID-19 cases in children at 138 UK hospitals. Children and young people have less severe acute covid-19 than adults. A systemic mucocutaneous-enteric symptom cluster was also identified in acute cases that shares features with MIS-C. This study provides additional evidence for refining the WHO MIS-C preliminary case definition. Children meeting the MIS-C criteria have different demographic and clinical features depending on whether they have acute SARS-CoV-2 infection (polymerase chain reaction positive) or are post-acute (antibody positive).

Derek Lowe on cold chain distribution and it's not a boring issue if you are a vaccine manufacturer! Read the comments as there are some cold chain experts weighing in.

There was a deluge of preprints today and I've tried to curate the most important ones.

MODELING

- Background: About 15 million people worldwide were affected by the Sars-Cov-2 infection, which already caused 600,000 deaths. This virus is mainly transmitted through exhalations from the airways of infected persons, so that Heating, Ventilation and Air Conditioning (HVAC) systems might play a role in spreading the infection in indoor environments. Methods: We modelled the role of HVAC systems in the diffusion of the contagion through a Computational Fluid Dynamics (CFD) simulations of cough at the Vatican State childrens hospital Bambino Gesu. Both waiting rooms and hospital rooms were modeled as indoor scenarios. A specific Infection-Index parameter was used to estimate the amount of contaminated air inhaled by each person present in the simulated indoor scenarios. The potential role of exhaust air ventilation systems placed above the coughing patients mouth was also assessed. Results: Our CFD-based simulations show that HVAC air-flow remarkably enhance infected droplets diffusion in the whole indoor environment within 25 seconds from the cough event, despite the observed dilution of saliva particles containing the virus. In the waiting room simulation, Infection-Index parameter increases the faster the higher the HVAC airflow. Greater flows of air conditioning correspond to greater diffusion of the infected droplets. The proper use of Local Exhaust Ventilation systems (LEV) simulated in the hospital room was associated to a complete reduction of infected droplets spreading from the patient s mouth in the first 0.5 seconds following the cough event. In the hospital room, the use of LEV system completely reduced the index computed for the patient hospitalized at the bed next to the spreader, with a decreased possibility of contagion. Conclusions: CFD-based simulations for indoor environment can be useful to optimize air conditioning flow and to predict the contagion risk both in hospitals/ambulatories and in other public/private settings. [**note: here is some work from Italy looking at the role of HVAC systems in the spread of SARS-CoV-2 in an enclosed environment. Set up of HVAC systems might be important in indoor spread of the virus.**]
  https://www.medrxiv.org/content/10.1101/2020.08.25.20181420v1

- Introduction: Recent outbreaks of COVID-19 in universities across the United States highlight the difficulties in containing the spread of COVID-19 on college campuses. While research has shown that mitigation strategies such as frequent student testing, contact tracing, and isolation of confirmed and suspected cases can detect early outbreaks, such mitigation strategies may have limited effectiveness if large outbreaks occur. A phased reopening is a practical intervention to limit early outbreaks, conserve institutional resources, and ensure proper safety protocols are in place before the return of additional students to campus. Methods: We develop dynamic compartmental transmission models of SARS-CoV-2 to assess the impact of a phased reopening and pre-arrival testing on minimizing outbreaks (measured by daily infections) and conserving university resources (measured by isolation bed capacity). We assume that one-third of the student population returns to campus each month as part of the phased reopening, and that pre-arrival testing removes 90% of infections at the semester start. We assume an on-campus population of N = 7500, an active COVID-19 prevalence of 2% at baseline, and that 60% of infected students require isolation for an average period of 11 days. We vary the reproductive number (Rt) between 1.25 and 4 to represent the effectiveness of alternative mitigation strategies throughout the semester, where Rt is constant or improving throughout the semester (ranging from 4 to 1.25). Results: Compared to pre-arrival testing only or neither intervention, phased reopening with pre-arrival testing reduced peak daily infections by 6% and 18% (Rt=1.25), 44% and 48% (Rt=2.5), 63% and 64% (Rt=4), and 72% and 74% (improving Rt), respectively, and reduced the proportion of on-campus beds needed for isolation from 10%-25% to 5%-9% across different values of Rt. Conclusion: Phased reopening with pre-arrival testing substantially reduces the peak number of daily infections throughout the semester and conserves university resources compared to strategies involving the simultaneous return of all students to campus. Phased reopenings allow institutions to improve safety protocols, adjust for factors that drive outbreaks, and if needed, preemptively move online before the return of additional students to campus, thus preventing unnecessary harm to students, institutional faculty and staff, and local communities. [**note: this model for college reopening comes from Clemson Univ and points to a phased opening as the best approach. No word on whether this will have any impact on the Clemson football program.**]
  https://www.medrxiv.org/content/10.1101/2020.08.25.20182030v1
- Background: Evaluate the correlation between U.S. state mandated social interventions and Covid-19 mortality using a retrospective analysis of Institute for Health Metrics and Evaluation (IHME) data. Methods: Twenty-seven (27) states in the United States were selected on June 17, 2020 from IHME data which had clearly defined and dated establishment of statewide mandates for social distancing measures to include: School closures, Prohibition on mass gatherings, business closures, stay at home orders, severe travel restrictions, and closure of non-essential businesses. The state Covid-19 mortality prevalence was defined as total normalized deaths to the peak daily mortality rate. The state mortality prevalence was correlated to the total number of mandates-days from their date of establishment to the peak daily mortality date. The slope of the maximum daily mortality rate was also correlated to mandate-days. Results: The standardized mortality per state to the initial peak mortality rate did not demonstrate a discernable correlation to the total mandate days (R2 = 0.000006, p= 0.995). The standardized peak mortality rate per state suggested a slight correlation to the total mandate days (R2 = 0.053,p=0.246), but was not statistically significant. There was a significant correlation between

standardized mortality and state population density (R2 = 0.524,p=0.00002). Conclusions: The analysis appears to suggest no mandate effective reduction in Covid-19 mortality nor a reduction in Covid-19 mortality rate to its defined initial peak when interpreting the mean-effect of the mandates as present in the data. A strong correlation to population density suggests human interaction frequency does affect the total mortality and maximum mortality rate. [**note: I am posting this abstract as the paper provides a counter-factual (IMO) analysis of state mandates and mortality. I briefly skimmed the paper and will let others pull apart the statistical analysis. I will not be surprised at all if some use this to argue against governmental mandates. As I have often noted, mandates are not just put in place to control mortality but also to minimize overburdening the healthcare system. I don't know of any studies done in this way that look at whether there is a correlation with mandates and hospitalization. That would be a useful study to do.]**
https://www.medrxiv.org/content/10.1101/2020.08.26.20182758v1

- After the COVID-19 outbreak, China immediately adopted stringent lockdown policies to contain the virus. Using comprehensive death records covering around 300 million Chinese people, we estimate the impacts of city and community lockdowns on non-COVID-19 mortality outside of Wuhan. Employing a difference-in-differences method, we find that lockdowns reduced the number of non-COVID-19 deaths by 4.9% (cardiovascular deaths by 6.2%, injuries by 9.2%, and non-COVID-19 pneumonia deaths by 14.3%). The health benefits are likely driven by significant reductions in air pollution, traffic, and human interactions. A back-of-the-envelope calculation shows that more than 32,000 lives could have been saved from non-COVID-19 diseases/causes during the 40 days of the lockdown on which we focus. The results suggest that the rapid and strict virus countermeasures not only effectively controlled the spread of COVID-19 but also brought about massive unintended public health benefits. These findings can help better inform policymakers around the world about the benefits and costs of city and community lockdowns policies in dealing with the COVID-19 pandemic. [**note: oh irony of ironies; just as I get finished writing notes to the above paper, the next one up in the queue is from China looking at the exact same issue!! It comes to a different conclusion. As for me, I'm still physical distancing and wearing a mask when I go out on errands; just doing my part to help out**]
https://www.medrxiv.org/content/10.1101/2020.08.28.20183699v1
- The revival of the global economy is being predicated on the Six-Foot Rule, a guideline that offers little protection from pathogen-bearing droplets sufficiently small to be continuously mixed through an indoor space. The importance of indoor, airborne transmission of COVID-19 is now widely recognized; nevertheless, no quantitative measures have been proposed to protect against it. In this article, we build upon models of airborne disease transmission in order to derive a safety guideline that would impose a precise upper bound on the cumulative exposure time, the product of the number of occupants and their time in an enclosed space. We demonstrate the manner in which this bound depends on the ventilation rate and dimensions of the room; the breathing rate, respiratory activity and face-mask use of its occupants; and the infectiousness of the respiratory aerosols, a disease-specific parameter that we estimate from available data. Case studies are presented, implications for contact tracing considered, and appropriate caveats enumerated. [**note: this is from MIT and has a good guideline at limiting indoor airborne transmission of SARS-CoV-2. It is a useful paper to download as it has**

references to case studies and past research.]
https://www.medrxiv.org/content/10.1101/2020.08.26.20182824v1

NEWLY REGISTERED CLINICAL TRIALS

- Don't you have enough reading to do today?😀

CLINICAL TRIAL RESULTS

- Background: It remains unclear whether COVID-19 is associated with psychiatric symptoms during or after the acute illness phase. Being affected by the disease exposes the individual to an uncertain prognosis and a state of quarantine. These factors can predispose individuals to the development of mental symptoms during or after the acute phase of the disease. There is a need for prospective studies assessing mental health symptoms in COVID-19 patients in the post-infection period. Methods: In this retrospective cohort study, nasopharyngeal swabs for COVID-19 tests were collected at patients homes under the supervision of trained healthcare personnel. Patients who tested positive for COVID-19 and were classified as mild cases (N=895) at treatment intake were further assessed for the presence of mental health disorders (on average, 56.6 days after the intake). We investigated the association between the number of COVID-19 symptoms at intake and depression, anxiety and PTSD, adjusting for previous mental health status, time between baseline and outcome, and other confounders. Multivariate logistic regression and generalized linear models were employed for categorical and continuous outcomes, respectively. Findings: Depression, anxiety and PTSD were reported by 26.2% (N=235), 22.4% (N=201), and 17.3% (N=155) of the sample. Reporting an increased number of COVID-related symptoms was associated with depression (aOR=1.059;95%CI=1.002-1.119), anxiety (aOR=1.072;95%CI=1.012-1.134), and PTSD (aOR=1.092;95%CI=1.024-1.166). Sensitivity analyses supported findings for both continuous and categorical measures. Interpretation: Exposure to an increased number of COVID-19 symptoms may predispose individuals to depression, anxiety and PTSD after the acute phase of the disease. These patients should be monitored for the development of mental health disorders after COVID-19 treatment discharge. Early interventions, such as brief interventions of psychoeducation on coping strategies, could benefit these individuals. [**note: from Columbia Univ, a look at some psychiatric symptoms accompanying recovery from mild COVID-19 cases. This is something that needs to be pursued further.**] https://www.medrxiv.org/content/10.1101/2020.08.25.20182113v1
- Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has led to the global coronavirus disease 2019 (COVID-19) pandemic. SARS-CoV-2 enters cells via angiotensin-Converting Enzyme 2 (ACE2) receptors, highly expressed in nasal epithelium with parallel high infectivity. We collected nasal swabs from anterior nares of 547 children, measured DNA methylation (DNAm), and tested differences at 15 ACE2 CpGs by sex, age, race/ethnicity and epigenetic age. ACE2 CpGs were differentially methylated by sex with 12 sites having lower DNAm (mean=12.71%) and 3 sites greater DNAm (mean=1.45%) among females. We observed differential DNAm at 5 CpGs for Hispanic females (mean absolute difference=3.22%) and 8 CpGs for Black males (mean absolute difference=1.33%), relative to white participants. Longer DNAm telomere length was associated with greater DNAm at 11 and 13 CpGs among males (mean absolute difference=7.86%) and females (mean absolute difference=8.21%), respectively. Nasal ACE2 DNAm differences could contribute to our understanding COVID-19 severity and

disparities. [**note: from one of the Harvard teaching hospitals, a broad look at DNA methylation in children. More interesting stuff regarding differences in sex and ethnicity. It will be interesting is this can also be linked to more severe outcomes.**]
https://www.medrxiv.org/content/10.1101/2020.08.25.20182105v1

