2020-07-27

Welcome to Week 19! The hits just keep on coming.

We are going to stay with pandemic performances for a little while longer. First up this week is troubled Portland who have the double whammy of COVID-19 and a questionable occupation force. Here is the Motown classic 'Ain't No Mountain High Enough': https://www.youtube.com/watch?v=RDekA8Bqc_0 We are all in this together as this group points out and we can surmount this virus. Here are the song's original interpreters: Marvin Gaye and Tammi Terrell (you have to overlook the horrible lip-synching): https://www.youtube.com/watch?v=IC5PL0XImjw

US COVID-19 STATISTICS - **Infection Rate: 1.3%; CFR: 3.4%** (IR up 0.1%; CFR down1%; **note:** the CFR for this current outbreak is hovering just above 1%)

The New York Times wonders if Manhattan will come back to life. There is also the sad story of this Houston family that tried to do all the right things but still became infected. How can we deal with the mountain of data on COVID-19 (**note: this is something your humble newsletter curator wonders about daily)?** Wow, here is a great idea for pandemic TV watching: Peter Falk's iconic 'Columbo'! I'll confess that this was one of my faves way back when along with Kojak.

Washington Post readers weigh in on the pandemic. Not much optimism here. About 4000 federal employees have been sickened by COVID-19 at work.

Kaiser Health News reports on a growing request from employers for COVID-19 liability waivers. Look for lawsuits to come out of this. Neighbors helping neighbors is always a good thing as this story of NYC points out.

STAT discusses the side effect profiles of the experimental COVID-19 vaccines and an opinion piece about the need for outreach as vaccines near commercial distribution.

It is a thankfully short day for preprints. Medical journals usually take the weekend off as well.

MODELING

- Background The number of proposed prognostic models for COVID-19, which aim to predict disease outcomes, is growing rapidly. It is not known whether any are suitable for widespread clinical implementation. We addressed this question by independent and systematic evaluation of their performance among hospitalised COVID-19 cases. Methods We conducted an observational cohort study to assess candidate prognostic models, identified through a living systematic review. We included consecutive adults admitted to a secondary care hospital with PCR-confirmed or clinically diagnosed community-acquired COVID-19 (1st February to 30th April 2020). We reconstructed candidate models as per their original descriptions and evaluated performance for their original intended outcomes (clinical deterioration or mortality) and time horizons. We assessed discrimination using the area under the receiver operating characteristic curve (AUROC), and calibration using calibration plots, slopes and calibration-in-the-large. We calculated net benefit compared to the default strategies of treating all and no patients, and

against the most discriminating predictor in univariable analyses, based on a limited subset of a priori candidates. Results We tested 22 candidate prognostic models among a cohort of 411 participants, of whom 180 (43.8%) and 115 (28.0%) met the endpoints of clinical deterioration and mortality, respectively. The highest AUROCs were achieved by the NEWS2 score for prediction of deterioration over 24 hours (0.78; 95% CI 0.73-0.83), and a novel model for prediction of deterioration <14 days from admission (0.78; 0.74-0.82). Calibration appeared generally poor for models that used probability outcomes. In univariable analyses, admission oxygen saturation on room air was the strongest predictor of in-hospital deterioration (AUROC 0.76; 0.71-0.81), while age was the strongest predictor of in-hospital mortality (AUROC 0.76; 0.71-0.81). No prognostic model demonstrated consistently higher net benefit than using the most discriminating univariable predictors to stratify treatment, across a range of threshold probabilities. Conclusions Oxygen saturation on room air and patient age are strong predictors of deterioration and mortality among hospitalised adults with COVID-19, respectively. None of the prognostic models evaluated offer incremental value for patient stratification to these univariable predictors. [**note: from the UK, this is an evaluation of clinical prognostic models for severe COVID-19 progression. Oxygen saturation and age were the strong predicition.**] https://www.medrxiv.org/content/10.1101/2020.07.24.20149815v1

NEWLY REGISTERED CLINICAL TRIALS

- Did not look today.

CLINICAL TRIAL RESULTS

- Background Coronavirus disease 2019 (COVID-19), caused by novel coronavirus SARS-CoV-2, has to date affected over 13.3 million globally. Although high rates of venous thromboembolism and evidence of COVID-19-induced endothelial dysfunction have been reported, the precise aetiology of the increased thrombotic risk associated with COVID-19 infection remains to be fully elucidated. Objectives Here, we assessed clinical platelet parameters and circulating platelet activity in patients with severe and non-severe COVID-19. Methods An assessment of clinical blood parameters in patients with severe COVID-19 disease (requiring intensive care), patients with non-severe disease (not requiring intensive care), general medical in-patients without COVID-19 and healthy donors was undertaken. Platelet function and activity were also assessed by secretion and specific marker analysis. Results We show that routine clinical blood parameters including increased MPV and decreased platelet:neutrophil ratio are associated with disease severity in COVID-19 upon hospitalisation and intensive care unit admission. Strikingly, agonist-induced ADP release was dramatically higher in COVID-19 patients compared with non-COVID-19 hospitalized patients and circulating levels of PF4, sP-selectin and TPO were also significantly elevated in COVID-19. Conclusion Distinct differences exist in routine full blood count and other clinical laboratory parameters between patients with severe and non-severe COVID-19. Moreover, we have determined that COVID-19 patients possess hyperactive circulating platelets. These data suggest that abnormal platelet reactivity may contribute to hypercoagulability in COVID-19. Further investigation of platelet function in COVID-19 may provide additional insights into the aetiology of thrombotic risk in this disease and may contribute to the optimisation of thrombosis prevention and treatment strategies. [**note:**

hyperactive circulating platelets may be the key to observed hypercoagulation in COVID-19.]
https://www.medrxiv.org/content/10.1101/2020.07.24.20156240v1

- Background: We recently delineated the functional biology of pathogenic and inflammation resolving synovial tissue macrophage clusters in rheumatoid arthritis (RA). Whilst RA is not a viral respiratory syndrome, it represents a pro-inflammatory cytokine-driven chronic articular condition often accompanied by cardiovascular and lung pathologies. We hypothesised that functionally equivalent macrophage clusters in the lung might govern inflammation and resolution of COVID-19 pneumonitis. Methods: To provide insight into the targetable functions of COVID-19 bronchoalveolar lavage (BALF) macrophage clusters, a comparative analysis of BALF macrophage single cell transcriptomics (scRNA-seq) with synovial tissue (ST) macrophage scRNA-seq and functional biology was performed. The function of shared BALF and ST MerTK inflammation-resolving pathway was confirmed with inhibitor in primary macrophage-synovial fibroblast co-cultures. Results: Distinct BALF FCN^{pos} and $FCN^{pos}SPP1^{pos}$ macrophage clusters emerging in severe COVID-19 patients were closely related to ST $CD48^{high}S100A12^{pos}$ and $CD48^{pos}SPP1^{pos}$ clusters driving synovitis in active RA. They shared transcriptomic profile and pathogenic mechanisms. Healthy lung resident alveolar $FABP4^{pos}$ macrophages shared a regulatory transcriptomic profile, including TAM (Tyro, Axl, MerTK) receptors pathway with synovial tissue $TREM2^{pos}$ macrophages that govern RA remission. This pathway was substantially altered in BALF macrophages of severe COVID-19. In vitro dexamethasone inhibited tissue inflammation via macrophages MerTK function. Conclusion: Pathogenesis and resolution of COVID-19 pneumonitis and RA synovitis might be driven by similar macrophage clusters and pathways. The MerTK-dependent anti-inflammatory mechanisms of dexamethasone, and the homeostatic function of TAM pathways that maintain RA in remission advocate the therapeutic MerTK agonism to ameliorate the cytokine storm and pneumonitis of severe COVID-19. [**note: this is a plausible linkage between rheumatoid arthritis and COVID-19}**
https://www.biorxiv.org/content/10.1101/2020.07.26.221572v1