- Rationale: whether systemic dissemination of SARS-CoV-2 has any impact on COVID-19 severity and also on the immunological alterations observed in this disease is largely unknown. Objectives: We determined the association of plasma SARS-CoV-2 RNA with clinical severity, laboratory findings and immunological parameters in a cohort of 250 patients with confirmed COVID-19 infection. Methods: Three groups of patients were studied: 50 outpatients, 100 hospitalised ward patients, and 100 critically ill. The association between plasma SARS-CoV-2 RNA and severity was evaluated using multivariate ordinal logistic regression analysis and Generalized Linear Model (GLM) analysis with a binomial distribution. The association between plasma SARS-CoV-2 RNA and laboratory parameters was evaluated using multivariate GLM with a gamma distribution. Measurements and Main Results: The prevalence of SARS-CoV-2-RNA viremia increased in parallel with severity of infection (22% in outpatients, 36 % in those hospitalised in wards, and 82% in those at the ICU). In hospitalised patients, the presence of SARS-CoV-2-RNA viremia was independently associated to critical illness: (adjusted OR= 8.30 [CI95%=4.21 - 16.34], $p < 0.001$). SARS-CoV-2-RNA viremia was an independent predictor of higher levels of ferritin, LDH and cytokines (involving CXCL10, CCL-2, IL-15, IL-10, IL-1ra and GCS-F), and lower of lymphocytes, monocytes and platelets counts Conclusions: SARS-CoV-2-RNA viremia is a robust marker of critical illness in COVID-19. Our findings support that hypercytokinemia in COVID-19 is a reactive event in response to the dissemination of viral material at the systemic level. [**note: this is from Spain and looks at viremia and associated hypercytokinemia and critical illness. No surprise that viremia is a robust marker of critical illness but it's good to have this confirmatory data.**]
https://www.medrxiv.org/content/10.1101/2020.08.25.20154252v1
- Infection by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been associated with leukopenia and uncontrolled inflammatory response in critically ill patients. A better comprehension of SARS-CoV-2-induced monocytes death is essential for the identification of therapies capable to control the hyper-inflammation and reduce viral replication in patients with COVID-19. Here, we show that SARS-CoV-2 induces inflammasome activation and cell death by pyroptosis in human monocytes, experimentally infected and in patients under intensive care. Pyroptosis was dependent on caspase-1 engagement, prior to IL-1beta production and inflammatory cell death. Monocytes exposed to SARS-CoV-2 downregulate HLA-DR, suggesting a potential limitation to orchestrate the immune response. Our results originally describe the mechanism by which monocytes, a central cellular component recruited from peripheral blood to respiratory tract, succumb in patients with severe 2019 coronavirus disease (COVID-19), and emphasize the need for identifying anti-inflammatory and antiviral strategies to prevent SARS-CoV-2-induced pyroptosis. [**note: from Brazil, a look at the inflammatory response and cell death of monocytes. The abstract does not mention that this can be prevented by atazaniavir but it is just an *in vitro* observation. They do have a clinical trial registered for this and several other antiviral agents: NCT04468087**]
https://www.medrxiv.org/content/10.1101/2020.08.25.20182055v1

- Background: During the current pandemic, a great effort is made to understand the COVID-19 and find an effective treatment. As of 17 August 2020, there is no specific drug or biologic agent which have been approved by the FDA for the prevention or treatment of COVID-19. Methods: We retrospectively analyzed the clinical and radiological findings of 211 COVID-19 in-patients that were treated between March - August 2020. Confirmation of a COVID-19 diagnosis was made according to a positive RT-PCR result with a consistent high-resolution-CT (HRCT) finding. Radiological images and the rate of clinical response of patients were investigated. Result: While 128 patients (58.7) did not develop pneumonia, the mild, moderate and severe pneumonia ratios were 28(13.2%), 31(18.7%) and 27(22.9%). 72 patients (34.1%) whose PCR tests were positive did not show any symptom and they were followed in isolation without treatment. 52 patients (24.6%) received hydroxychloroquine plus azithyromycine, 57 patients (27%) received favipiravir and 30 patients (14.2%) received favipiravir plus dexamethosone as the first line of treatment. 63.1% of pneumonia patients who received hydroxychloroquine plus azithyromycine, 28.3% of patients who received favipravir and 10% of patients who received favipravir plus dexamethasone showed a failure of treatment. Conclusion: The pulmonary infiltrates of COVID-19 are not infective; therefore, the characteristic of the disease should be described as COVID-19 pneumonitis instead of pneumonia. The favipiravir plus dexamethasone seems to be the only drug combination to achieve the improvement of radiological presentation and clinical symptoms in COVID-19 pneumonia patients. [**note: this is from Turkey and looks at clinical and radiological findings and two drug treatments. HCQ + azithromycin shows no treatment effect (no surprise here) while the antiviral favipravir + dexamethasone did (was this more a result of the steroid?) It's the first treatment effect of favipravir that I've seen though there are a number of registered trials ongoing.**]
  https://www.medrxiv.org/content/10.1101/2020.08.25.20181388v1
- Background. Angiotensin converting enzyme (ACE) type 2 is the receptor of SARSCoV-2 for entry into lungs cells. Because ACE-2 may be modulated by ACE inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs), there is concern that patients treated with ACEIs and ARBs are at higher risk for COVID-19 infection. Aim. This study sought to analyze the association of COVID-19 with previous treatment with ACEI and ARB. Methods. We retrospectively reviewed 684 consecutive patients hospitalized for suspected COVID-19 pneumonia and tested by PCR. Patients were split into 2 groups, whether (group 1, n=484) or not (group 2, n=250) COVID-19 was confirmed. Multivariate adjusted comparisons included a propensity score analysis. Results Age was 63.6 ± 18.7 years, and 302(44%) were female. Hypertension was present in 42.6% and 38.4% patients of group 1 and 2, respectively (P=0.28). A treatment with ARBs (20.7% versus 12.0%, respectively, OR 1.92, 95% confidence interval [1.23-2.98], p=0.004) was more frequent in patients of group 1 than in group 2. No difference was found for treatment with ACEIs (12.7% vs 15.7%, respectively, OR 0.81 [0.52-1.26], p=0.35). Propensity score matched multivariate logistic regression confirmed a significant association between COVID-19 and a previous treatment with ARBs (adjusted OR 2.18 [1.29-3.67], p=0.004). Significant interaction between ARBs and ACEIs for the risk of COVID-19 was observed in patients aged>60, women, and hypertensive patients. Conclusion . This study suggests that ACEIs and ARBs are not similarly associated with the COVID-19. In this retrospective series, patients with COVID-19 pneumonia received more frequently a previous treatment with ARBs, than patients without COVID-19. [**note: this is an observational study of ACEi and ARBs in patients with diagnosed COVID-19**

pneumonia. The information on ARBs seems to contradict some other observational studies. There are a small number of clinical trials to see if such drugs are protective but I have not seen any results.] https://www.medrxiv.org/content/10.1101/2020.08.30.20182451v1

- There is a pressing need for an in-depth understanding of immunity to SARS-CoV-2. Here we investigated T cell recall responses to fully glycosylated Spike trimer, recombinant N protein as well as to S, N, M and E peptide pools in the early convalescent phase. All subjects showed SARS-CoV-2-specific T cell responses to at least one antigen. SARS-CoV-2-specific CD4+ T cells were primarily of the central memory phenotype and exhibited a lower IFN-[gamma] to TNF-[alpha] ratio compared to influenza-specific responses of the same donors, independent of disease severity. SARS-CoV-2-specific T cells were less multifunctional than influenza-specific T cells, particularly in severe cases, potentially suggesting exhaustion. High IL-10 production was noted in response to N protein, possibly contributing to immunosuppression, with potential implications for vaccine design. We observed granzyme B+/IFN-[gamma] CD4+ and CD8+ proliferative responses to peptide pools in most individuals, with CD4+ responses predominating over CD8+ responses. Peripheral T follicular helper responses to S or N strongly correlated with serum neutralization assays as well as RBD-specific IgA. Overall, T cell responses to SARS-CoV-2 are robust, however, CD4+ Th1 responses predominate over CD8+ responses and are more inflammatory with a weaker Tfh response than influenza-specific CD4+ responses, potentially contributing to COVID-19 disease. [**note: this Toronto study looks at the difference in T cell response between SARS-CoV-2 and influenza.**]
  https://www.medrxiv.org/content/10.1101/2020.08.27.20183319v1
- Introduction: On the basis of the preliminary report from the RECOVERY trial, the use of dexamethasone or alternative corticosteroids (CS) is currently recommended in severe COVID-19 patients requiring supplemental oxygen. However, last updated recommendations have not taken a position either for or against the use of other immunomodulators such as tocilizumab (TCZ), with or without CS, since results are still limited. Methods: From March 17 to April 7, 2020, a real-world observational retrospective analysis was conducted at our 750-bed university hospital to study the characteristics and risk factors for mortality in patients with severe COVID-19 treated with TCZ, with or without CS, in addition to standard of care (SOC). Data were obtained from routine clinical practice, stored in electronic medical records. The main outcome was all-cause in-hospital mortality. Results: A total of 1,092 COVID-19 patients were admitted during the study period. Of them, 186 (17%) were treated with TCZ, of which 129 (87.8%) in combination with CS. Of the total 186, 155 (83.3 %) patients were receiving non-invasive ventilation when TCZ, with or without CS was initiated. Mean time from symptoms onset and hospital admission to TCZ use was 12 (SD 4.3) and 4.3 days (SD 3.4), respectively. Overall, 147 (79%) survived and 39 (21%) died. By multivariate analysis, mortality was associated with older age (HR=1.09, p<0.001), chronic heart failure (HR=4.4, p=0.003), and chronic liver disease (HR=4.69, p=0.004). The use of CS, in combination with TCZ, was the main protective factor against mortality (HR=0.26, p<0.001) in such severe COVID-19 patients receiving TCZ. No serious superinfections were observed after a 30-day follow-up. Conclusions: In severe COVID-19 patients receiving TCZ due to systemic host-immune inflammatory response syndrome, the use of CS in combined therapy with TCZ, was the main protective factor against in-hospital mortality. [**note: this is from Spain showing the utility of corticosteroids with tocilizumab provided clinical benefit when looking at mortality. There are some tocilizumab trials still going on but**

the sponsoring company is not pursuing this because of the earlier controlled trial that showed no clinical benefit.]
https://www.medrxiv.org/content/10.1101/2020.08.31.20182428v1

- Objective: To estimate the prevalence of disability and anxiety in Covid-19 survivors at discharge from hospital and analyze relative risk by exposures. Design: Multi-center retrospective cohort study. Setting: Twenty-eight hospitals located in eight provinces of China. Methods: A total of 432 survivors with laboratory-confirmed SARS CoV-2 infection participated in this study. At discharge, we assessed instrumental activities of daily living (IADL) with Lawton's IADL scale, dependence in activities of daily living (ADL) with the Barthel Index, and anxiety with Zung's self-reported anxiety scale. Exposures included comorbidity, smoking, setting (Hubei vs. others), disease severity, symptoms, and length of hospital stay. Other risk factors considered were age, gender, and ethnicity (Han vs. Tibetan). Results: Prevalence of at least one IADL problem was 36.81% (95% CI: 32.39-41.46). ADL dependence was present in 16.44% (95% CI: 13.23-20.23) and 28.70% (95% CI: 24.63- 33.15) were screened positive for clinical anxiety. Adjusted risk ratio (RR) of IADL limitations (RR 2.48, 95% CI: 1.80-3.40), ADL dependence (RR 2.07, 95% CI 1.15-3.76), and probable clinical anxiety (RR 2.53, 95% CI 1.69-3.79) were consistently elevated in survivors with severe Covid-19. Age was an additional independent risk factor for IADL limitations and ADL dependence; and setting (Hubei) for IADL limitations and anxiety. Tibetan ethnicity was a protective factor for anxiety but a risk factor for IADL limitations. Conclusion: *A significant proportion of Covid-19 survivors had disability and anxiety at discharge from hospital. Health systems need to be prepared for an additional burden resulting from rehabilitation needs of Covid-19 survivors.* [**note: here is a follow up study from China on recovered COVID-19 patients. I'm seeing more papers on PTSD resulting from COVID-19 and don't know what to make of it right now.**] https://www.medrxiv.org/content/10.1101/2020.08.26.20182246v1

DRUG DEVELOPMENT

- An increasing body of literature describes the role of host factors in COVID-19 pathogenesis. There is a need to combine diverse, multi-omic data in order to evaluate and substantiate the most robust evidence and inform development of future therapies. We conducted a systematic review of experiments identifying host factors involved in human betacoronavirus infection (SARS-CoV-2, SARS-CoV, MERS-CoV, seasonal coronaviruses). Gene lists from these diverse sources were integrated using Meta-Analysis by Information Content (MAIC). This previously described algorithm uses data-driven gene list weightings to produce a comprehensive ranked list of implicated host genes. 5,418 genes implicated in human betacoronavirus infection were identified from 32 datasets. The top ranked gene was *PPIA*, encoding cyclophilin A. Pharmacological inhibition with cyclosporine in vitro exerts antiviral activity against several coronaviruses including SARS-CoV. Other highly-ranked genes included proposed prognostic factors (*CXCL10*, *CD4*, *CD3E*) and investigational therapeutic targets (*IL1A*) for COVID-19, but also previously overlooked genes with potential as therapeutic targets. Gene rankings also inform the interpretation of COVID-19 GWAS results, implicating *FYCO1* over other nearby genes in a disease-associated locus on chromosome 3. Pathways enriched in gene rankings included T-cell receptor signalling, protein processing, and viral infections. We identified limited overlap of our gene list with host genes implicated in ARDS (innate immune and inflammation genes) and Influenza A virus infection (RNA-binding and ribosome-associated

genes). We will continue to update this dynamic ranked list of host genes as the field develops, as a resource to inform and prioritise future studies. Updated results are available at https://baillielab.net/maic/covid19 [**note: here is an enterprising group from Scotland that are maintaining a website that might be of use for drug development**]