DRUG DEVELOPMENT

- Due to the lack of protective immunity of humans towards the newly emerged SARS-CoV-2, this virus has caused a massive pandemic across the world resulting in hundreds of thousands of deaths. Thus, a vaccine is urgently needed to contain the spread of the virus. Here, we describe Newcastle disease virus (NDV) vector vaccines expressing the spike protein of SARS-CoV-2 in its wild type or a pre-fusion membrane anchored format. All described NDV vector vaccines grow to high titers in embryonated chicken eggs. In a proof of principle mouse study, we report that the NDV vector vaccines elicit high levels of antibodies that are neutralizing when the vaccine is given intramuscularly. Importantly, these COVID-19 vaccine candidates protect mice from a mouse-adapted SARS-CoV-2 challenge with no detectable viral titer and viral antigen in the lungs. [**note: the vaccine vectors keep on coming. Here is Newcastle disease virus being used. The drawback here is the need for growing it in embryonated chicken eggs. If implemented, it would compete for seasonal influenza vaccine production.b]**
https://www.biorxiv.org/content/10.1101/2020.07.26.221861v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The spike protein of SARS-CoV-2 is known to enable viral invasion into human cells through direct binding to host receptors including ACE2. An alternate entry receptor for the virus was recently proposed to be basigin/CD147. These early studies have already prompted a clinical trial and multiple published hypotheses of the role of this host receptor in viral infection and pathogenesis. We sought to independently characterize the basigin-spike protein interaction. After conducting several lines of experiments, we report that we are unable to find evidence supporting the role of basigin as a putative spike-binding receptor. Recombinant forms of both the entire ectodomain and S1 domain of the SARS-CoV-2 spike protein that directly bind ACE2 do not interact with basigin expressed on the surface of human cells. Using specialized assays tailored to detect receptor interactions as weak or weaker than the proposed basigin-spike binding, we report no evidence for direct binding of the viral spike to either of the two common isoforms of basigin. Given the pressing need for clarity on which targets of SARS-CoV-2 may lead to promising therapeutics, we present these findings to allow more informed decisions about the translational relevance of this putative mechanism in the race to understand and treat COVID-19. [**note: this points to a potential blind alley for research**] https://www.biorxiv.org/content/10.1101/2020.07.25.221036v1

DIAGNOSTIC DEVELOPMENT

- It's Monday and nothing new here.

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2020-07-28

This is the greatest pandemic video collage that I've seen to date. It will take something really special to top it. Here are a bunch and I mean a bunch of Chicagoans singing and riffing off 'Sweet Home Chicago.' The performers are identified at the end of the video and try to see how many you can identify (I got 10 of them that were the easiest). Hard blues will put a smile on your face: https://www.youtube.com/watch?v=V7-Nxgwa2iM

US COVID-19 STATISTICS - **Infection Rate: 1.3%; CFR: 3.4%** (IR unchanged; CFR unchanged; **note:** the CFR for this current outbreak is moved up to 2%)

I listened/watched an interesting discussion between Russ Roberts and Nassim Taleb on Roberts's show Econtalk. Taleb is well known for his book 'The Black Swan' that discusses 'fat tale' events that are inadequately addressed by conventional statistical methods. Taleb goes into why a pandemic fits into this category and how to properly assess risk. This is highly recommended: https://www.youtube.com/watch?v=CyOsUbK8PiE is the best way to understand the concepts as Taleb has a couple of graphs that he presents. There is also a 'debate' between Taleb and John Ioannidis worth looking at. I've comment on Ioannidis in the past and I think he needs to study up on the Rumsfeld Paradigm as there are things we cannot readily get data on even with all the research going on.

The Atlantic's Derek Thompson on the scourge of hygiene theater. I think he is probably right; we are focusing on minimal risk things rather than those that pose a real one.

The New York Times has an investigative story on Dr. Sapan Desai who was a principal co-author on two discredited COVID-19 database reports. We will see how this small school district in Georgia makes out with in-class instruction and no mask mandate. Speaking of masks, there is this story on how masks can reduce the viral dose making the disease less severe. HERE is a link to the preprint. Maybe I will email a copy to Governor Kemp in Georgia and the head of the school board of Jefferson, GA. Nah, let them do the experiment for the rest of us. Why we should be doing lots of tests even with imperfect kits; makes good sense!!

The Washington Post is pessimistic on the major league baseball season as 14 players on the Miami team test positive for COVID-19. The Toronto Blue Jays cannot play any home games because of the travel embargo Canada has imposed on the US. Both mRNA vaccines from Moderna and Pfizer enter large scale trials. Pfizer may have an edge as they are in 150 sites world-wide. Things are not going well in Hong Kong both politically and COVID-19 wise. YIKES! Now I know why my stock in Kontoor Brands is tanking (they make Wrangler and Lee Jeans). Come on loyal readers, buy a pair of jeans today!!! Finally, last but not least from the WaPo – President Trump should listen to his BFF Boris Johnson's message on how to avoid the worst from COVID-19!

One would expect aspiring physicians, in this case anesthesiologists, to know better.

STAT provides yet another reason for avoiding Facebook! Zuckerberg – clean this stuff up!! My BFF, Zeke Emanuel questions whether the US can actually distribute a COVID-19 vaccine. I hope they do a better job of this than getting diagnostic tests out. Finally from STAT, *an interesting discussion of contact tracing apps*.

JAMA hit the ground running on Monday! Houston Methodist hospital did a careful surveillance study of healthcare workers. Those dealing directly with COVID-19 patients had a higher rate of infection (5.4%) compared to those not facing COVID-19 patients (0.6%). This seems to me a low level of infection compared to some other hospital system reports. Here is a Swiss model for viral aerosol emission from simulated emissions (**I am not sure how relevant this is**). In this mathematical modeling study, breathing and coughing by a simulated individual with COVID-19 were estimated to release large numbers of viruses in a poorly ventilated room with a coughing person. However, the estimated infectious risk posed by a person with typical viral load who breathes normally was low, and only few people with very high viral load posed an infection risk in a poorly ventilated closed environment. Here is a paper on work-related absence due to illness in mid-April coincident with the height of the early pandemic. Work absence due to illness rose to record levels. We had no data on what illnesses caused absences. Although the confidential survey offered respondents 13 non–illnesses-related options—including child care—as the reason for work absence, some jobholders who stayed home to care for children or others may have attributed their absenteeism to their own illness. Publicity around COVID-19 may have caused workers with non–COVID-19–related symptoms, or anxiety, to stay home. Increasing options for telecommuting and economic duress, however, might have the opposite effect. Similarly, reductions in other viral illnesses due to social distancing, and decreased injuries and air-pollution-related illnesses, might have cut work absences from non–COVID-19 illnesses. Finally, here is a paper looking all of the clinical trials registered on the NIH database through May 19 (**this is the database that I use for posting new clinical trials**). They found 1551 registered trials. They categorized reported outcomes and graded studies using the 2011 Oxford Centre for Evidence-Based Medicine (OCEBM) level of evidence framework. This cross-sectional study found that despite the marked rise in

COVID-19 studies, only 29.1% of those registered in ClinicalTrials.gov have the potential to result in OCEBM level 2 evidence. Of the RCTs, only 29.3% are placebo-controlled, blinded studies. Global decline in new cases could also stall enrollment. Even before results are known, most studies likely will not yield meaningful scientific evidence at a time when rapid generation of high-quality knowledge is critical. [**note: this has been my own observation as well. Lots of junk trials are being registered and it is doubtful that many will meet the enrollment standards}**

Phew! There was a lot to read yesterday and this morning. Do have a look at the first modeling paper on intense disease surveillance. The paper from Uruguay offers a good model for other nations to emulate. Some very interesting drug discovery papers today.

MODELING

- Background The rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the associated coronavirus disease 2019 (COVID-19) have precipitated a global pandemic heavily challenging our social behavior, economy, and healthcare infrastructure. Public health practices currently represent the primary interventions for managing the spread of the pandemic. We hypothesized that frequent, longitudinal workplace disease surveillance would represent an effective approach to controlling SARS-CoV-2 transmission among employees and their household members, reducing potential economic consequences and loss of productivity of standard isolation methods, while providing new insights into viral-host dynamics. Methodology and Findings On March 23, 2020 a clinical study (OCIS-05) was initiated at a small Southern California organization. Results from the first 3 months of the ongoing study are presented here. Study participants (27 employees and 27 household members) consented to provide frequent nasal or oral swab samples that were analyzed by RT-qPCR for SARS-CoV-2 RNA using CDC protocols. Only participants testing negative were allowed to enter the "safe zone" workplace facility. Optional blood samples were collected at baseline and throughout the 3-month study. Serum virus-specific antibody concentrations (IgG, IgM, and IgA) were measured using a selective, sensitive, and quantitative ELISA assay developed in house. A COVID-19 infection model, based on traditional SEIR compartmental models combined with Bayesian non-linear mixed models and modern machine learning, was used to predict the number of employees and household members who would have become infected in the absence of workplace surveillance. Two study participants were found to be infected by SARS-CoV-2 during the study. One subject, a household member, tested positive clinically by RT-qPCR prior to enrollment and experienced typical COVID-19 symptoms that did not require hospitalization. While on study, the participant was SARS-CoV-2 RNA positive for at least 71 days and had elevated virus-specific antibody concentrations (medians: IgM, 9.83 ug mL-1; IgG, 11.5 ug mL-1; IgA, 1.29 ug mL-1) in serum samples collected at three timepoints. A single, unrelated employee became positive for SARS-CoV-2 RNA over the course of the study, but remained asymptomatic with low associated viral RNA copy numbers. The participant did not have detectable serum IgM and IgG concentrations, and IgA concentrations decayed rapidly (half-life: 1.3 d). The employee was not allowed entry to the safe zone workplace until testing negative three consecutive times over 7 d. No other employees or household members contracted COVID-19 over the course of the study. Our model predicted that under the current prevalence in Los Angeles County

without surveillance intervention, up to 7 employees (95% CI = 3-10) would have become infected with at most 1 of them requiring hospitalizations and 0 deaths. Conclusions Our clinical study met its primary objectives by using intense longitudinal testing to provide a safe work environment during the COVID-19 pandemic, and elucidating SARS-CoV-2 dynamics in recovering and asymptomatic participants. The surveillance plan outlined here is scalable and transferrable. The study represents a powerful example on how an innovative public health initiative can be dovetailed with scientific discovery. [**note: OK, this is a very small trial but somebody had to get the ball rolling. This shows the impact of frequent testing in keeping workplaces open. As I have noted the US needs to change "shop 'til you drop" to "test the heck out of COVID until we are all safe" Enough said about this paper, good work!**]
https://www.medrxiv.org/content/10.1101/2020.07.25.20160812v1

- Background Social distancing measures, including school closures, are being used to control SARS-CoV-2 transmission in many countries. Once "lockdown" has driven incidence to low levels, selected activities are being permitted. Re-opening schools is a priority because of the welfare and educational impact of closures on children. However, the impact of school re-opening needs to be considered within the context of other measures. Methods We use social contact data from the UK to predict the impact of social distancing policies on the reproduction number. We calibrate our tool to the COVID-19 epidemic in the UK using publicly available death data and Google Community Mobility Reports. We focus on the impact of re-opening schools against a back-drop of wider social distancing easing. Results We demonstrate that pre-collected social contact data, combined with incidence data and Google Community Mobility Reports, is able to provide a time-varying estimate of the reproduction number (R). From an pre-control setting when R=2.7 (95%CI 2.5, 2.9), we estimate that the minimum reproduction number that can be achieved in the UK without limiting household contacts is 0.45 (95%CI:0.41-0.50); in the absence of other changes, preventing leisure contacts has a smaller impact (R=2.0,95%CI:1.8-2.4) than preventing work contacts (R=1.5,95%CI:1.4-1.7). We find that following lockdown (when R=0.7 (95% CI 0.6, 0.8)), opening primary schools in isolation has a modest impact on transmission R=0.83 (95%CI:0.77-0.90) but that high adherence to other measures is needed. Opening secondary schools as well as primary school is predicted to have a larger overall impact (R=0.95,95%CI:0.85-1.07), however transmission could still be controlled with effective contact tracing. Conclusions *Our findings suggest that primary school children can return to school without compromising transmission, however other measures, such as social distancing and contract tracing, are required to control transmission if all age groups are to return to school. Our tool provides a mapping from policies to the reproduction number and can be used by policymakers to compare the impact of social-easing measures, dissect mitigation strategies and support careful localized control strategies*. [**note: this model for school reopening is from a group of UK researchers. They argue that younger children may return but if all age groups do social distancing measures need to be put in place.**]
https://www.medrxiv.org/content/10.1101/2020.07.25.20156471v1
- Objective: To analyze the vertical distribution of six cities in Henan Province,China from January 21, 2020 to June17, 2020: Xinyang City (including Gushi County), Nanyang City (including Dengzhou City), Zhumadian City (including XincaiCounty), Zhengzhou City (including Gongyi City), Puyang City and Anyang City (including Hua County) corona virus disease 2019(COVID-19) epidemiological characteristics and local prevention and control measures.Methods: Data were

collected and analyzed through the COVID-19 information published on the official websites of health commissions of Henan Province and six cities.Results: As of June 17, 2020, the cumulative incidence rate of COVID-19 in Henan province was 1.33/100,000, the cumulative cure rate was 98.27%, the cumulative mortality rate was 1.73%, the age range of diagnosed cases was 5days-85years old, and the male to female ratio was 1.09:1.The confirmed cases of COVID-19 in Henan province were mainly imported cases from Hubei, accounting for 87.74%, of which the highest number was 70.50% in Zhumadian. The contact cases and local cases increased in a fluctuating manner over time.Significance: In this paper, epidemiological characteristics of COVID-19 in Henan province from the outbreak to the effective control within 60 days were analyzed, and effective and distinctive prevention and control measures in various cities were summarized, so as to provide a favorable reference for the further formulation and implementation of epidemic prevention and control and a valuable theoretical basis for effectively avoiding the second outbreak. [**note: epidemiological study of Henan Province in China. It is a useful paper to take a look at as the area was able to control the spread of the virus. Most of the illness was in patients between 35-70 years of age. Mortality rate was still significant at 1.73%**] https://www.medrxiv.org/content/10.1101/2020.07.25.20161844v1

- Objectives -- To investigate the relation of severe COVID-19 to prior drug prescribing. Design -- Matched case-control study (REACT-SCOT) based on record linkage to hospital discharges since June 2015 and dispensed prescriptions issued in primary care during the last 240 days. Setting -- Scottish population. Main outcome measure -- Severe COVID-19, defined by entry to critical care or fatal outcome. Participants -- All 4272 cases of severe COVID-19 in Scotland since the start of the epidemic, with 36948 controls matched for age, sex and primary care practice. Results -- Severe COVID-19 was strongly associated with the number of non-cardiovascular drug classes dispensed. This association was strongest in those not resident in care homes, in whom the rate ratio (95% CI) associated with dispensing of 12 or more drug classes versus none was 10.8 (8.7, 13.2), and was not accounted for by treatment of conditions designated as conferring increased risk. Of 17 drug classes postulated at the start of the epidemic to be "medications compromising COVID", all were associated with increased risk of severe COVID-19. The largest effect was for antipsychotic agents: rate ratio 4.14 (3.39, 5.07). Other drug classes with large effects included proton pump inhibitors (rate rato 2.19 (1.70, 2.80) for >= 2 defined daily doses/day), opioids (3.62 (2.65, 4.94) for >= 50 mg morphine equivalent/day) and gabapentinoids. These associations persisted after adjusting for covariates, and were stronger with recent than with non-recent exposure. Conclusions -- Severe COVID-19 is associated with polypharmacy and with drugs that cause sedation, respiratory depression or dyskinesia, have anticholinergic effects or affect the gastrointestinal system. These associations are not easily explained by co-morbidity. Although the evidence for causality is not conclusive, these results support existing guidance on reducing overprescribing of these drug classes and limiting inappropriate polypharmacy as a potential means of reducing COVID-19 risk [**note: from Scotland, the impact on polypharmacy and severe COVID-19**] https://www.medrxiv.org/content/10.1101/2020.07.23.20160747v1
- Background: South America has become the new epicenter of the COVID-19 pandemic with more than 1.1M reported cases and >50,000 deaths (June 2020). Conversely, Uruguay stands out as an outlier managing this health crisis with remarkable success. Methods: We developed a molecular diagnostic test to detect SARS-CoV-2. This methodology was transferred to research institutes, public hospitals and academic laboratories all around the country, creating a COVID-

19 diagnostic lab network. Uruguay also implemented active epidemiological surveillance following the Test, Trace and Isolate (TETRIS) strategy coupled to real-time genomic epidemiology. Results: Three months after the first cases were detected, the number of positive individuals reached 826 (23 deaths, 112 active cases and 691 recovered). The Uruguayan strategy was based in a close synergy established between the national health authorities and the scientific community. In turn, academia rapidly responded to develop national RT-qPCR tests. Consequently, Uruguay was able to perform ~1,000 molecular tests per day in a matter of weeks. The COVID-19 diagnostic lab network performed more than 54% of the molecular tests in the country. This, together with real-time genomics, were instrumental to implement the TETRIS strategy, helping to contain domestic transmission of the main outbreaks registered so far. Conclusions: Uruguay has successfully navigated the first trimester of the COVID-19 health crisis in South America. A rapid response by the scientific community to increase testing capacity, together with national health authorities seeking out the support from the academia were fundamental to successfully contain, until now, the COVID-19 outbreak in the country. [**note: maybe this is the country the US ought to study in terms of implementing good public health measures! My new mantra: "Why can't the US be more like Uruguay?"**]
https://www.medrxiv.org/content/10.1101/2020.07.24.20161802v1