- There is dire need for an effective and affordable vaccine against SARS-CoV-2 to tackle the ongoing pandemic. In this study, we describe a modular virus-like particle vaccine candidate displaying the SARS-CoV-2 spike glycoprotein receptor-binding domain (RBD) using SpyTag/SpyCatcher technology (RBD-SpyVLP). Low doses of RBD-SpyVLP in a prime-boost regimen induced a strong neutralising antibody response in mice and pigs that was superior to convalescent human sera. We evaluated antibody quality using ACE2 blocking and neutralisation of cell infection by pseudovirus or wild-type SARS-CoV-2. Using competition assays with a monoclonal antibody panel, we showed that RBD-SpyVLP induced a polyclonal antibody response that recognised all key epitopes on the RBD, reducing the likelihood of selecting neutralisation-escape mutants. The induction of potent and polyclonal antibody responses by RBD-SpyVLP provides strong potential to address clinical and logistic challenges of the COVID-19 pandemic. Moreover, RBD-SpyVLP is highly resilient, thermostable and can be lyophilised without losing immunogenicity, to facilitate global distribution and reduce cold-chain dependence. [**note: another week and another vaccine prototype. This one is quite interesting in that it is thermostable and can be lyophilized!! If you read Derek Lowe's piece on cold chain issues you right away know why this is important. I'm going to read more about this technology which is new to me**]
https://www.biorxiv.org/content/10.1101/2020.08.31.275701v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Reports of new-onset diabetes and diabetic ketoacidosis in individuals with COVID-19 have led to the hypothesis that SARS-CoV-2, the virus that causes COVID-19, is directly cytotoxic to pancreatic islet beta cells. This model would require binding and entry of SARS-CoV-2 into host cells via cell surface co-expression of ACE2 and TMPRSS2, the putative receptor and effector protease, respectively. To define ACE2 and TMPRSS2 expression in the human pancreas, we examined six transcriptional datasets from primary human islet cells and assessed protein expression by immunofluorescence in pancreata from donors with and without diabetes. ACE2 and TMPRSS2 transcripts were low or undetectable in pancreatic islet endocrine cells as determined by bulk or single cell RNA sequencing, and neither protein was detected in alpha or beta cells from any of these donors. Instead, ACE2 protein was expressed in the islet and exocrine tissue microvasculature and also found in a subset of pancreatic ducts, whereas TMPRSS2 protein was restricted to ductal cells. The absence of ACE2 and TMPRSS2 co-expression in islet endocrine cells makes it unlikely that SARS-CoV-2 directly affects pancreatic islet beta cells. [**note: onset diabetes linked to COVID-19 seems not to be a result of infection of pancreatic islet beta cells.**] https://www.biorxiv.org/content/10.1101/2020.08.31.275719v1

DIAGNOSTIC DEVELOPMENT

- Developing and deploying new diagnostic tests is difficult, but the need to do so in response to a rapidly emerging pandemic such as COVID-19 is crucially important for an effective response. In the early stages of a pandemic, laboratories play a key role in helping health care providers and

public health authorities detect active infection, a task most commonly achieved using nucleic acid-based assays. While the landscape of diagnostics is rapidly evolving, polymerase chain reaction (PCR) remains the gold-standard of nucleic acid-based diagnostic assays, in part due to its reliability, flexibility, and wide deployment. To address a critical local shortage of testing capacity persisting during the COVID-19 outbreak, our hospital set up a molecular based laboratory developed test (LDT) to accurately and safely diagnose SARS-CoV-2. We describe here the process of developing an emergency-use LDT, in the hope that our experience will be useful to other laboratories in future outbreaks and will help to lower barriers to fast and accurate diagnostic testing in crisis conditions. [**note: want to help out and set up your own hospital based testing capacity? These Mass General and Broad Institute researchers have the plan for you to follow!.**] https://www.medrxiv.org/content/10.1101/2020.08.26.20157297v1

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2020-09-02

Today is a special treat day for the Lisztomania fans. No, I'm not going to screen the great Ken Russell film, though it is worth seeking out if only for the high camp of watching The Who's Roger Daltrey as the amorous pianist. Rather I have a piece that Liszt wrote for both solo piano and then also voice and piano. It is one of the three Petrarch Sonnets that he set, in this case Sonnet 123; 'I vidi in terra angelici costumi'. The solo piano works are part of his Years of Pilgrimage group composed in 1838. Here is Marc-André Hamelin playing the solo version: https://www.youtube.com/watch?v=Qb9qJ_lLXlc and here are soprano Elizabeth Caballero and pianist Elaine Rinaldi in the vocal version: https://www.youtube.com/watch?v=uTcX-WiHrbI

According to The Washington Post, the US is not joining the global effort to develop, manufacture and equitably distribute a COVID-19 vaccine in part because of the WHO involvement in this program. I can attest to this story about the pandemic real estate boom. Houses in our inside the beltway neighborhood are selling the day they hit the market. Don't know whether it's above the asking price. Apple and Google are expanding coronavirus warning software. I think this is a good thing. It's not the time for bathroom jokes (I was really tempted 😁) but as this WaPo op-ed notes, it's time to consider how toilets might be spreading COVID-19. There is also a study from China from the Annals of Internal Medicine on this a little further down. There may be a great business opportunity in retrofitting toilets without lids to make them COVID-19 compliant. Time for the environmental engineers to come up with a good plumbing code!

Here is one COVID-19 long hauler's experience. Not fun at all.

Kaiser Health News discusses the issues related to a COVID-19 vaccine roll out.

The Annals of Internal Medicine have a study from China on the probable evidence of fecal aerosol transmission of SARS-CoV-2 in a high rise building along with an accompanying editorial.

The Atlantic on wearing a mask and not talking. As the old saying goes, "shut up already!"

STAT discusses vaccine development and a possible fall approval. We need more physician-scientists!

The Lancet has a commentary regarding research needs to better understand long term health effects of COVID-19, the 'long-hauler' syndrome. Here is an in depth article on measuring mobility to monitor travel and physical distancing intervention using a cell phone.

The New England Journal of Medicine has a report on humoral immune response to SARS-CoV-2 in Iceland and an accompanying editorial on the power of antibody surveillance. There is a letter from Yale researchers comparing nasopharyngeal swab and saliva collection methods for viral assays.

I has an interesting vaccine paper in yesterday's newsletter but forgot to put a link in about the company Spy Biotech. Their approach to vaccine development is quite interesting and there is a nice video on the site.

MODELING

- We have performed detailed modeling of the COVID-19 epidemic within the State of Illinois at the population level, and within the University of Illinois at Urbana-Champaign at a more detailed level of description that follows individual students as they go about their educational and social activities. We ask the following questions: (1) How many COVID-19 cases are expected to be detected by entry screening? (2) Will this initial bump in cases be containable using the mitigation steps being undertaken at UIUC? Our answers are: (1) Assuming that there are approximately 45,000 students returning to campus in the week beginning August 15, 2020, our most conservative estimate predicts that a median of 270 ± 90 (minimum-maximum range) COVID-19 positive cases will be detected by entry screening. The earliest estimate for entry screening that we report was made on July 24th and predicted 198 ± 90 (68% CI) positive cases. (2) If the number of returning students is less, then our estimate just needs to be scaled proportionately. (3) This initial bump will be contained by entry screening initiated isolation and contact tracing, and once the semester is underway, by universal masking, a hybrid teaching model, twice-weekly testing, isolation, contact tracing, quarantining and the use of the Safer Illinois exposure notification app. [**note: here is detailed work by researchers at Univ of Illinois regarding opening of their 45K student campus.**]
  https://www.medrxiv.org/content/10.1101/2020.08.29.20184473v1
- Contact tracing is increasingly being used to combat COVID-19, and digital implementations are now being deployed, many of them based on Apple and Google's Exposure Notification System. These systems are new and are based on smartphone technology that has not traditionally been used for this purpose, presenting challenges in understanding possible outcomes. In this work, we use individual-based computational models to explore how digital exposure notifications can be used in conjunction with non-pharmaceutical interventions, such as traditional contact tracing and social distancing, to influence COVID-19 disease spread in a population. Specifically, we use a representative model of the household and occupational structure of three counties in the state of Washington together with a proposed digital exposure notifications deployment to quantify impacts under a range of scenarios of adoption, compliance, and mobility. In a model in which 15% of the population participated, we found that digital exposure notification systems could reduce infections and deaths by approximately 8% and 6%, effectively complementing traditional contact tracing. We believe this can serve as guidance to health authorities in Washington state and beyond on how exposure notification systems can complement traditional public health interventions to suppress the spread of COVID-19. [**note: this is from**

the folks at Google and shows how their digital exposure notification can be used to impact COVID-19 spread within a population. Unfortunately the state of Maryland does not have an app yet but I will be checking back as I want to be part of the solution!]
https://www.medrxiv.org/content/10.1101/2020.08.29.20184135v1

- The coronavirus (COVID-19) pandemic was particularly invasive in Italy during the period of March to the end of April 2020 then displayed a significant decrease both in the number of infections and in the seriousness of illness throughout the summer of 2020. In this discussion, we measure the seriousness of the disease by the ratio of Intensive Care Units (ICU) spaces occupied by COVID-19 patients and the number of still Active Cases (AC) each month from April to August 2020. We also use the ratio between the number of Deaths (D) and the number of Active Cases. What clearly emerges, from rigorous statistical analysis, is a progressive decrease of both the ratios, indicating progressive mitigation of the disease. This is particularly evident when comparing March-April with July-August; during the summer period the two ratios have become roughly 18 times lower. We test such sharp decreases against possible bias in counting active cases, and we confirm their statistical significance. We then interpret such evidence in terms of the well-known seasonality of the human immune system and the virus-inactivating effect of stronger UV rays in the summer. [**note: this models the evolution of COVID-19 in Italy from the initial outbreak to the quiescent summer period.**]
https://www.medrxiv.org/content/10.1101/2020.08.29.20184127v1
- The zoonotic origin of the SARS-CoV-2 pandemic is still unknown. Animal experiments have shown that non-human primates, cats, ferrets, hamsters, rabbits and bats can be infected by SARS-CoV-2. In addition, SARS-CoV-2 RNA has been detected in felids, mink and dogs in the field. Here, we describe an in-depth investigation of outbreaks on 16 mink farms and humans living or working on these farms, using whole genome sequencing. We conclude that the virus was initially introduced from humans and has evolved, most likely reflecting widespread circulation among mink in the beginning of the infection period several weeks prior to detection. At the moment, despite enhanced biosecurity, early warning surveillance and immediate culling of infected farms, there is ongoing transmission between mink farms with three big transmission clusters with unknown modes of transmission. We also describe the first animal to human transmissions of SARS-CoV-2 in mink farms. [**note: from The Netherlands. I don't plan on becoming a mink farmer and don't understand why they are still raised in captivity. I thought mink coats were a relic of the past.**]
https://www.biorxiv.org/content/10.1101/2020.09.01.277152v1

NEWLY REGISTERED CLINICAL TRIALS

- Yes, you are correct that I did not check this today. However, I did get a nice note from one of my readers telling me that a company they do work for has just started enrolling patients in a clinical trial of brequinar, an oral antiviral agent. Good luck, as we desperately need one of these!

CLINICAL TRIAL RESULTS

- Background: Passive immunotherapy with convalescent plasma (CP) is a potential treatment for COVID-19 for which evidence from controlled clinical trials is lacking. Methods: We conducted a multi-center, randomized clinical trial in patients hospitalized for COVID-19. All patients received

standard of care treatment, including off-label use of marketed medicines, and were randomized 1:1 to receive one dose (250-300 mL) of CP from donors with IgG anti-SARS-CoV-2. The primary endpoint was the proportion of patients in categories 5, 6 or 7 of the COVID-19 ordinal scale at day 15. Results: The trial was stopped after first interim analysis due to the fall in recruitment related to pandemic control. With 81 patients randomized, there were no patients progressing to mechanical ventilation or death among the 38 patients assigned to receive plasma (0%) versus 6 out of 43 patients (14%) progressing in control arm. Mortality rates were 0% vs 9.3% at days 15 and 29 for the active and control groups, respectively. No significant differences were found in secondary endpoints. At inclusion, patients had a median time of 8 days (IQR, 6-9) of symptoms and 49,4% of them were positive for anti-SARS-CoV-2 IgG antibodies. Conclusions: *Convalescent plasma could be superior to standard of care in avoiding progression to mechanical ventilation or death in hospitalized patients with COVID-19. The strong dependence of results on a limited number of events in the control group prevents drawing firm conclusions about CP efficacy from this trial*. (Funded by Instituto de Salud Carlos III; NCT04345523). [**note: this is the first result from a controlled trial of convalescent plasma that I've seen. Unfortunately, as you can see from the abstract they could not meet the enrollment number because of the drop in cases resulting from pandemic control measures in Spain. It may work but more patients were clearly needed.**]
https://www.medrxiv.org/content/10.1101/2020.08.26.20182444v2