- In this paper, we compare the inference regarding the effectiveness of the various non-pharmaceutical interventions (NPIs) for COVID-19 obtained from two SIR models, both produced by the Imperial College COVID-19 Response Team. One model was applied to European countries and published in Nature, concluding that complete lockdown was by far the most effective measure and 3 million deaths were avoided in the examined countries. The Imperial College team applied a different model to the USA states. Here, we show that inference is not robust to model specification and indeed changes substantially with the model used for the evolution of the time-varying reproduction number. Applying to European countries the model that the Imperial College team used for the USA states shows that complete lockdown has no or little impact, since it was introduced typically at a point when the time-varying reproduction number was already very low. We also show that results are not robust to the inclusion of additional follow-up data. [**note: speaking of the good Professor Ioannidis, here is a paper he is a coauthor on about comparing two models. I still don't think he gets it. The clampdown is needed to keep the healthcare system from collapsing. I'll just refer folks back to the Taleb post at the beginning of today's newsletter.**]
https://www.medrxiv.org/content/10.1101/2020.07.22.20160341v1
- The Covid-19 pandemic affects mortality directly through infection as well as through changes in the social, environmental and healthcare determinants of health. The impacts on mortality are likely to vary across countries in magnitude, timing, and age and sex composition. Here, we applied an ensemble of 16 Bayesian probabilistic models to vital statistics data, by age group and sex, to consistently and comparably estimate the impacts of the first phase of the pandemic on all-cause mortality for 17 industrialised countries. The models accounted for factors that affect death rates including seasonality, temperature, and public holidays, as well as for medium-long-term secular trends and the dependency of death rates in each week on those in preceding week(s). From mid-February through the end of May 2020, an estimated 202,900 (95% credible interval 179,400-224,900) more people died in these 17 countries than would have had the pandemic not taken place. Nearly three quarters of these excess deaths occurred

in England and Wales, Italy and Spain, where less than half of the total population of these countries live. When all-cause mortality is considered, the total number of deaths, deaths per 100,000 people, and relative increase in deaths were similar between men and women in most countries. Further, in many countries, the balance of excess deaths changed from male-dominated early in the pandemic to being equal or female-dominated later on. Taken over the entire first phase of the pandemic, there was no detectable rise in all-cause mortality in New Zealand, Bulgaria, Hungary, Norway, Denmark and Finland and for women in Austria and Switzerland (posterior probability of an increase in deaths <90%). Women in Portugal and men in Austria experienced relatively small increases in all-cause mortality, with posterior probabilities of 90-99%. For men in Switzerland and Portugal, and both sexes in the Netherlands, France, Sweden, Belgium, Italy, Scotland, Spain and England and Wales, all-cause mortality increased as a result of the pandemic with a posterior probability >99%. After accounting for population size, England and Wales and Spain experienced the highest death toll, nearly 100 deaths per 100,000 people; they also had the largest relative (percent) increase in deaths (37% (95% credible interval 30-44) in England and Wales; 38% (31-44) in Spain). New Zealand, Bulgaria, Hungary, Norway, Denmark and Finland experienced changes in deaths that ranged from possible slight declines to increases of no more than 5%. The large impact in England and Wales stems partly from having experienced (together with Spain) the highest weekly increases in deaths, more than doubling in some weeks, and having had (together with Sweden) the longest duration when deaths exceeded levels that would be expected in the absence of the pandemic. The heterogeneous magnitude and character of the excess deaths due to the Covid-19 pandemic reflect differences in how well countries have managed the pandemic (e.g., timing, extent and adherence to lockdowns and other social distancing measures; effectiveness of test, trace and isolate mechanisms), and the resilience and preparedness of the health and social care system (e.g., effective facility and community care pathways; minimising spread of infection within hospitals and care homes, and between them and the community). [**note: this is a large all-cause mortality study of the COVID-19 pandemic in 17 industrialized countries. Australia, Germany, Greece, US, Japan, South Korea, and Taiwan were excluded as they could not obtain age- and sex-specific data.**]
https://www.medrxiv.org/content/10.1101/2020.07.26.20161570v1

NEWLY REGISTERED CLINICAL TRIALS

- The first-in-human Phase I study component will evaluate two dose levels of RAPA-501-ALLO off the shelf cells in patients with post-intubation, stage 3 COVID-19 disease, with key endpoints of safety, biologic and potential disease-modifying effects. The randomized, double-blind, placebo-controlled Phase 2 study component will evaluate infusion of RAPA-501 ALLO off the shelf cells or a control infusion, with the primary endpoint assessing whether RAPA-501 cells reduce 30-day mortality. RAPA-501-ALLO cells are generated from healthy volunteers, cryopreserved, banked, and are then available for off-the-shelf therapy anytime. During manufacturing, T cells are "reprogrammed" ex vivo using a novel, patented 6-day two-step process that involves T cell de-differentiation and subsequent re-differentiation towards the two key anti-inflammatory programs, the TREG and Th2 pathways, thus creating a "hybrid" product. The hybrid phenotype inhibits inflammatory pathways operational in COVID-19, including modulation of multiple cytokines and chemokines, which attract inflammatory cells into tissue for initiation of multi-

organ damage. The hybrid TREG and Th2 phenotype of RAPA-501-ALLO cells cross-regulates Th1 and Th17 populations that initiate hyperinflammation of COVID-19. RAPA-501 immune modulation occurs in a T cell receptor independent manner, thus permitting off-the-shelf cell therapy. Finally, in experimental models of viral pneumonia and ARDS, TREG cells mediate a protective effect on the lung alveolar tissue. Because of this unique mechanism of action that involves both anti-inflammatory and tissue protective effects, the allogeneic RAPA-501 T cell product is particularly suited for evaluation in the setting of post-intubation, Stage 3 COVID-19. [**note: sponsor is Rapa Therapeutics which is right up the road from where I live! One of my neighbors works at this company.**] NCT04482699

CLINICAL TRIAL RESULTS

- Context: Originally developed for the treatment of human immunodeficiency virus (HIV), the antiviral combination lopinavir/ritonavir (LPV/r) is being investigated for use against coronavirus disease (COVID-19). We present a case series raising safety and efficacy concerns in COVID-19 affected patients. Methods: We measured LPV trough concentrations in 12 patients treated at our center and reviewed their clinical charts for side effects known to occur in HIV patients. Results: Compared to established LPV trough concentrations in HIV treated patients, concentrations in COVID-19 affected patients were 3-fold greater (20.64 +/- 10.14 mcg/mL versus 6.25 mcg/mL). In addition, cholestasis and dyslipidemia toxicity thresholds were exceeded in 12/12 and 11/12 patients respectively. No patients achieved the presumed therapeutic concentration. The side effects noted were mainly gastrointestinal symptoms (5/12, 42%), electrolytes imbalances (4/12, 33%), liver enzyme disturbances (5/12, 42%), and triglyceride elevations (2/12, 17%). Conclusion: None of our patients reached presumed therapeutic LPV concentrations despite experiencing side effects and exceeding cholestasis and dyslipidemia toxicity thresholds. This raises concerns for the safety and efficacy of LPV/r. Clinicians should consider closely monitoring for side effects and not necessarily attribute them to COVID-19 itself. [**note: from Montreal, a cautionary safety note about the use of lopinavir/ritonavir for COVID-19. I've not seen any efficacy reports with this combination but there are enough clinical trials that the warning is warranted.**] https://www.medrxiv.org/content/10.1101/2020.07.23.20153932v1
- Multiple clinical studies are ongoing to assess whether existing vaccines may afford protection against SARS-CoV-2 infection through trained immunity. In this exploratory study, we analyze immunization records from 137,037 individuals who received SARS-CoV-2 PCR tests. We find that polio, Hemophilus influenzae type-B (HIB), measles-mumps-rubella (MMR), varicella, pneumococcal conjugate (PCV13), geriatric flu, and hepatitis A / hepatitis B (HepA-HepB) vaccines administered in the past 1, 2, and 5 years are associated with decreased SARS-CoV-2 infection rates, even after adjusting for geographic SARS-CoV-2 incidence and testing rates, demographics, comorbidities, and number of other vaccinations. Furthermore, age, race/ethnicity, and blood group stratified analyses reveal significantly lower SARS-CoV-2 rate among black individuals who have taken the PCV13 vaccine, with relative risk of 0.45 at the 5 year time horizon (n: 653, 95% CI: (0.32, 0.64), p-value: 6.9e-05). These findings suggest that additional pre-clinical and clinical studies are warranted to assess the protective effects of existing non-COVID-19 vaccines and explore underlying immunologic mechanisms. We note that the findings in this study are preliminary and are subject to change as more data becomes

available and as further analysis is conducted. [**note: maybe we really don't need a COVID-19 vaccine. Let's just vaccinate everyone regular with the ones we already have. Let the anti-vaxxers fend for themselves!!!**]
https://www.medrxiv.org/content/10.1101/2020.07.27.20161976v1

DRUG DEVELOPMENT

- Many COVID-19 patients demonstrate lethal respiratory complications caused by cytokine release syndrome (CRS). Multiple cytokines have been implicated in CRS, but TNFSF14 (LIGHT) has not been previously measured in this setting. In this study, we observed significantly elevated serum LIGHT levels in hospitalized COVID-19 patients as compared to healthy age and gender matched control patients. The assay detected bioavailable LIGHT unbound to the inhibitor Decoy receptor-3 (DcR3). Bioavailable LIGHT levels were elevated in patients both on and off ventilatory support, with a trend toward higher levels in patients requiring mechanical ventilation. In hospitalized patients over the age of 60, who exhibited a mortality rate of 82%, LIGHT levels were significantly higher (p=0.0209) in those who died compared to survivors. As previously reported, interleukin 6 (IL-6) levels were also elevated in these patients with significantly (p=0.0076) higher levels observed in patients who died vs. survivors, paralleling the LIGHT levels. Although attempts to block IL-6 binding to its receptor have shown limited effect in COVID-19 CRS, neutralization of LIGHT may prove to be more effective owing to its more central role in regulating antiviral immune responses. [**note: here is another part of the cytokine pathway that might be a target for therapy. The company sponsoring this research is Cerecor, Inc. whose Chief Scientific Officer is Garry Neil who I worked with a lot when I was at PhRMA and he was on the Executive Committee overseeing our division!**]
https://www.medrxiv.org/content/10.1101/2020.07.27.20152892v1
- The recent global pandemic caused by the new coronavirus SARS-CoV-2 presents an urgent need for new therapeutic candidates. While the importance of traditional in silico approaches such as QSAR in such efforts in unquestionable, these models fundamentally rely on structural similarity to infer biological activity and are thus prone to becoming trapped in the very nearby chemical spaces of already known ligands. For novel and unprecedented threats such as COVID-19 much faster and efficient paradigms must be devised to accelerate the identification of new chemical classes for rapid drug development. Here we report the development of a new biological activity-based modeling (BABM) approach that builds on the hypothesis that compounds with similar activity patterns tend to share similar targets or mechanisms of action. In BABM, compound activity profiles established on massive scale across multiple assays are used as signatures to predict compound activity in a new assay or against a new target. We first trained and validated this approach by identifying new antiviral lead candidates for Zika and Ebola based on data from ~0.5 million compounds screened against ~2,000 assays. BABM models were then applied to predict ~300 compounds not previously reported to have activity for SARS-CoV-2, which were then tested in a live virus assay with high (>30%) hit rates. The most potent compounds showed antiviral activities in the nanomolar range. These potent confirmed compounds have the potential to be further developed in novel chemical space into new anti-SARS-CoV-2 therapies. These results demonstrate unprecedented ability using BABM to predict novel structures as chemical leads significantly beyond traditional methods, and its application in rapid drug discovery response in a global public health crisis. [**note: this is another approach**

to drug discovery modeling from NIH. The investigators used the tool to screen 500K compounds, identifying just over 300 compounds as potential anti-SARS-CoV-2 leads. 100 were tested *in vitro,* with some good compounds with activity at very low concentrations. You will need to download the paper to see the drug candidates as there are too many to list here. Also note that there are supplementary tables not in the PDF.]
https://www.biorxiv.org/content/10.1101/2020.07.27.223578v1

- Efficacious interventions are urgently needed for the treatment of COVID-19. Here, we report a monoclonal antibody (mAb), MW05, showing high SARS-CoV-2 neutralizing activity by disrupting the interaction of receptor binding domain (RBD) with angiotensin-converting enzyme 2 (ACE2) receptor. Crosslinking of Fc with FcγRIIB mediates antibody-dependent enhancement (ADE) activity by MW05. This activity was eliminated by introducing the LALA mutation to the Fc region (MW05/LALA). Most importantly, potent prophylactic and therapeutic effects against SARS-CoV-2 were observed in rhesus monkeys. A single dose of MW05/LALA completely blocked the infection of SARS-CoV-2 in a study of its prophylactic effect and totally cleared SARS-CoV-2 in three days in a treatment setting. These results pave the way for the development of MW05/LALA as an effective strategy for combating COVID-19. {**note: from China, animal data showing a single dose of an mAb blocked SARS-CoV-2 infection and totally cleared the virus in a treatment setting.**] https://www.biorxiv.org/content/10.1101/2020.07.26.222257v1
- The main protease (Mpro) of SARS-CoV-2, the pathogen responsible for the COVID-19 pandemic, is a key antiviral drug target. While most SARS-CoV-2 Mpro inhibitors have a gamma-lactam glutamine surrogate at the P1 position, we recently discovered several Mpro inhibitors have hydrophobic moieties at the P1 site, including calpain inhibitors II/XII, which are also active against human cathepsin L, a host-protease that is important for viral entry. To determine the binding mode of these calpain inhibitors and establish a structure-activity relationship, we solved X-ray crystal structures of Mpro in complex with calpain inhibitors II and XII, and three analogues of GC-376, one of the most potent Mpro inhibitors in vitro. The structure of Mpro with calpain inhibitor II confirmed the S1 pocket of Mpro can accommodate a hydrophobic methionine side chain, challenging the idea that a hydrophilic residue is necessary at this position. Interestingly, the structure of calpain inhibitor XII revealed an unexpected, inverted binding pose where the P1 prime pyridine inserts in the S1 pocket and the P1 norvaline is positioned in the S1 prime pocket. The overall conformation is semi-helical, wrapping around the catalytic core, in contrast to the extended conformation of other peptidomimetic inhibitors. Additionally, the structures of three GC-376 analogues UAWJ246, UAWJ247, and UAWJ248 provide insight to the sidechain preference of the S1 prime, S2, S3 and S4 pockets, and the superior cell-based activity of the aldehyde warhead compared with the alpha-ketoamide. Taken together, the biochemical, computational, structural, and cellular data presented herein provide new directions for the development of Mpro inhibitors as SARS-CoV-2 antivirals. [**note: some good work looking for inhibitors to the virus main proteas (Mpro). We still need more drug candidates with reasonable structure activity relationships.**]
https://www.biorxiv.org/content/10.1101/2020.07.27.223727v1
- Targeted therapeutics for the treatment of coronavirus disease 2019 (COVID-19), especially severe cases, are currently lacking. As macrophages have unique effector functions as a first-line defense against invading pathogens, we genetically armed human macrophages with chimeric antigen receptors (CARs) to reprogram their phagocytic activity against SARS-CoV-2. After

investigation of CAR constructs with different intracellular receptor domains, we found that although cytosolic domains from MERTK (CAR_{MERTK}) did not trigger antigen-specific cellular phagocytosis or killing effects, unlike those from MEGF10, FcRγ and CD3ζ did, these CARs all mediated similar SARS-CoV-2 clearance in vitro. Notably, we showed that CAR_{MERTK} macrophages notably reduced the virion load without upregulation of proinflammatory cytokine expression. These results suggest that CAR_{MERTK} drives an "immunologically silent" scavenger effect in macrophages and pave the way for further investigation of CARs for the treatment of individuals with COVID-19, particularly those with severe cases at a high risk of hyperinflammation. [**note: this is a cool approach from China altering human macrophages to reprogram activity against SARS-CoV-2. I would really like to see how this does in the clinic.**]
https://www.biorxiv.org/content/10.1101/2020.07.26.222208v1