- Importance The healthcare demand created by the COVID-19 pandemic was far beyond the hospital surge capacity in many countries, resulting in possible negative influence on prognosis of other severe diseases, such as cardiovascular disease (CVD). Objective To assess the impact of the COVID-19 outbreak on CVD-related hospitalizations and mortality. Design Community-based prospective cohort study. Setting the UK Biobank population. Participants 421,717 UK Biobank participants who were registered in England and alive on December 1st 2019. Main outcomes and measures The primary outcome of interest was CVD death, as deaths with CVD as a cause of death according to the death registers. We retrieved information on hospitalizations with CVD as the primary diagnosis based on the UK Biobank hospital inpatient data. The study period was between December 1st 2019 and May 30th 2020, and we used the same calendar period of the three preceding years as the reference period. Standardized mortality/incidence ratios (SMRs/SIRs) with 95% confidence intervals were used to estimate the relative risk of CVD outcomes during the study period, compared with the reference period, to control for seasonal variations and aging of the study population. Results We observed a distinct increase in CVD-related deaths in March and April 2020 as compared to the corresponding months of the three preceding years. The observed number of CVD death (n=217) was almost doubled in April, compared with the expected number (n=120), corresponding to an SMR of 1.81 (95% CI 1.58-2.06). We observed a sharp decline of CVD hospitalization in March (n=841) and April (n=454), compared with the expected number (n=1208 for March and 1026 for April), leading to an SIR of 0.70 (95% CI 0.65-0.74) for March and 0.44 (95% CI 0.40-0.48) for April. There was also a clear increase of death, but a clear decrease of hospitalization, in March and April for all the five major subtypes of CVD. Conclusions We observed a distinct excess in CVD deaths in the beginning of the COVID-19 outbreak in the UK Biobank population. In addition to CVD complications of SARS-CoV-2 infections, the reduced hospital capacity might have contributed to the observed excess CVD deaths. [**note: study is from China but using UK data and shows**

excess cardiovascular mortality in the beginning of the COVID-19 outbreak. Reduced hospital capacity might be one contribution along with SARS-CoV-2 infection.]
https://www.medrxiv.org/content/10.1101/2020.08.29.20184317v1

- Background: Patients with coronavirus disease 19 (COVID-19) are at high risk for thrombotic arterial and venous occlusions, while lung histopathology often reveals fibrin-based occlusion of small vessels in patients who succumb to the disease. At the same time, bleeding complications have been observed in some patients. Better understanding the balance between coagulation and fibrinolysis will help inform optimal approaches to thrombosis prophylaxis and potential utility of fibrinolytic-targeted therapies. Objective: To evaluate fibrinolysis among a large cohort of hospitalized COVID-19 patients. Patients and methods: 118 hospitalized COVID-19 patients and 30 healthy controls were included in the study. We measured plasma antigen levels of tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) and performed spontaneous clot lysis assays. Results: We found markedly elevated levels of tPA and PAI-1 among patients hospitalized with COVID-19. Both factors demonstrated a strong correlation with neutrophil counts and markers of neutrophil activation, but not with D-dimer. High levels of tPA and PAI-1 were associated with worse respiratory status. High levels of tPA, in particular, were also strongly correlated with mortality and with a significant enhancement in spontaneous ex vivo clot lysis. Conclusion: *While both tPA and PAI-1 are elevated among COVID-19 patients, extremely high levels of tPA enhance spontaneous fibrinolysis and are significantly associated with mortality in some patients. These data indicate that fibrinolytic homeostasis in COVID-19 is complex with a subset of patients expressing a balance of factors that may favor fibrinolysis and suggests that further study of tPA as a potential biomarker is warranted*. [**note: more information on coagulation and fibrinolysis in hospitalized COVID-19 patients. Some interesting finding, in particular high levels of tPA that correlate with mortality.**]
https://www.medrxiv.org/content/10.1101/2020.08.29.20184358v1

DRUG DEVELOPMENT

- There is an urgent need for anti-viral agents that treat SARS-CoV-2 infection. The shortest path to clinical use is repurposing of drugs that have an established safety profile in humans. Here, we first screened a library of 1,900 clinically safe drugs for inhibiting replication of OC43, a human beta-coronavirus that causes the common-cold and is a relative of SARS-CoV-2, and identified 108 effective drugs. We further evaluated the top 26 hits and determined their ability to inhibit SARS-CoV-2, as well as other pathogenic RNA viruses. 20 of the 26 drugs significantly inhibited SARS-CoV-2 replication in human lung cells (A549 epithelial cell line), with EC50 values ranging from 0.1 to 8 micromolar. We investigated the mechanism of action for these and found that masitinib, a drug originally developed as a tyrosine-kinase inhibitor for cancer treatment, strongly inhibited the activity of the SARS-CoV-2 main protease 3CLpro. X-ray crystallography revealed that masitinib directly binds to the active site of 3CLpro, thereby blocking its enzymatic activity. Mastinib also inhibited the related viral protease of picornaviruses and blocked picornaviruses replication. Thus, our results show that masitinib has broad anti-viral activity against two distinct beta-coronaviruses and multiple picornaviruses that cause human disease and is a strong candidate for clinical trials to treat SARS-CoV-2 infection. [**note: yet another drug repurposing study with some data to back it up. Many of the identified drugs are anti-psychotic and anti-allergic drugs that share a similar tricyclic structure, in agreement with the**

previous identification of olanzapine (a tricyclic anti-depressant) as an anti-SARS-CoV-2 drug. Masitinib looks like the best of the group in that it blocks the main protease.]
https://www.biorxiv.org/content/10.1101/2020.08.31.274639v1

- SARS-CoV-2 enters cells via ACE-2, which binds the spike protein with moderate affinity. Despite a constant background mutational rate, the virus must retain binding with ACE2 for infectivity, providing a conserved constraint for SARS-CoV-2 inhibitors. To prevent mutational escape of SARS-CoV-2 and to prepare for future related coronavirus outbreaks, we engineered a de novo trimeric ACE2 (T-ACE2) protein scaffold that binds the trimeric spike protein with extremely high affinity (KD < 1 pM), while retaining ACE2 native sequence. T-ACE2 potently inhibits all tested pseudotyped viruses including SARS-CoV-2, SARS-CoV, eight naturally occurring SARS-CoV-2 mutants, two SARSr-CoVs as well as authentic SARS-CoV-2. The cryo-EM structure reveals that T-ACE2 can induce the transit of spike protein to three-up RBD conformation upon binding. T-ACE2 thus represents a promising class of broadly neutralizing proteins against SARS-CoVs and mutants. [**note: another cool invention of a trimeric ACE2 protein scaffold that binds coronavirus as a decoy so it doesn't attach to the cellular receptor. There are several other similar approaches that I've posted on this in earlier newsletters.**]
https://www.biorxiv.org/content/10.1101/2020.08.31.274704v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Nothing today

DIAGNOSTIC DEVELOPMENT

- Nothing today

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2020-09-03

Sonny and Cher? Why not? No further comments other than I was a senior in high school when this came out: https://www.youtube.com/watch?v=PkmBjHwzyMw (now some of you must be considering that I just might be suffering from severe COVID Cabin Fever; your diagnosis is obviously correct). Then there is the final time they sung the song: https://www.youtube.com/watch?v=c4EaFzRVjlM

Let us take a moment to remember pitching great Tom Seaver who passed away as a result of complications from COVID-19. Seaver had a stellar career and perfect biomechanics in his pitching motion.

US COVID-19 STATISTICS - **Infection Rate: 1.8%; CFR: 3.0%** (IR no change; CFR no change; **note:** the CFR for this current outbreak continues to hover at 2%)

Just a quick editorial note today. There are lots of stories circulating about various conspiracies about the true number of COVID-19 fatalities. Some public officials seem to be buying into these. I could spend all day posting links but that would be a waste of my and your time. If you want to go down that rabbit hole, Google is your friend and you will be besieged with all types of weirdness that is likely more screwy than anything the great gonzo journalist, Hunter Thompson, ever wrote about (for the uninitiated, 'Fear and Loathing in Las Vegas' is a good place to start). In these times it is also worth remembering Thomas Pynchon's proverbs for paranoids that appeared in 'Gravity's Rainbow'. In

particular, I find #3 quite useful, "*if they can get you asking the wrong questions, they don't have to worry about the answers*." There are unsubstantiated rumors that Mr. Pynchon is a regular reader of this newsletter.

On the vaccine front, it looks like we *may* have a vaccine available for early fall. The New York Times links to three documents, HERE, HERE, & HERE. My read of this is that it will be the two mRNA vaccines judging from the cold storage requirements in the second document. Both Dr. Hahn (FDA commish) and Dr. Fauci have been quoted as saying a vaccine would be available early based on overwhelmingly positive data. There is a FDA Vaccine Advisory Committee meeting scheduled for late October and I assume that no vaccine will get released without its blessing. I had some timelines for the Phase 3 trials in a recent newsletter and I don't think that the trials would be finished by that date. We will have to wait and see. Even if a vaccine is released, availability would be limited. Historian Rick Perlstein writes about the Swine Flu vaccine release in 1976. I was a post-doc at Cornell and remember standing in a long line to get my shot. A critical care MD asks whether convalescent plasma helps patients. It's a valid question. The Times also covers Dr. Scott Atlas, the new advisor to President Trump on COVID-19 matters.

The Washington Post has a story on how contact tracing in South Korea operates. The first COVID-19 death resulting from last month's Sturgis SD motorcycle rally was announced. The WaPo also covers the new research on steroid use in treating COVID-19. Here is story of the politics/science (maybe the two can never be separated) on an early vaccine 'approval' (whatever that might mean. Will the postponed Tokyo Olympic games take place next summer? This article has a possible answer. Don't know what to read during the pandemic? This WaPo piece surveys what others are reading. I am reading Carl Hiassen's just published book, "Squeeze Me." It is full of gut-splitting humor and a pretty good caricature of the current POTUS (his Secret Service code name is Mastadon!). Reviews are HERE & HERE.

Atul Gawande weighs in on the testing mess in The New Yorker.

JAMA have the motherload of papers on steroid treatment for COVID-19. Three scientific papers HERE, HERE & HERE; s meta-analysis from the WHO Rapid Evidence Appraisal group, and finally a commentary. Low dose hydrocortisone did was not efficacious in treating severe COVID-19 but the trial was stopped early and with not enough patients enrolled. The second study from Brazil looked at IV dexamethasone for moderate and severe ARDS accompanying COVID-19. In this trial days alive and free from mechanical ventilation were significantly higher. The third trial, also with hydrocortisone in severe COVID-19 was also stopped early though there was a suggestion of clinical efficacy.

STAT report that the Sanofi/GSK COVID-19 vaccine is beginning clinical trials. This uses an established technology used for Sanofi's Flublok vaccine with an adjuvant produced by GSK. Here is an opinion piece as to whether placebo arms are needed in vaccine trials.

Kaiser Health News has an important article on the conundrum hospitals face regarding the use of convalescent plasma. Should they continue randomized controlled clinical trials which would mean having a placebo arm? As the old saying goes, "it's complicated!"

Do take a look at the modelling paper I have linked; it offers an optimistic take on the second wave.

MODELING

- Background Recent reports based on conventional SEIR models suggest that the next wave of the COVID-19 pandemic in the UK could overwhelm health services, with fatalities that far exceed the first wave. These models suggest non-pharmaceutical interventions would have limited impact without intermittent national lockdowns and consequent economic and health impacts. We used Bayesian model comparison to revisit these conclusions, when allowing for heterogeneity of exposure, susceptibility, and viral transmission. Methods We used dynamic causal modelling to estimate the parameters of epidemiological models and, crucially, the evidence for alternative models of the same data. We compared SEIR models of immune status that were equipped with latent factors generating data; namely, location, symptom, and testing status. We analysed daily cases and deaths from the US, UK, Brazil, Italy, France, Spain, Mexico, Belgium, Germany, and Canada over the period 25-Jan-20 to 15-Jun-20. These data were used to estimate the composition of each country's population in terms of the proportions of people (i) not exposed to the virus, (ii) not susceptible to infection when exposed, and (iii) not infectious when susceptible to infection. Findings Bayesian model comparison found overwhelming evidence for heterogeneity of exposure, susceptibility, and transmission. Furthermore, both lockdown and the build-up of population immunity contributed to viral transmission in all but one country. Small variations in heterogeneity were sufficient to explain the large differences in mortality rates across countries. The best model of UK data predicts a second surge of fatalities will be much less than the first peak (31 vs. 998 deaths per day. 95% CI: 24-37)--substantially less than conventional model predictions. The size of the second wave depends sensitively upon the loss of immunity and the efficacy of find-test-trace-isolate-support (FTTIS) programmes. Interpretation A dynamic causal model that incorporates heterogeneity of exposure, susceptibility and transmission suggests that the next wave of the SARS-CoV-2 pandemic will be much smaller than conventional models predict, with less economic and health disruption. This heterogeneity means that seroprevalence underestimates effective herd immunity and, crucially, the potential of public health programmes. [**note: catchy paper titles always catch my eye, and "Dark matter, second waves and epidemiological modelling" is no exception. At first I thought it was a paper on surfing as those of us who engaged in this past time always knew the second wave would be better than the first. Alas, it's about COVID-19. It uses dynamic causal modeling and comes to an optimistic conclusion regarding herd immunity. It's a worthwhile read and I hope the authors are correct. Researchers are from University College in London.**] https://www.medrxiv.org/content/10.1101/2020.09.01.20185876v1