- Remdesivir, an investigational broad-spectrum antiviral agent, has shown in vitro activity against SARS-CoV-2. To maximize direct delivery to the target site, the lungs, we aim to develop remdesivir as a dry powder for inhalation using thin film freezing (TFF). TFF produces a brittle matrix of nanostructured aggregates that can be sheared into respirable low-density microparticles upon aerosolization from a passive dry powder inhaler. In vitro aerodynamic testing demonstrated that drug loading and excipient type affected the aerosol performance of remdesivir. Remdesivir combined with optimal excipients (e.g. Captisol, mannitol, lactose, leucine) exhibited suitable aerosol performance (up to 92.4% FPF and 0.86 micron MMAD). Remdesivir was amorphous after the TFF process, which we hypothesize will provide a benefit for drug dissolution once administered to the lungs. Neither the organic/water processing cosolvent or the rapid freezing rate used during the TFF process affected the chemical stability of remdesivir during processing. In conclusion, TFF is a suitable technology for producing remdesivir dry powder formulations suitable for pulmonary administration. [**note: here is an important new method for preparing remdesivir for powder inhalation. There is a trial going on but I don't know if this method is used in drug prep.**]
https://www.biorxiv.org/content/10.1101/2020.07.26.222109v1
- The Corona Virus Disease 2019 (COVID-19) pandemic caused by the Severe Acute Respiratory Syndrome Related Coronavirus 2 (SARS-CoV-2) is a global health emergency. As only very limited therapeutic options are clinically available, there is an urgent need for the rapid development of safe, effective, and globally available pharmaceuticals that inhibit SARS-CoV-2 entry and ameliorate COVID-19. In this study, we explored the use of small compounds acting on the homeostasis of the endolysosomal host-pathogen interface, to fight SARS-CoV-2 infection. We find that fluoxetine, a widely used antidepressant and a functional inhibitor of acid sphingomyelinase (FIASMA), efficiently inhibits the entry and propagation of SARS-CoV-2 in the cell culture model without cytotoxic effects. Mechanistically, fluoxetine induced both impaired endolysosomal acidification and the accumulation of cholesterol within the endosomes. As the FIASMA group consists of a large number of small compounds that are well-tolerated and widely used for a broad range of clinical applications, exploring these licensed pharmaceuticals may offer a variety of promising antivirals for host-directed therapy to counteract SARS-CoV-2 and COVID 19. [**note: Prozac for all!!! Keep a level head and avoid COVID-19. I don't think much of this mechanism. Lots of weird compounds interact this way, even HCQ and chasing after them for SARS-CoV-2 is likely a dead end.**]
https://www.biorxiv.org/content/10.1101/2020.07.27.222836v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Pandemic SARS-CoV-2 has infected over 10 million people and caused over 500,000 mortalities. Vaccine development is in urgent need to stop the pandemic. Despite great progresses on SARS-CoV-2 vaccine development, the efficacy of the vaccines remains to be determined. Deciphering the interactions of the viral epitopes with their elicited neutralizing antibodies in the convalescent COVID-19 population inspires the vaccine development. In this study, we devised a peptide array composed of 20-mer overlapped peptides of spike (S), membrane (M) and envelope (E) proteins, and performed a screening with 120 COVID-19 convalescent serums and 24 non-COVID-19 serums. We identified five SARS-CoV-2-specific dominant epitopes that reacted with above 40% COVID-19 convalescent serums. Epitopes in the receptor-binding domain (RBD) of S ill reacted with the convalescent serums. Of note, two peptides non-specifically interacted with most of the non-COVID-19 serums. Neutralization assay indicated that only five serums completely blocked viral infection at the dilution of 1:200. By using a peptide-compete neutralizing assay, we found that three dominant epitopes partially competed the neutralization activity of several convalescent serums, suggesting antibodies elicited by these epitopes played an important role in neutralizing viral infection. The epitopes we identified in this study may serve as vaccine candidates to elicit neutralizing antibodies in most vaccinated people or specific antigens for SARS-CoV-2 diagnosis. [**note: work from China on defining epitopes of SARS-CoV-2**]
  https://www.medrxiv.org/content/10.1101/2020.07.25.20161869v1
- Development of effective strategies to detect, treat, or prevent COVID-19 requires a robust understanding of the natural immune response to SARS-CoV-2, including the cellular response mediated by T cells. We used an unbiased, genome-wide screening technology, termed T-Scan, to identify specific epitopes in SARS-CoV-2 that are recognized by the memory CD8+ T cells of 25 COVID-19 convalescent patients, focusing on epitopes presented by the six most prevalent Human Leukocyte Antigen (HLA) types: A*02:01, A*01:01, A*03:01, A*11:01, A*24:02, and B*07:02. For each HLA type, the patients' T cells recognized 3-8 immunodominant epitopes that are broadly shared among patients. Remarkably, 94% of screened patients had T cells that recognized at least one of the three most dominant epitopes for a given HLA, and 53% of patients had T cells that recognized all three. Subsequent validation studies in 18 additional A*02:01 patients confirmed the presence of memory CD8+ T cells specific for the top six identified A*02:01 epitopes, and single-cell sequencing revealed that patients often have >5 different T cell clones targeting each epitope, but that the same T cell receptor Valpha and Vbeta regions are predominantly used to recognize these epitopes, even across patients. In total, we identified 29 shared epitopes across the six HLA types studied. T cells that target most of these immunodominant epitopes (27 of 29) do not cross-react with the endemic coronaviruses that cause the common cold, and the epitopes do not occur in regions with high mutational variation. Notably, only 3 of the 29 epitopes we identified reside in the spike protein, highlighting the need for new classes of vaccines that are designed to elicit a broader CD8+ T cell response. [**note: and another paper on epitopes, this time looking at CD8+ T cells. Company doing this work is TScan Therapeutics.**]
  https://www.medrxiv.org/content/10.1101/2020.07.24.20161653v1
- IGHV3-53-encoded neutralizing antibodies are commonly elicited during SARS-CoV-2 infection and target the receptor-binding domain (RBD) of the spike (S) protein. Such IGHV3-53 antibodies

generally have a short CDR H3 due to structural constraints in binding the RBD (mode A). However, a small subset of IGHV3-53 antibodies to the RBD contain a longer CDR H3. Crystal structures of two IGHV3-53 neutralizing antibodies here demonstrate that a longer CDR H3 can be accommodated in a different binding mode (mode B). These two classes of IGHV3-53 antibodies both target the ACE2 receptor binding site, but with very different angles of approach and molecular interactions. Overall, these findings emphasize the versatility of IGHV3-53 in this common antibody response to SARS-CoV-2, where conserved IGHV3-53 germline-encoded features can be combined with very different CDR H3 lengths and light chains for SARS-CoV-2 RBD recognition and virus neutralization. [**note: this is intriguing in that an antibody can have two binding modes.**] https://www.biorxiv.org/content/10.1101/2020.07.26.222232v1

- A high-resolution understanding of the antibody response to SARS-CoV-2 is important for the design of effective diagnostics, vaccines and therapeutics. However, SARS-CoV-2 antibody epitopes remain largely uncharacterized, and it is unknown whether and how the response may cross-react with related viruses. Here, we use a multiplexed peptide assay ('PepSeq') to generate an epitope-resolved view of reactivity across all human coronaviruses. PepSeq accurately detects SARS-CoV-2 exposure and resolves epitopes across the Spike and Nucleocapsid proteins. Two of these represent recurrent reactivities to conserved, functionally-important sites in the Spike S2 subunit, regions that we show are also targeted for the endemic coronaviruses in pre-pandemic controls. At one of these sites, we demonstrate that the SARS-CoV-2 response strongly and recurrently cross-reacts with the endemic virus hCoV-OC43. Our analyses reveal new diagnostic and therapeutic targets, including a site at which SARS-CoV-2 may recruit common pre-existing antibodies and with the potential for broadly-neutralizing responses. [**note: more good work on epitope analysis. There appears to be some cross reactivity with an endemic coronavirus. It's still unclear whether this may offer a clue about mild COVID-19 symptoms versus progression to severe COVID-19.**] https://www.biorxiv.org/content/10.1101/2020.07.27.222943v1
- The coronavirus disease 2019 (COVID-19) caused by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has quickly spread worldwide and has infected more than ten million individuals. One of the typical features of COVID-19 is that both type I and III interferon (IFN)-mediated antiviral immunity are suppressed. However, the molecular mechanism by which SARS-CoV-2 evades this antiviral immunity remains elusive. Here, we report that the SARS-CoV-2 membrane (M) protein inhibits the production of type I and III IFNs induced by the cytosolic dsRNA-sensing pathway of RIG-I/MDA-5-MAVS signaling. The SARS-CoV2 M protein also dampens type I and III IFN induction stimulated by Sendai virus infection or poly (I:C) transfection. Mechanistically, the SARS-CoV-2 M protein interacts with RIG-I, MAVS, and TBK1 and prevents the formation of a multi-protein complex containing RIG-I, MAVS, TRAF3, and TBK1, thus impeding IRF3 phosphorylation, nuclear translocation, and activation. Consequently, the ectopic expression of the SARS-CoV2 M protein facilitates the replication of vesicular stomatitis virus (VSV). Taken together, the SARS-CoV-2 M protein antagonizes type I and III IFN production by targeting RIG-I/MDA-5 signaling, which subsequently attenuates antiviral immunity and enhances viral replication. This study provides insight into the interpretation of the SARS-CoV-2-induced antiviral immune suppression and sheds light on the pathogenic mechanism of COVID-19. [**note: another nefarious way the SARS-CoV-2 virus puts a damper on the immune response.**] https://www.biorxiv.org/content/10.1101/2020.07.26.222026v1

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus 2019 (COVID-19). No treatment is available. Micro-RNAs (miRNAs) in mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) are potential novel therapeutic agents because of their ability to regulate gene expression by inhibiting mRNA. Thus, they may degrade the RNA genome of SARS-CoV-2. EVs can transfer miRNAs to recipient cells and regulate conditions within them. MSC-EVs harbor major therapeutic miRNAs that play important roles in the biological functions of virus-infected host cells. Here, we examined their potential impact on viral and immune responses. MSC-EVs contained 18 miRNAs predicted to interact directly with the 3' UTR of SARS-CoV-2. These EVs suppressed SARS-CoV-2 replication in Vero E6 cells. In addition, five major miRNAs suppressed virus activity in a luciferase reporter assay by binding the 3' UTR. MSC-EVs showed strong regenerative effects and potent anti-inflammatory activity which may prevent lethal cytokine storms. We confirmed that EVs regulated inflammatory responses by several cell types, including human brain cells that express the viral receptor ACE2, suggesting that the brain may be targeted by SARS-CoV-2. miRNAs in MSC-EVs have several advantages as therapeutic agents against SARS-CoV-2: 1) they bind specifically to the viral 3' UTR, and are thus unlikely to have side effects; 2) because the 3' UTR is highly conserved and rarely mutates, MSC-EV miRNAs could be used against novel variants arising during viral replication; and 3) unique cargoes carried by MSC-EVs can have diverse effects, such as regenerating damaged tissue and regulating immunity. [**note: from South Korea, a possible use of stem cells with micro-RNAs that could degrade the viral RNA genome.**]
  https://www.biorxiv.org/content/10.1101/2020.07.27.190561v1

DIAGNOSTIC DEVELOPMENT

- Large-scale serosurveillance of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) will only be possible if serological tests are sufficiently reliable, rapid and inexpensive. Current assays are either labour-intensive and require specialised facilities (e.g. virus neutralization assays), or expensive with suboptimal specificity (e.g. commercial ELISAs). Bead-based assays offer a cost-effective alternative and allow for multiplexing to test for antibodies of other pathogens. Here, we compare the performance of four antigens for the detection of SARS-CoV-2 specific IgG antibodies in a panel of sera that includes both severe (n=40) and mild (n=52) cases, using a neutralization and a Luminex bead-based assay. While we show that neutralising antibody levels are significantly lower in mild than in severe cases, we demonstrate that a combination of recombinant nucleocapsid protein (NP), receptor-binding domain (RBD) and the whole spike protein (S1S2) results in a highly sensitive (96%) and specific (99%) bead-based assay that can detect IgG antibodies in both groups. Although S1-specific IgG levels correlate most strongly with neutralizing antibody levels, they fall below the detection threshold in 10% of the cases in our Luminex assay. In conclusion, our data supports the use of RBD, NP and S1S2 for the development of SARS-CoV-2 serological bead-based assays. Finally, we argue that low antibody levels in mild/asymptomatic cases might complicate the epidemiological assessment of large-scale surveillance studies. [**note: this is another take on a multiplex assay but look at the caveat regarding low antibody levels in mild/asymptomatic cases.**]
  https://www.medrxiv.org/content/10.1101/2020.07.25.20161943v1

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2020-07-29

Today's pandemic choral presentation comes from Canada. The virtual choir does a nice rendition of Leonard Cohen's Hallelujah, another iconic piece: https://www.youtube.com/watch?v=XIDim9aYWds For a solo version here is Jeff Buckley another one who died too young: https://www.youtube.com/watch?v=y8AWFf7EAc4

US COVID-19 STATISTICS - **Infection Rate: 1.3%; CFR: 3.4%** (IR unchanged; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

The Washington Post notes that Twitter management deleted some posts from certain political figures on how everyone has the HCQ story wrong. I guess by 'wooden stake' campaign against this Zombie drug is not working. There is professor at Yale who published an '*opinion*' piece in Newsweek that we should start using HCQ. These types of stunts do a great disservice to the cause of public health. I am not going to belabor the point about whether HCQ works either as a treatment or prophylactic, that is for well-designed clinical trials (including observational ones) to determine. For me the evidence is clear about treatment and the few small amounts of data on prophylaxis also point to no utility. We will have to wait for the large Duke study on 15K healthcare workers to really know if prophylaxis works or not. I don't know where that trial stands in terms of enrollment or if the DSMB has had any interim look at the data. Unknowns related to pandemics cause a lot of people to grasp at straws. As I have noted, I think this is a Zombie drug.

No surprise in this Washington Post story that young people are infecting older family members in shared homes. Reopening of schools may have a marked impact here.

The New York Times has a good overview of the current state of the pandemic in the US. New York City's contact tracing program may be dysfunctional. Can large retailers who have implemented mask policies really enforce them? Here is a snapshot of infections on US college campuses. Finally, a very nice article on how coronavirus pool testing works and where it can be implemented.

Kaiser Health News has a story on how churches in Colorado Springs are handling reopening with and without mask guidance for parishioners.

Derek Lowe has a very interesting piece on the Pfizer/BioNTech vaccine selection process.

Please note that when I talk about DIY (do it yourself) clinical trials, this is meant to be humorous. It goes without saying that anything you read in my newsletters should not be taken as medical advice of any kind.

MODELING

- The COVID-19 pandemic has posed a huge challenge to healthcare systems and their personnel worldwide. The study of the impact of SARS-CoV-2 infection among healthcare workers, through prevalence studies, will let us know viral expansion, individuals at most risk and the most exposed areas. The aim of this study is to gauge the impact of SARS-CoV-2 pandemic in our hospital workforce and identify groups and areas at increased risk. Methods and Findings. This is a cross-sectional and longitudinal study carried out on healthcare workers based on molecular

and serological diagnosis of SARS-CoV-2 infection. Of the 3013 HCW invited to participate, finally 2439 (80.9%) were recruited, including 674 (22.4%) who had previously consulted at the OHS for confirmed exposure and/or presenting symptoms suggestive of COVID-19. A total of 411 (16.9%) and 264 (10.8%) healthcare workers were SARS-CoV-2 IgG and rRT-PCR positive, respectively. The cumulative prevalence considering all studies (IgG positive HCW and/or rRT-PCR positive detection) has been 485 (19.9%). SARS-CoV-2 IgG-positive patients in whom the virus was not detected were 221 (9.1%); up to 151 of them (68.3%) did not report any compatible symptoms nor consult at the OHS for this reason. Men became more infected than women (25% vs 18.5%, p=0.0009), including when data were also classified by age. COVID-19 cumulative prevalence among the HCW assigned to medical departments was higher (25.2%) than others, as well as among medical staff (25.4%) compared with other professional categories (p<0.01). Conclusions. Global impact of the COVID-19 pandemic on HCW of our centre has been 19.9%. Doctors and medical services personnel have had the highest prevalence of SARS-CoV-2 infection, but many of them have not presented compatible symptoms. This emphasizes the performance of continuous surveillance methods of the most exposed health personnel and not only based on the appearance of symptoms. [**note: another good study of healthcare worksers in the Madrid area. Cumulative infection was close to 20%.**]
https://www.medrxiv.org/content/10.1101/2020.07.26.20162529v1