NEWLY REGISTERED CLINICAL TRIALS

- A randomized, open-label, 2 arm, pilot trial of Lambda 180 mcg administered subcutaneously once weekly, for up to two weeks (2 injections at most), in addition to standard supportive care, compared to standard supportive care alone, in a population of COVID-19 infected patients. patients will be randomized according to 1:1 ratio to one of the 2 trial arms: Lambda 180 mcg S.C + standard care (intervention arm) or standard care only (control arm). [**note: this is an Israeli trial of pegylated Interferon-lamda**] NCT04534673
- his is an open-label, randomized, multi-centre pilot study where hospitalized subjects will be randomized in a 2:1 ratio to receive Isoquercetin (IQC-950AN) in addition to standard of care or standard of care only for 28 days following confirmation of a COVID-19 infection. [**note: this is a Canadian trial of flavonoid that comes from various plant sources.**] NCT04536090

- A clinical study to assess the efficacy and safety of oral tafenoquine compared to placebo in patients with mild to moderate COVID 19 disease. [**note: this is another anti-malarial drug; the sponsor is 60 Degrees Pharmaceuticals**] NCT04533347
- In this first-in-humans dose escalation study, AZD7442 (AZD8895 + AZD1061) will be evaluated for safety, tolerability, pharmacokinetics, and generation of anti-drug antibodies (ADAs). The study is intended to enable future studies of AZD7442's efficacy in preventing and treating COVID-19. AZD7442 is a combination of two mAbs derived from convalescent patients with SARS-CoV-2 infection. Discovered by Vanderbilt University Medical Center and licensed to AstraZeneca in June 2020, the mAbs were optimised by AstraZeneca with half-life extension and reduced Fc receptor binding. [**note: these are two mAbs that AstraZeneca are putting into trial.**] NCT04507256
- The Clinical trial aim to evaluate the effectiveness and safety of the administration of the intravenous prostaglandin E1 analog in the reduction of mortality and complications of patients with COVID-19 diagnosis. Therefore the investigators propose an open randomized clinical trial in the Fundación Santa Fe de Bogota NCT04536363

CLINICAL TRIAL RESULTS

- It is important to understand the temporal trend of pediatric severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral load to estimate the transmission potential of children in schools and communities. We determined differences in SARS-CoV-2 viral load dynamics between nasopharyngeal samples of infected asymptomatic and symptomatic children. The daily cycle threshold values of SARS-CoV-2 in the nasopharynx of a cohort of infected children were collected for analysis. Among 17 infected children, 10 (58.8%) were symptomatic. Symptomatic children, when compared to asymptomatic children, had higher viral load (mean cycle threshold on day 7 of illness 28.6 versus 36.7, p = 0.02). Peak SARS-CoV-2 viral loads occured around days 2-3 of illness/days of diagnosis in infected children. After adjusting for the estimated date of infection, the higher SARS-CoV-2 viral loads in symptomatic children remained. We postulate that symptomatic SARS-CoV-2-infected children may have higher transmissibility than asymptomatic children. *As peak viral load in infected children occurred in the early stage of illness, viral shedding and transmission in the pre-symptomatic phase probable. Our study highlights the importance of screening for SARS-CoV-2 in children with epidemiological risk factors, even when they are asymptomatic in order to improve containment of the virus in the community, including educational settings*. [**note: this is a useful study on viral nasopharynx in infected children**. We still need to understand whtether these children can infect others and to what extent.]
  https://www.medrxiv.org/content/10.1101/2020.08.31.20185488v1
- SARS-CoV-2 infection has a high transmission level. At the present time there is not a specific treatment approved but it is known that, in vitro, chloroquine and hydroxychloroquine can inhibit the coronavirus. Objective: verifying if patients with autoimmune diseases that are on treatment with HCQ have less incidence and severity on COVID-19. Material and methods: this is a retrospective cohort study. The exposed cohort was formed by individuals with autoimmune diseases with HCQ treatment. The control cohort was randomly selected using the Health Card database. To deal with confounding variables and evaluate the effect of HCQ on the incidence and severity of SARS-CoV-2 infection, propensity score matching was used. Risk difference and

paired percentage difference between exposed and non-exposed groups was estimated. Results: 919 individuals formed the exposed cohort and 1351 the control cohort. After matching, there were 690 patients on each group. During the time of the study, in the exposed group there were 42 (6.1%) individuals with suspected COVID-19, 12(1.7%) with confirmed COVID-19 and 3(0.4%) were hospitalized. In the control group there were 30(4.3%) individuals with suspected COVID-19, 13(1.9%) with confirmed COVID-19 and 2(0.3%) were hospitalized. The risk difference between each cohort was: 0.017(-0.05-0.04) for suspected COVID-19; -0.014(-0.015-0.012) for confirmed COVID-19 and 0.001(-0.007-0.007) for hospitalized patients. There were not significant differences. Conclusion: there is no difference neither on the incidence nor on the severity of COVID-19 between patients with autoimmune diseases with HCQ treatment and patients that do not take HCQ. [**note: I was wondering when someone would do this observational study. This is from Spain and looks at patients on HCQ for treatment of autoimmune diseases. In looking at a matching cohort there was no prophylactic effect of HCQ. As I have said, it is a Zombie drug.**]
https://www.medrxiv.org/content/10.1101/2020.08.31.20185314v1

- Immune system dysfunction is paramount in Coronavirus disease 2019 (COVID-19) severity and fatality rate. Mucosal-Associated Invariant T (MAIT) cells are innate-like T cells involved in mucosal immunity and protection against viral infections. Here, we studied the immune cell landscape, with emphasis on MAIT cells, in a cohort of 182 patients including patients at various stages of disease activity. A profound decrease of MAIT cell counts in blood of critically ill patients was observed. These cells showed a strongly activated and cytotoxic phenotype that positively correlated with circulating pro-inflammatory cytokines, notably IL-18. MAIT cell alterations markedly correlated with disease severity and patient mortality. SARS-CoV-2-infected macrophages activated MAIT cells in a cytokine-dependent manner involving an IFNα-dependent early phase and an IL-18-induced later phase. Therefore, altered MAIT cell phenotypes represent valuable biomarkers of disease severity and their therapeutic manipulation might prevent the inflammatory phase involved in COVID-19 aggravation. [**note: from France, yet another potential biomarker and possible treatment site.**]
https://www.medrxiv.org/content/10.1101/2020.08.31.20185082v1
- Multisystem inflammatory syndrome in children (MIS-C) presents with fever, inflammation and multiple organ involvement in individuals under 21 years following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. To identify genes, pathways and cell types driving MIS-C, we sequenced the blood transcriptomes of MIS-C cases, pediatric cases of coronavirus disease 2019, and healthy controls. We define a MIS-C transcriptional signature partially shared with the transcriptional response to SARS-CoV-2 infection and with the signature of Kawasaki disease, a clinically similar condition. By projecting the MIS-C signature onto a co-expression network, we identified disease gene modules and found genes downregulated in MIS-C clustered in a module enriched for the transcriptional signatures of exhausted $CD8^+$ T-cells and $CD56^{dim}CD57^+$ NK cells. Bayesian network analyses revealed nine key regulators of this module, including *TBX21*, a central coordinator of exhausted $CD8^+$ T-cell differentiation. Together, these findings suggest dysregulated cytotoxic lymphocyte response to SARS-Cov-2 infection in MIS-C. [**note: more good work from Mt. Sinai on multisystem inflammatory syndrome in children. Cytotoxic lymphocytes are dysregulated.**]
https://www.medrxiv.org/content/10.1101/2020.08.29.20182899v1

DRUG DEVELOPMENT

- SARS-CoV-2 vaccines are advancing into human clinical trials, with emphasis on eliciting high titres of neutralising antibodies against the viral spike (S). However, the merits of broadly targeting S versus focusing antibody onto the smaller receptor binding domain (RBD) are unclear. Here we assessed prototypic S and RBD subunit vaccines in homologous or heterologous prime-boost regimens in mice and non-human primates. We find S is highly immunogenic in mice, while the comparatively poor immunogenicity of RBD was associated with limiting germinal centre and T follicular helper cell activity. Boosting S-primed mice with either S or RBD significantly augmented neutralising titres, with RBD-focussing driving moderate improvement in serum neutralisation. In contrast, both S and RBD vaccines were comparably immunogenic in macaques, eliciting serological neutralising activity that generally exceed levels in convalescent humans. These studies confirm recombinant S proteins as promising vaccine candidates and highlight multiple pathways to achieving potent serological neutralisation. [**note: from Australia some basic research on vaccines**]
  https://www.biorxiv.org/content/10.1101/2020.09.01.278630v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- A spike protein mutation D614G became dominant in SARS-CoV-2 during the COVID-19 pandemic. However, the mutational impact on viral spread and vaccine efficacy remains to be defined. Here we engineer the D614G mutation in the SARS-CoV-2 USA-WA1/2020 strain and characterize its effect on viral replication, pathogenesis, and antibody neutralization. The D614G mutation significantly enhances SARS-CoV-2 replication on human lung epithelial cells and primary human airway tissues, through an improved infectivity of virions with the spike receptor-binding domain in an 'up' conformation for binding to ACE2 receptor. Hamsters infected with D614 or G614 variants developed similar levels of weight loss. However, the G614 virus produced higher infectious titers in the nasal washes and trachea, but not lungs, than the D614 virus. The hamster results confirm clinical evidence that the D614G mutation enhances viral loads in the upper respiratory tract of COVID-19 patients and may increases transmission. For antibody neutralization, sera from D614 virus-infected hamsters consistently exhibit higher neutralization titers against G614 virus than those against D614 virus, indicating that (i) the mutation may not reduce the ability of vaccines in clinical trials to protect against COVID-19 and (ii) therapeutic antibodies should be tested against the circulating G614 virus before clinical development. [**note: more work on the dominant mutation of SARS-CoV-2 (maybe we should call this the European Virus as the mutation appeared there**😉) **Anyway the good news is that vaccines and antibodies should work against both strains.**]
  https://www.biorxiv.org/content/10.1101/2020.09.01.278689v1
- The recent outbreak of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), has led to a worldwide pandemic. One week after initial symptoms develop, a subset of patients progresses to severe disease, with high mortality and limited treatment options. To design novel interventions aimed at preventing spread of the virus and reducing progression to severe disease, detailed knowledge of the cell types and regulating factors driving cellular entry is urgently needed. Here we assess the expression patterns in genes required for COVID-19 entry into cells and replication, and their

regulation by genetic, epigenetic and environmental factors, throughout the respiratory tract using samples collected from the upper (nasal) and lower airways (bronchi). Matched samples from the upper and lower airways show a clear increased expression of these genes in the nose compared to the bronchi and parenchyma. Cellular deconvolution indicates a clear association of these genes with the proportion of secretory epithelial cells. Smoking status was found to increase the majority of COVID-19 related genes including ACE2 and TMPRSS2 but only in the lower airways, which was associated with a significant increase in the predicted proportion of goblet cells in bronchial samples of current smokers. Both acute and second hand smoke were found to increase ACE2 expression in the bronchus. Inhaled corticosteroids decrease ACE2 expression in the lower airways. No significant effect of genetics on ACE2 expression was observed, but a strong association of DNA- methylation with ACE2 and TMPRSS2- mRNA expression was identified in the bronchus. [**note: maybe this doesn't fit here but I don't have a good category. This is from a large multi-national group looking viral receptor gene expression in upper and lower airways.**]
https://www.medrxiv.org/content/10.1101/2020.08.31.20169946v1

- While cross-reactive T cells epitopes of SARS-CoV-2 and seasonal/common cold human coronaviruses (hCoVs) have been reported in individuals unexposed to SARS-CoV-2, potential antibody-based cross-reactivity is incompletely understood. Here, we have probed for high resolution antibody binding against all hCoVs represented as 1,539 peptides with a phage-displayed antigen library. We detected broad serum antibody responses against peptides of seasonal hCoVs in up to 75% of individuals. Recovered COVID-19 patients exhibited distinct antibody repertoires targeting variable SARS-CoV-2 epitopes, and could be accurately classified from unexposed individuals (AUC=0.96). *Up to 50% of recovered patients also mounted antibody responses against unique epitopes of seasonal hCoV-OC43, that were not detectable in unexposed individuals. These results indicate substantial interindividual variability and antibody cross-reactivity between hCoVs from the direction of SARS-CoV-2 infections towards seasonal hCoVs. Our accurate high throughput assay allows profiling preexisting antibody responses against seasonal hCoVs cost-effectively and could inform on their protective nature against SARS-CoV-2*. [**note: this is from Israel and provides the beginning of a look at cross-reactivity of antibodies produced against various coronaviruses.**]
https://www.medrxiv.org/content/10.1101/2020.09.01.20182220v1

DIAGNOSTIC DEVELOPMENT

- The ongoing coronavirus disease 2019 (COVID-19) pandemic calls for a method to rapidly and conveniently evaluate neutralizing antibody (NAb) activity in patients. Here, an up-conversion phosphor technology-based point-of-care testing (UPT-POCT) and a microneutralization assay were employed to detect total antibodies against the receptor-binding domain of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein and NAb activity in COVID-19 patients' sera, respectively, in order to determine if UPT-POCT could be used as a surrogate method for rapid evaluation of serum NAb activity in COVID-19 patients. In total, 519 serum samples from 213 recovered and 99 polymerase chain reaction re-positive (RP) COVID-19 patients were used in this report. We found that UPT-POCT reporting values correlated highly with NAb titers from 1:4 to 1:1024, with a correlation coefficient $r = 0.9654$ ($P < 0.001$), as well as protection rate against RP ($r = 0.9886$, $P < 0.0001$). As a significant point for reducing re-

positive rate, UPT-POCT values of 4.380, corresponding to NAb titer of 1:64, may be appropriate as an indicator for evaluating high efficiency of protection. This study demonstrates that the quantitative lateral flow based UPT-POCT, could be used to rapidly evaluate NAb titer, which is of importance for assessing vaccine immunization efficacy, herd immunity, and screening patient plasma for high NAbs. [**note: from China a rapid test for neutralizing antibodies in COVID-19 patients.**] https://www.medrxiv.org/content/10.1101/2020.09.01.20185447v1

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2020-09-04

Antonio Carlos Jobim was one of the great composers of the 20th century. He played as important a role as anyone for the popularity of bossa nova which was quickly picked up by numerous American jazz players. Here is a live film of 'Wave' with Oscar Peterson, Joe Pass and Niels-Henning Orsted Pederson: https://www.youtube.com/watch?v=vUuB4UYe8nM Here is the vocal version by NOVA: https://www.youtube.com/watch?v=TBHQ-CJLPi4

US COVID-19 STATISTICS - **Infection Rate: 1.9; CFR: 3.0%** (IR up 0.1%; CFR no change; **note:** the CFR for this current outbreak continues to hover at 2%)

The Penn State athletic doctor notes that one third of the COVID-19 positive Big Ten athletes have myocarditis, yet some college football programs are going ahead with the season. It may be time for players to get woke. The WaPo offers a clarification that the doc did not conduct the research but heard about the results from a colleague.

The Washington Post notes that the party of the state's governor is important in terms of mask requirements. There was preprint on this that I read yesterday but I did not post it since this was something so obvious that it did not warrant mention. If you want to read the paper, a link is in this article. YIKES! The pandemic is ruining our sleep (cornoasomnia). Isn't this what afternoon naps are supposed to cure?

Gettysburg College has instituted an all student quarantine because of COVID-19 outbreak. Fraternities and sororities at Indiana University are experiencing high levels of COVID-19 with some under quarantine. The university has told the 2600 students who live in them to move out and go home if possible. University of Illinois who have one of the most aggressive COVID-19 testing programs is seeing rising numbers of cases. The college is under a two-week lockdown. I could go on with other stories such as University of South Carolina who have had 1000 cumulative cases since reopening. I think I will only focus on Mitch Daniels's Purdue as he was outspoken about reopening. According to the Purdue COVID-19 dashboard, Purdue has conducted 3110 tests in the last seven days with 83 students and four employees testing positive. I will periodically update this information.

In an interview on National Public Radio, Moncef Slaoui, the chief advisor for the US vaccine program, said it was unlikely but not impossible that a vaccine could be available by the end of October.

Speaking of vaccines, Derek Lowe has his usual good roundup of where things stand. Do scroll down and read the comment by 'FrankN' who raises some good points about the need for cold chain storage with the lipid/mRNA vaccines. As a former lipid biochemist, I can attest to the issues he raises about separation.

The New England Journal of Medicine has the results of the Novavax early vaccine trial. This is a protein/adjuvant preparation.

Science have a perspective on the use of variants of ACE2 as a molecular trap for SARS-CoV-2. This is being looked at by several different groups and I am aware of one trial of recombinant native ACE2 being registered. HERE is the original paper.

MODELING

- Universal masking the health care setting and in the community to contain the spread of SARS-CoV-2 has been recently recommended by the WHO, but supporting data are rare. The City of Jena was the first community in Germany to issue an order on mandatory public masking. Here, we report the development of the number of novel infections in our hospital and the city of Jena after implementation of universal masking in our hospital and the city. [**note; this is from the moderately sized city of Jena in Germany who implemented a mandatory public masking law. They had a comparison city that implemented their own masking law four weeks later. Jena saw no new COVID-19 case whereas Erfurt continued to observe case until their citizens masked up. As the authors of the paper note, this is an observational study and an ethical controlled trial on mask use versus non-mask likely could not be done. I think this nice study closes out the question of whether masks work. I do not plan to cite any future papers on this topic as for me the question is settled!!! MASK UP WHEN YOU GO OUT. 'Nuff said.**] https://www.medrxiv.org/content/10.1101/2020.09.02.20187021v1

NEWLY REGISTERED CLINICAL TRIALS

- Hey, I checked yesterday!!

CLINICAL TRIAL RESULTS

- Background Since 1920, a decrease in serum cholesterol has been identified as a marker of severe pneumonia. We have assessed the performance of serum apolipoprotein-A1, the main transporter of HDL-cholesterol, to identify the early spread of coronavirus disease 2019 (Covid-19) in the general population and its diagnostic performance for the Covid-19. Methods We compared the daily mean serum apolipoprotein-A1 during the first 34 weeks of 2020 in a population that is routinely followed for a risk of liver fibrosis risk in the USA (212,297 sera) and in France (20,652 sera) in relation to a local increase in confirmed cases, and in comparison to the same period in 2019 (266,976 and 28,452 sera, respectively). We prospectively assessed the sensitivity of this marker in an observational study of 136 consecutive hospitalized cases and retrospectively evaluated its specificity in 7,481 controls representing the general population. Results The mean serum apolipoprotein-A1 levels in the survey populations began decreasing in January 2020, compared to the same period in 2019. This decrease was highly correlated with the daily increase in confirmed Covid-19 cases in the following 34 weeks, both in France and USA, including the June and mid-July recovery periods in France. Apolipoprotein-A1 at the 1.25 g/L cutoff had a sensitivity of 90.6% (95%CI84.2-95.1) and a specificity of 96.1% (95.7-96.6%) for the diagnosis of Covid-19. The area under the characteristics curve was 0.978 (0.957-0.988), and outperformed haptoglobin and liver function tests. The adjusted risk ratio of apolipoprotein-A1 for survival without transfer to intensive care unit was 5.61 (95%CI 1.02-31.0;P=0.04).

Conclusion Apolipoprotein-A1 could be a sentinel of the pandemic in existing routine surveillance of the general population. NCT01927133, CER-2020-14. [**note: another possible marker for COVID-19 risk. It is interesting that they were able to use data being compiled for another use to see if there was a correlation to COVID-19.**]
https://www.medrxiv.org/content/10.1101/2020.09.01.20186213v1

- Background Recent evidence has established a beneficial effect of systemic corticosteroids for treatment of moderate-to-severe COVID-19. However, it is unknown if inhaled corticosteroid use is associated with reduced morbidity of the disease. Methods In a nationwide cohort of hospitalized SARS-CoV-2 test-positive individuals in Denmark, we estimated the 30-day hazard ratio of intensive care unit (ICU) admission or death among users of inhaled corticosteroids (ICS) compared with users of non-ICS inhalers (β2-agonist/muscarinic-antagonists), or non-users of ICS, with Cox regression adjusted for age, sex, and other confounders. We repeated these analyses among influenza test-positive patients during 2010-2018. Results Among 2,180 hospitalized SARS-CoV-2 patients, 282 were admitted to ICU and 421 died within 30 days. ICS use was associated with a hazard ratio of 1.25 (95% CI [CI], 0.60 to 2.61) for ICU admission and 0.84 (95% CI, 0.54 to 1.31) for death compared with non-ICS inhaler use. Compared with no ICS use, the hazard ratio of ICU admission or death was 1.22 (95% CI, 0.77 to 1.94) and 1.05 (95% CI, 0.75 to 1.47), respectively. Among 10,279 hospitalized influenza patients, the hazard ratios were 1.43 (95% CI, 0.89 to 2.30) and 1.11 (95% CI, 0.85 to 1.46) for ICU admission, and 0.80 (95% CI, 0.63 to 1.01) and 1.03 (95% CI, 0.87 to 1.22) for death compared with non-ICS inhaler use and no ICS use, respectively. Conclusions Our results do not support an effect of inhaled corticosteroid use on COVID-19 morbidity, however we can only rule out moderate-to-large reduced or increased risks. [**note: from Denmark, an observational study showing inhaled corticosteroid use is likely not useful.**]
https://www.medrxiv.org/content/10.1101/2020.09.03.20187278v1
- Background: COVID-19 may differentially impact people with obesity. We aimed to describe and compare the demographics, comorbidities, and outcomes of obese patients with COVID-19 to those of non-obese patients with COVID-19, or obese patients with seasonal influenza. Methods: We conducted a cohort study based on outpatient/inpatient care, and claims data from January to June 2020 from the US, Spain, and the UK. We used six databases standardized to the OMOP common data model. We defined two cohorts of patients diagnosed and/or hospitalized with COVID-19. We created corresponding cohorts for patients with influenza in 2017-2018. We followed patients from index date to 30 days or death. We report the frequency of socio-demographics, prior comorbidities, and 30-days outcomes (hospitalization, events, and death) by obesity status. Findings: We included 627 044 COVID-19 (US: 502 650, Spain: 122 058, UK: 2336) and 4 549 568 influenza (US: 4 431 801, Spain: 115 224, UK: 2543) patients. The prevalence of obesity was higher among hospitalized COVID-19 (range: 38% to 54%) than diagnosed COVID-19 (30% to 47%), or diagnosed/hospitalized influenza (15% to 48%) patients. Obese hospitalized COVID-19 patients were more often female and younger than non-obese COVID-19 patients or obese influenza patients. Obese COVID-19 patients were more likely to have prior comorbidities, present with cardiovascular and respiratory events during hospitalization, require intensive services, or die compared to non-obese COVID-19 patients. Obese COVID-19 patients were also more likely to require intensive services or die compared to obese influenza patients, despite presenting with fewer comorbidities. Interpretation: We show

that obesity is more common among COVID-19 than influenza patients, and that obese patients present with more severe forms of COVID-19 with higher hospitalization, intensive services, and fatality than non-obese patients. These data are instrumental for guiding preventive strategies of COVID-19 infection and complications [**note: this is a very large cohort study on COVID-19 patients (627K) with and without obesity in the US, Spain and the UK. Findings substantiate what he have learned from smaller studies, obese people are at greater risk. Many of the authors are from the OHDSI project!!!**]
https://www.medrxiv.org/content/10.1101/2020.09.02.20185173v1

- Importance: Clinical biomarkers that accurately predict mortality are needed for the effective management of patients with severe COVID-19 illness. Objective: To determine whether D-dimer levels after anticoagulation treatment is predictive of in-hospital mortality. Design: Retrospective study using electronic health record data. Setting: A large New York City hospital network serving a diverse, urban patient population. Participants: Adult patients hospitalized for severe COVID-19 infection who received therapeutic anticoagulation for thromboprophylaxis between February 25, 2020 and May 31, 2020. Exposures: Mean and trend of D-dimer levels in the 3 days following the first therapeutic dose of anticoagulation. Main Outcomes: In-hospital mortality versus discharge. Results: 1835 adult patients (median age, 67 years [interquartile range, 57-78]; 58% male) with PCR-confirmed COVID-19 who received therapeutic anticoagulation during hospitalization were included. 74% (1365) of patients were discharged and 26% (430) died in hospital. The study cohort was divided into four groups based on the mean D-dimer levels and its trend following anticoagulation initiation, with significantly different in-hospital mortality rates ($p<0.001$): 49% for the high mean-increase trend (HI) group; 27% for the high-decrease (HD) group; 21% for the low-increase (LI) group; and 9% for the low-decrease (LD) group. Using penalized logistic regression models to simultaneously analyze 67 variables (baseline demographics, comorbidities, vital signs, laboratory values, D-dimer levels), post-anticoagulant D-dimer groups had the highest adjusted odds ratios (ORadj) for predicting in-hospital mortality. The ORadj of in-hospital death among patients from the HI group was 6.58 folds (95% CI 3.81-11.16) higher compared to the LD group. The LI (ORadj: 4.06, 95% CI 2.23-7.38) and HD (ORadj: 2.37; 95% CI 1.37-4.09) groups were also associated with higher mortality compared to the LD group. Conclusions and Relevance: D-dimer levels and its trend following the initiation of anticoagulation have high and independent predictive value for in-hospital mortality. This novel prognostic biomarker should be incorporated into management protocols to guide resource allocation and prospective studies for emerging treatments in hospitalized COVID-19 patients. [**note: another paper from Mt. Sinai! D-dimer levels are a predictor of mortality and the trend following initiation of anti-coagulant therapy is important.**]
https://www.medrxiv.org/content/10.1101/2020.09.02.20180984v1

DRUG DEVELOPMENT

- A self-transcribing and replicating RNA (STARR) based vaccine (LUNAR-COV19) has been developed to prevent SARS-CoV-2 infection. The vaccine encodes an alphavirus-based replicon and the SARS-CoV-2 full length spike glycoprotein. Translation of the replicon produces a replicase complex that amplifies and prolong SARS-CoV-2 spike glycoprotein expression. A single prime vaccination in mice led to robust antibody responses, with neutralizing antibody titers increasing up to day 60. Activation of cell mediated immunity produced a strong viral antigen

specific CD8+ T lymphocyte response. Assaying for intracellular cytokine staining for IFN-gamma; and IL-4 positive CD4+ T helper lymphocytes as well as anti-spike glycoprotein IgG2a/IgG1 ratios supported a strong Th1 dominant immune response. Finally, single LUNAR-COV19 vaccination at both 2 microgram and 10 microgram doses completely protected human ACE2 transgenic mice from both mortality and even measurable infection following wild-type SARS-CoV-2 challenge. Our findings collectively suggest the potential of Lunar-COV19 as a single dose vaccine. [**note: this vaccine was covered in Derek Lowe's blog post above. It is from Arcturus Therapeutics**] https://www.biorxiv.org/content/10.1101/2020.09.03.280446v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Recent studies have characterized the single-cell immune landscape of host immune response of coronavirus disease 2019 (COVID-19), specifically focus on the severe condition. However, the immune response in mild or even asymptomatic patients remains unclear. Here, we performed longitudinal single-cell transcriptome sequencing and T cell/B cell receptor sequencing on 3 healthy donors and 10 COVID-19 patients with asymptomatic, moderate, and severe conditions. We found asymptomatic patients displayed distinct innate immune responses, including increased CD56briCD16- NK subset, which was nearly missing in severe condition and enrichment of a new Th2-like cell type/state expressing a ciliated cell marker. Unlike that in moderate condition, asymptomatic patients lacked clonal expansion of effector CD8+ T cells but had a robust effector CD4+ T cell clonal expansion, coincide with previously detected SARS-CoV-2-reactive CD4+ T cells in unexposed individuals. Moreover, NK and effector T cells in asymptomatic patients have upregulated cytokine related genes, such as IFNG and XCL2. Our data suggest early innate immune response and type I immunity may contribute to the asymptomatic phenotype in COVID-19 disease, which could in turn deepen our understanding of severe COVID-19 and guide early prediction and therapeutics. [**note: from China, this is a study of the immune landscape in various stages of COVID-19 disease. Of interest it the difference in asymptomatic patients.**] https://www.biorxiv.org/content/10.1101/2020.09.02.276865v1

DIAGNOSTIC DEVELOPMENT

- The 2019 SARS CoV-2 (COVID-19) pandemic has highlighted the need for rapid and accurate tests to diagnose acute infection and immune response to infection. A multiplexed assay built on grating-coupled fluorescent plasmonics (GC-FP) was shown to have 100% selectivity and sensitivity (n = 23) when measuring serum IgG levels against three COVID-19 antigens (spike S1, spike S1S2, and the nucleocapsid protein). The entire assay takes less than 30 min, making it highly competitive with well-established ELISA and immunofluorescence assays. GC-FP is quantitative over a large dynamic range, providing a linear response for serum titers ranging from 1:25 to 1:1,600, and shows high correlation with both ELISA and a Luminex-based microsphere immunoassay (MIA) (Pearson $r > 0.9$). Compatibility testing with dried blood spot samples (n = 63) demonstrated 100% selectivity and 86.7% sensitivity. A machine learning (ML) model was trained to classify dried blood spot samples for prior COVID-19 infection status, based on the combined antibody response to S1, S1S2, and Nuc antigens. The ML model yielded 100% selectivity and 80% sensitivity and demonstrated a higher stringency than diagnosis with a single antibody-antigen response. The platform is flexible and will readily accommodate IgG, IgM, and IgA. Further, the assay uses sub-nanogram quantities of capture ligand and is thus

readily modified to include additional antigens, which is shown by the addition of RBD in later iterations of the test. The combination of rapid, multiplexed, and quantitative detection for both blood serum and dried blood spot samples makes GC-FP an attractive approach for COVID-19 antibody testing. [**note: this looks like a cool technology and was developed by Ciencia, Inc.** https://www.medrxiv.org/content/10.1101/2020.09.02.20187070v2

- Expanding testing capabilities is integral to managing the further spread of SARS-CoV-2 and developing reopening strategies, particularly in regards to identifying and isolating asymptomatic and pre-symptomatic individuals. Central to meeting testing demands are specimens that can be easily and reliably collected and laboratory capacity to rapidly ramp up to scale. We and others have demonstrated that high and consistent levels of SARS-CoV-2 RNA can be detected in saliva from COVID-19 inpatients, outpatients, and asymptomatic individuals. As saliva collection is non-invasive, extending this strategy to test pooled saliva samples from multiple individuals could thus provide a simple method to expand testing capacity. However, hesitation towards pooled sample testing arises due to the dilution of positive samples, potentially shifting weakly positive samples below the detection limit for SARS-CoV-2 and thereby decreasing the sensitivity. Here, we investigated the potential of pooling saliva samples by 5, 10, and 20 samples prior to RNA extraction and RT-qPCR detection of SARS-CoV-2. Based on samples tested, we conservatively estimated a reduction of 7.41%, 11.11%, and 14.81% sensitivity, for each of the pool sizes, respectively. Using these estimates we modeled anticipated changes in RT-qPCR cycle threshold to show the practical impact of pooling on results of SARS-CoV-2 testing. *In tested populations with greater than 3% prevalence, testing samples in pools of 5 requires the least overall number of tests. Below 1% however, pools of 10 or 20 are more beneficial and likely more supportive of ongoing surveillance strategies*. [**note: this is from Yale who were one of the first to work on saliva testing for SARS-CoV-2. It presents a pool testing strategy based on the background of infection in the population.**] https://www.medrxiv.org/content/10.1101/2020.09.02.20183830v1
- Rapid, sensitive, and precise multiplexed assays for serological analysis during candidate COVID-19 vaccine development would streamline clinical trials. The VaxArray Coronavirus (CoV) SeroAssay quantifies IgG antibody binding to 9 pandemic, potentially pandemic, and endemic human CoV spike antigens in 2 hours with automated results analysis. IgG antibodies in serum bind to the CoV spike protein capture antigens printed in a microarray format and are labeled with a fluorescent anti-species IgG secondary label. The assay demonstrated excellent lower limits of quantification ranging from 0.3 to 2.0 ng/mL and linear dynamic ranges of 76 to 911-fold. Average precision of 11% CV and accuracy (% recovery) of 92.5% over all capture antigens were achieved over 216 replicates representing 3 days and 3 microarray lots. Clinical performance on 263 human serum samples (132 SARS-CoV-2 negatives and 131 positives based on donor-matched RT-PCR and/or date of collection) produced 98.5% PPA (sensitivity) and 100% NPA (specificity). [**note: this is from a Colorado company InDevR and may prove useful in measuring vaccine response.**] https://www.medrxiv.org/content/10.1101/2020.09.03.20179598v1
- The true prevalence and population seropositivity of SARS-CoV-2 infection remains unknown, due to the number of asymptomatic infections and limited access to high-performance antibody tests. To control the COVID-19 pandemic it is crucial to understand the true seroprevalence, but not every region has access to extensive centralized PCR and serology testing. Currently

available rapid antibody tests lack the accuracy needed for recommendation by health authorities. To fill this gap, we analyzed and validated the clinical performance of a new point-of-care SARS-CoV-2 Rapid Antibody Assay, a chromatographic immunoassay for qualitative detection of IgM/IgG antibodies for use in near-patient settings. Analysis was performed using 42 Anti-SARS-Cov-2 positive (CoV+) and 92 Anti-SARS-Covid-2 negative (CoV-) leftover samples from before December 2019, using the Elecsys® Anti-SARS-CoV-2 as the reference assay. Analytical specificity was tested using leftover samples from individuals with symptoms of common cold collected before December 2019. The SARS-CoV-2 Rapid Antibody Test was 100.0% (95% CI 91.59-100.00) sensitive and 96.74% (95% CI 90.77-99.32) specific with an assay failure rate of 0.00%. No cross-reactivity was observed against the common cold panel. Method comparison was additionally conducted by two external laboratories, using 100 CoV+/275 CoV- samples, also comparing whole blood versus plasma matrix. The comparison demonstrated for plasma 96.00% positive/96.36% negative percent agreement with the Elecsys Anti-SARS-CoV-2 and overall 99.20% percent agreement between whole blood and EDTA plasma. The SARS-CoV-2 Rapid Antibody Test demonstrated similar clinical performance to the manufacturer's data and to a centralized automated immunoassay, with no cross-reactivity to common cold panels. [**note: this is a new rapid point of care COVID-19 antibody test from from a Korean company and the study and validation work is done by Roche Diagnostics.**]
https://www.medrxiv.org/content/10.1101/2020.09.01.20180687v1

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2020-09-05

Fleetwood Mac were great from the mid-1970s to once Lindsey Buckingham and Stevie Nicks joined the group. When they left about a decade later, the band wasn't quite the same. There were reunions of a sort but the original magic was never captured. Here is Rhiannon live from 1976: https://www.youtube.com/watch?v=wgmRb3MlpHQ Just good rock and roll!

US COVID-19 STATISTICS - **Infection Rate: 1.9; CFR: 3.0%** (IR no change; CFR no change; **note:** the CFR for this current outbreak continues to hover at 2%)

According to The Washington Post, the Institute for Health Metrics and Evaluation at the Univ of Washington does not have good news in terms of COVID-19 fatalities. Cooler weather and more indoors time could boost viral transmission this fall and winter with deaths in the US reaching 410,000. More on whether there will be an early vaccine approval. While it is a good time to get a passport you cannot go anywhere because of COVID-19.

New York City parents are exhausted according to The New York Times but will they send their kids back to school when they open? The Times links to this paper that shows vaping is really not good for young people in terms of contracting COVID-19. This is not a surprise. Vaccine companies are making a pledge to fully conduct pre-approval safety studies of new COVID-19 vaccines.

According to my Yahoo news feed, Labor Day revelers are heading to Florida in large numbers. I hope they are masking up and keeping physical distance from others. You cannot make stories like this up!

The famous Mustang Ranch brothel in Nevada is eligible for a small business grant under the coronavirus relief package.

The Guardian paints a discouraging picture of the coming cold weather in the US and likely COVID-19 outbreaks.

The Lancet has a paper on early safety and immunogencity results of the Russian adenovirus based COVID-19 vaccine. Antibody titer was not as high as with the Moderna or AstraZeneca/Oxford vaccines. However, researchers note that the vaccine is highly immunogenic and induces strong humoral and cellular immune responses in 100% of healthy adult volunteers, with antibody titres in vaccinated participants higher than those in convalescent plasma. There is also a paper from Brazil on whether azithromycin added to standard of care provides a benefit in treating severe COVID-19. Adding azithromycin to standard of care treatment (which included hydroxychloroquine) did not improve clinical outcomes. Our findings do not support the routine use of azithromycin in combination with hydroxychloroquine in patients with severe COVID-19. There is an accompanying editorial on this finding.

MODELING

- As prospects for eradicating CoV-2 dwindle, we are faced with the question of how the severity of CoV-2 disease may change in the years ahead. Will CoV-2 continue to be a pathogenic scourge that, like smallpox or measles, can be tamed only by ongoing vaccination, or will it join the ranks of mild endemic human coronaviruses (HCoVs)? Our analysis of immunological and epidemiological data on HCoVs shows that infection-blocking immunity wanes rapidly, but disease-reducing immunity is long-lived. We estimate the relevant parameters and incorporate them into a new epidemiological model framework which separates these different components of immunity. *Our model recapitulates both the current severity of CoV-2 and the relatively benign nature of HCoVs; suggesting that once the endemic phase is reached, CoV-2 may be no more virulent than the common cold. The benign outcome at the endemic phase is contingent on the virus causing primary infections in children. We predict a very different outcome were a CoV like MERS (that causes severe disease in children) to become endemic. These results force us to re-evaluate control measures that rely on identifying and isolating symptomatic infections, and reconsider ideas regarding herd immunity and the use of immune individuals as shields to protect vulnerable groups*. [**note: this is an interesting paper from Emory on possible outcomes of the pandemic. It is worth reading in full to grasp the nuanced argument.**] https://www.medrxiv.org/content/10.1101/2020.09.03.20187856v1
- Abstract Introduction: Prior to the diagnosis of the first SARS-CoV2 patient in Florida, the Miami Dade Fire Rescue developed and implemented its return-to-work protocol based on guidelines from the CDC and Florida Fire Chiefs Association. As of February 17, 2020, all asymptomatic employees exposed to PCR-confirmed positive SARS-CoV2 individuals would be excluded from work for 14 days and report absence of symptoms to a delegated supervisor every 24 hours. We postulated that if COVID-19 transmission rate continues at the current rate in the absence of systemic vaccination strategy for SARS-CoV2, then a safer and more efficient return-to-work policy is needed for exposed first responders who are identified as low-risk for disease transmission. Objectives: We sought to establish a safe and shortened return-to-work protocol to maintain our workforce. We evaluated the utility of serological antibody testing in predicting

negative seroconversion of first responders at 7 days post low-risk exposure to confirmed COVID-19 individuals. Methods: All exposed, asymptomatic employees underwent serology testing for SARS-CoV2 one week after the initial exposure. Participants who were serologically negative had follow-up RT-PCR within 24 hours and serology testing 14 days after the initial serological test. Results: Overall, of the 71 firefighters who have had documented exposures to SARS-CoV2 positive individuals in the fire rescue agency, 41 of 71 had initially negative serology studies. Of the 41 patients with negative serology studies, 20 voluntarily underwent confirmatory PCR testing within one day after serology testing and all 20 participants were negative. Subsequently, out of the 20 participants who underwent serology and PCR testing, 10 participants followed up and underwent repeat serology testing 14 days after exposure and all 10 participants had negative repeat serology tests. The other ten who chose not to retest remained asymptomatic 14 days after exposure. Conclusions: Although serology testing has limitations, it correlated with negative prediction of disease in low-risk participants with exposures in this study. Serology testing may offer a feasible, alternative return-to-work strategy for fire agencies. Keywords: COVID-19, SARS-CoV-2, Rapid IgM-IgG Combined test, Point-of-Care Testing, Return-to-Work protocol [**note: this is an approach from Miami Dade county in Florida about a return to work strategy for fire fighters suing serology testing.**] https://www.medrxiv.org/content/10.1101/2020.09.03.20187823v1

- Background: Decisions around US college and university operations will affect millions of students and faculty amidst the COVID-19 pandemic. We examined the clinical and economic value of different COVID-19 mitigation strategies on college campuses. Methods: We used the Clinical and Economic Analysis of COVID-19 interventions (CEACOV) model, a dynamic microsimulation that tracks infections accrued by students and faculty, accounting for community transmissions. Outcomes include infections, $/infection-prevented, and $/quality-adjusted-life-year ($/QALY). Strategies included extensive social distancing (ESD), masks, and routine laboratory tests (RLT). We report results per 5,000 students (1,000 faculty) over one semester (105 days). Results: Mitigation strategies reduced COVID-19 cases among students (faculty) from 3,746 (164) with no mitigation to 493 (28) with ESD and masks, and further to 151 (25) adding RLTq3 among asymptomatic students and faculty. ESD with masks cost $168/infection-prevented ($49,200/QALY) compared to masks alone. Adding RLTq3 ($10/test) cost $8,300/infection-prevented ($2,804,600/QALY). If tests cost $1, RLTq3 led to a favorable cost of $275/infection-prevented ($52,200/QALY). No strategies without masks were cost-effective. Conclusion: Extensive social distancing with mandatory mask-wearing could prevent 87% of COVID-19 cases on college campuses and be very cost-effective. Routine laboratory testing would prevent 96% of infections and require low cost tests to be economically attractive. [**note: this paper would have been more useful a month or so ago as universities were making plans to reopen. It's still an interesting read in terms of evaluating the economic impact.**] https://www.medrxiv.org/content/10.1101/2020.09.03.20187062v1

NEWLY REGISTERED CLINICAL TRIALS

- I was more interested in watching the Ajax – Augsburg soccer match than scouring the NIH database.

CLINICAL TRIAL RESULTS

- Background and aims: Immune dysregulation caused by SARS-CoV-2 infection is thought to play a pathogenic role in COVID-19. SARS-CoV-2 can infect a variety of host cells, including intestinal epithelial cells. We sought to characterize the role of the gastrointestinal immune system in the pathogenesis of the inflammatory response associated with COVID-19. Methods: We measured cytokines, inflammatory markers, viral RNA, microbiome composition and antibody responses in stool and serum samples from a prospectively enrolled cohort of 44 hospitalized COVID-19 patients. Results: SARS-CoV-2 RNA was detected in stool of 41% of patients and was found more frequently in patients with diarrhea than those without (16[44%] vs 5[19%], p=0.06). Patients who survived had lower median viral genome copies than those who did not (p=0.021). Compared to uninfected controls, COVID-19 patients had higher median fecal levels of IL-8 (166.5 vs 286.5 pg/mg; p=0.05) and lower levels of fecal IL-10 (678 vs 194 pg/mg; p<0.001) compared to uninfected controls. Stool IL-23 was higher in patients with more severe COVID-19 disease (223.8 vs 86.6 pg/mg; p=0.03) and *we find evidence of intestinal virus-specific IgA responses, which was associated with more severe disease. Fecal cytokines and calprotectin levels were not correlated with gastrointestinal symptoms or with the level of virus detected. Conclusions: Although SARS-CoV-2 RNA was detectable in the stools of COVID-19 patients and select individuals had evidence for a specific mucosal IgA response, intestinal inflammation was limited, even in patients presenting with gastrointestinal symptoms*. [**note: more from the busy workers at Mt. Sinai; an interesting finding that looking at fecal markers for COVID-19, intestinal inflammation was limited.**]
  https://www.medrxiv.org/content/10.1101/2020.09.03.20183947v1
- The pathophysiology of COVID-19 associated thrombosis seems to be multifactorial, involving interplay between cellular and plasmatic elements of the hemostasis. We hypothesized that COVID-19 is accompanied by platelet apoptosis with subsequent alteration of the coagulation system. We investigated depolarization of mitochondrial inner transmembrane potential (ΔΨm), cytosolic calcium (Ca2+) concentration, and phosphatidylserine (PS) externalization by flow cytometry. Platelets from intensive care unit (ICU) COVID-19 patients (n=21) showed higher ΔΨm depolarization, cytosolic Ca2+ concentration and PS externalization, compared to healthy controls (n=18) and COVID-19 non-ICU patients (n=4). Moreover significant higher cytosolic Ca2+ concentration and PS was observed compared to septic ICU control group (ICU control). In ICU control group (n=5; non-COVID-19 ICU) cytosolic Ca2+ concentration and PS externalization was comparable to healthy control, with an increase in ΔΨm depolarization. Sera from ICU COVID-19 patients induced significant increase in apoptosis markers (ΔΨm depolarization, cytosolic Ca2+ concentration and PS externalization) compared to healthy volunteer and septic ICU control. Interestingly, immunoglobulin G (IgG) fractions from COVID-19 patients induced an Fc gamma receptor IIA dependent platelet apoptosis (ΔΨm depolarization, cytosolic Ca2+ concentration and PS externalization). *Enhanced PS externalization in platelets from ICU COVID-19 patients was associated with increased sequential organ failure assessment (SOFA) score (r=0.5635) and D-Dimer (r=0.4473). Most importantly, patients with thrombosis had significantly higher PS externalization compared to those without. The strong correlations between apoptosis markers and increased D-Dimer levels as well as the incidence of thrombosis may indicate that antibody-mediated platelet apoptosis potentially contributes to sustained increased thromboembolic risk in ICU COVID-19 patients*. [**note: severe COVID-19 is associated with**

increased antibody-mediated platelet apotosis.]
https://www.medrxiv.org/content/10.1101/2020.09.03.20187286v1

DRUG DEVELOPMENT

- Like the SARS-CoV, SARS-CoV-2 also employs a receptor-binding motif (RBM) of its envelope spike protein for binding the host angiotensin-converting enzyme 2 (ACE2) to gain viral entry. Currently, extensive efforts are being made to produce vaccines against a surface fragment of a SARS-CoV-2, such as the spike protein, in order to boost protective antibody responses. It was previously unknown how spike protein-targeting antibodies would affect innate inflammatory responses to SARS-CoV-2 infections. Here we generated a highly purified recombinant protein corresponding to the RBM of SARS-CoV-2, and used it to screen for cross-reactive monoclonal antibodies (mAbs). We found two RBM-binding mAbs that competitively inhibited its interaction with human ACE2, and specifically blocked the RBM-induced GM-CSF secretion in both human monocyte and murine macrophage cultures. Our findings have suggested a possible strategy to prevent SARS-CoV-2-elicited cytokine storm, and provided a potentially useful criteria for future assessment of innate immune-modulating properties of various SARS-CoV-2 vaccines. [**note: this is the development of two monoclonosls and they find that GM-CSF secretion is also blocked suggesting a strategy to prevent cytokine storm.**]
https://www.biorxiv.org/content/10.1101/2020.09.04.280081v1
- The COVID-19 outbreak caused by SARS-CoV-2 has created an unprecedented health crisis since there is no coronavirus vaccine in the market due to the novelty of this virus. Therefore, SARS-CoV-2 vaccines have become very important to reduce morbidity and mortality. At this point, inactivated vaccines are important because the straightforward process of existing infrastructure used for several licensed human vaccines can be used for SARS-CoV-2. Inactive vaccines provide an antigenic presentation similar to that when they encounter invasive virus particles of the immune system. In this study, in vitro and in vivo safety and efficacy analyzes of lyophilized vaccine candidates inactivated by gamma-irradiation were performed. Our candidate OZG-3861 version 1 (V1) is an inactivated SARS-CoV-2 virus vaccine, and SK-01 version 1 (V1) is the GM-CSF adjuvant added vaccine candidate. We applied the candidates intradermal to BALB/c mice to assess the toxicity and immunogenicity of the OZG-3861 V1 and SK-01 V1. Here, we report our preliminary results in vaccinated mice. When considered in terms of T and B cell responses, it was observed that especially the vaccine models containing GM-CSF as an adjuvant caused significant antibody production with neutralization capacity in absence of the antibody-dependent enhancement feature. Another finding showed that the presence of adjuvant is more important in T cell response rather than B cell. The vaccinated mice showed T cell response upon restimulation with whole inactivated SARS-CoV-2 or peptide pool. This study encouraged us to start the challenge test using infective SARS-CoV-2 viruses and our second version of gamma-irradiated inactivated vaccine candidates in humanized ACE2+ mice. [**note: Turkey enters the vaccine development race with this irradiated inactive vaccine. Here are animal results!**] https://www.biorxiv.org/content/10.1101/2020.09.04.277426v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- While severe acute disease has been linked to an expansion of antibody-secreting plasmablasts, we sought to identify B cell responses that correlated with positive clinical outcomes in

convalescent patients. We characterized the peripheral blood B cell immunophenotype and plasma antibody responses in 40 recovered non-hospitalized COVID-19 subjects that were enrolled as donors in a convalescent plasma treatment study. We observed a significant negative correlation between the frequency of peripheral blood memory B cells and the duration of symptoms for convalescent subjects. Memory B cell subsets in convalescent subjects were composed of classical CD24+ class-switched memory B cells, but also activated CD24-negative and natural unswitched CD27+ IgD+ IgM+ subsets. Memory B cell frequency was significantly correlated with both IgG1 and IgM responses to the SARS-CoV-2 spike protein receptor binding domain (RBD). IgM+ memory, but not switched memory, directly correlated with virus-specific antibody responses, and remained stable over time. *Our findings suggest that the frequency of memory B cells is a critical indicator of disease resolution, and that IgM+ memory B cells play an important role in SARS-CoV-2 immunity*. [**note: more work on the immune response in recovered patients.B]**
https://www.medrxiv.org/content/10.1101/2020.09.04.20187724v1

DIAGNOSTIC DEVELOPMENT

- Nothing today

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2020-09-06

For reflection Sunday here is a live concert from the Temple of Dendur at Metropolitan Museum of Art featuring Rhiannon Giddens with Francesco Turrisi. It's full of some very good Americana and world music and Ms. Giddens addresses some particularly thorny questions (such as performance in blackface) throughout. Enjoy this one, I did: https://www.youtube.com/watch?v=Ignhso0iv9U

Spy vs. Spy the old Mad Magazine strip; no it isn't back but The New York Times reports on high level espionage as Russia and China work hard to steal vaccine secrets. Given all the nice vaccine work from China it is unclear why they even need to bother with the cloak and dagger stuff. Parents get time off and then employees without children complain about this at some tech companies. College towns are the new centers of the COVID-19 pandemic.

The Washington Post discusses the confluence of the coming influenza season with the current COVID-19 pandemic. Time to schedule a flu shot. Let us remember Paola De Simone who passed away the other day during a virtual lecture at a Buenos Aires university. She had been suffering the after effects of COVID-19. Go to college and prepare to get tested for COVID-19 at regular intervals. Money quote, Lior Pachter of Cal Tech who did a study of university plans said the data suggested many colleges were clinging to "an unrealistic belief, a kind of fiction, that people would come back to campus and not get sick." Here is another in the WaPo oral history of the pandemic, an Arizona single mom who is trying to balance a lot of things. Even this rural southwest Virginia county cannot escape SARS-CoV-2.

It's a very short newsletter this morning as there are no worthwhile preprints posted. I did check the clinical trial registry and have a couple for you. We are still waiting for clinical trial results from several antiviral studies. Enjoy your Sunday off, I certainly will!

MODELING

- Nothing today.

NEWLY REGISTERED CLINICAL TRIALS

- To evaluate whether time-to-improvement is significantly better in IMU-838 plus Oseltamivir (IONIC Intervention) and standard care vs. Oseltamivir and standard care in adult subjects with coronavirus disease (COVID-19). The IONIC Protocol describes an overarching trial design to provide reliable evidence on the efficacy of IMU-838 (vidofludimus calcium) when delivered in combination with an antiviral therapy (Oseltamivir) [IONIC Intervention] for confirmed or suspected COVID-19 infection in adult patients receiving usual standard of care. [**note: this is a UK based trial of an orally bioavailable inhibitor of dihydroorotate dehydrogenase (DHODH), with potential anti-inflammatory, immunomodulating and anti-viral activities and an antiviral against influenza.**] NCT04516915
- This study is to assess the safety, tolerability, pharmacodynamics, and pharmacokinetics of Niclosamide (DWRX2003) following escalating doses of DWRX2003 administered as an intramuscular injection in healthy volunteers. [**note: this is a Korean study of a drug used to treat tapeworm infections.**] NCT04524052

CLINICAL TRIAL RESULTS

- Nothing today

DRUG DEVELOPMENT

- Nothing today

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Nothing today

DIAGNOSTIC DEVELOPMENT

- Nothing today