- It has become increasingly clear that the COVID-19 epidemic is characterized by overdispersion whereby the majority of the transmission is driven by a minority of infected individuals. Such a strong departure from the homogeneity assumptions of traditional well-mixed compartment model is usually hypothesized to be the result of short-term super-spreader events, such as individual's extreme rate of virus shedding at the peak of infectivity while attending a large gathering without appropriate mitigation. However, heterogeneity can also arise through long-term, or persistent variations in individual susceptibility or infectivity. Here, we show how to incorporate persistent heterogeneity into a wide class of epidemiological models, and derive a non-linear dependence of the effective reproduction number R_e on the susceptible population fraction S. Persistent heterogeneity has three important consequences compared to the effects of overdispersion: (1) It results in a major modification of the early epidemic dynamics; (2) It significantly suppresses the herd immunity threshold; (3) It significantly reduces the final size of the epidemic. We estimate social and biological contributions to persistent heterogeneity using data on real-life face-to-face contact networks and age variation of the incidence rate during the COVID-19 epidemic, and show that empirical data from the COVID-19 epidemic in New York City (NYC) and Chicago and all 50 US states provide a consistent characterization of the level of persistent heterogeneity. Our estimates suggest that the hardest-hit areas, such as NYC, are close to the persistent heterogeneity herd immunity threshold following the first wave of the epidemic, thereby limiting the spread of infection to other regions during a potential second wave of the epidemic. Our work implies that general considerations of persistent heterogeneity in addition to overdispersion act to limit the scale of pandemics. [**note: from University of Illinois, another paper on how to model for herd immunity this time focusing on persistent heterogeneity. I found this a useful paper to read and they are candid about the efforts needed to suppress outbreaks (contact tracing & control of superspreading events). They do note that their estimates for herd immunity are at the low end of the range. They don't**

explicitly give a number that I can see but it appears to be about 30%.)]
https://www.medrxiv.org/content/10.1101/2020.07.26.20162420v1

- Background: The current COVID-19 pandemic has led to a surge of research activity. While this research provides important insights, the multitude of studies results in an increasing segmentation of information. To ensure comparability across projects and institutions, standard datasets are needed. Here, we introduce the "German Corona Consensus Dataset" (GECCO), a uniform dataset that uses international terminologies and health IT standards to improve interoperability of COVID-19 data. Methods: Based on previous work (e.g., the ISARIC-WHO COVID-19 case report form) and in coordination with experts from university hospitals, professional associations and research initiatives, data elements relevant for COVID-19 research were collected, prioritized and consolidated into a compact core dataset. The dataset was mapped to international terminologies, and the Fast Healthcare Interoperability Resources (FHIR) standard was used to define interoperable, machine-readable data formats. Results: A core dataset consisting of 81 data elements with 281 response options was defined, including information about, for example, demography, anamnesis, symptoms, therapy, medications or laboratory values of COVID-19 patients. Data elements and response options were mapped to SNOMED CT, LOINC, UCUM, ICD-10-GM and ATC, and FHIR profiles for interoperable data exchange were defined. Conclusion: GECCO provides a compact, interoperable dataset that can help to make COVID-19 research data more comparable across studies and institutions. The dataset will be further refined in the future by adding domain-specific extension modules for more specialized use cases. [**note: good approach to data standardization. Why do I keep seeing this stuff come from outside the US?**]
https://www.medrxiv.org/content/10.1101/2020.07.27.20162636v1
- Despite the significant morbidity and mortality caused by the 2019 novel coronavirus disease (COVID-19), our understanding of basic disease epidemiology remains limited. This study aimed to describe key patient characteristics, comorbidities, treatments, and outcomes of a large U.S.-based cohort of patients hospitalized with COVD-19 using electronic health records (EHR). METHODS: We identified patients in the Optum De-identified COVID-19 EHR database who had laboratory-confirmed COVID-19 or a presumptive diagnosis between 20 February 2020 and 6 June 2020. We included hospitalizations that occurred 7 days prior to, or within 21 days after, COVID-19 diagnosis. Among hospitalized patients we describe the following: vital statistics and laboratory results on admission, relevant comorbidities (using diagnostic, procedural, and revenue codes), medications (NDC, HCPC codes), ventilation, intensive care unit (ICU) stay, length of stay (LOS), and mortality. RESULTS: We identified 76,819 patients diagnosed with COVID-19, 16,780 of whom met inclusion criteria for COVID-related hospitalization. Over half the cohort was over age 50 (74.5%), overweight or obese (72.1%), or had hypertension (58.1%). At admission, 29.1% of patients presented with fever (>38 C) and 30.6% had low oxygen saturation (<90%). Among the 16,099 patients with complete hospital records, we observed that 58.9% had hypoxia, 23.4% had an ICU stay during hospitalization, 18.1% were ventilated, and 16.2% died. The median LOS was 6 days (IQR: 4, 11). CONCLUSIONS: To our knowledge, this is the largest descriptive study of patients hospitalized with COVID-19 in the United States. We report summary statistics of key clinical outcomes that provide insights to better understand COVID-19 disease epidemiology. [**note: this is a large epidemiological study using an electronic**

health record dtabase. I find it interesting that it was done by a biopharma company, Genentech.] https://www.medrxiv.org/content/10.1101/2020.07.17.20156265v1

NEWLY REGISTERED CLINICAL TRIALS

- Did not look today.

CLINICAL TRIAL RESULTS

- The prolonged mechanical ventilation required by patients with severe COVID-19 is expected to result in significant Intensive Care Unit - Acquired Weakness (ICUAW) in many of the survivors. However, in our post-COVID-19 follow up clinic we have found that, as well as the anticipated global weakness related to loss of muscle mass, a significant proportion of these patients also have disabling focal neurological deficits relating to an axonal mononeuritis multiplex. Amongst the 69 patients with severe COVID-19 that have been discharged from the intensive care units in our hospital, we have seen 11 individuals (16%) with such neuropathies. In many instances, the multi-focal nature of the weakness in these patients was initially unrecognised as symptoms were wrongly assumed to simply relate to "critical illness neuropathy". *While mononeuropathy is well recognised as an occasional complication of intensive care, our experience suggests that such deficits are common and frequently disabling in patients recovering from COVID-19*. [**note: something else for clinicians to look out for in patients recovering from severe COVID-19.** https://www.medrxiv.org/content/10.1101/2020.07.19.20149898v1
- D-dimer concentration has been used to identify candidates for intensified anticoagulant treatment for both venous thromboembolism prevention and mitigation of the microthrombotic complications associated with COVID-19. Thromboelastography (TEG) maximum amplitude (MA) has been validated as an indicator of hypercoagulability and MA ≥ 68 mm has been utilized as a marker of hypercoagulability in other conditions. We evaluated the relationship between coagulation, inflammatory, and TEG parameters in patients with COVID-19 on extracorporeal membrane oxygenation (ECMO). Methods: We performed a single center retrospective analysis of consecutive patients that received ECMO for the treatment of COVID-19. TEG, inflammatory, and coagulation markers were compared in patients with and without thrombotic complications. Correlation tests were performed to identify the coagulation and inflammatory markers that best predict hypercoagulability as defined by an elevated TEG MA. Results: 168 TEGs were available in 24 patients. C-reactive protein and fibrinogen were significantly higher in patients that developed a thrombotic event versus those that did not (p=0.038 and p=0.043 respectively). D-dimer was negatively correlated with TEG MA (p<0.001) while fibrinogen was positively correlated (p<0.001). A fibrinogen > 441 mg/dL had a sensitivity of 91.2% and specificity of 85.7% for the detection of MA ≥ 68 mm. Conclusions: In critically ill patients with COVID-19, D-dimer concentration had an inverse relationship with hypercoagulability as measured by TEG MA. D-dimer elevation may reflect severity of COVID-19 related sepsis rather than designate patients likely to benefit from anticoagulation. Fibrinogen concentration may represent a more useful marker of hypercoagulability in this population. [**note: this is a very useful finding for severe COVID-19 treatment and whether to use anticoagulants. D-Dimer elevation may not be the good clinical measurement to use.**] https://www.medrxiv.org/content/10.1101/2020.07.27.20162842v1

- COVID-19 is complicated by acute lung injury, and death in some individuals. It is caused by SARS-CoV-2 that requires the ACE2 receptor and serine proteases to enter airway epithelial cells (AECs). Objective: To determine what factors are associated with ACE2 expression particularly in patients with asthma and chronic obstructive pulmonary disease (COPD). Methods: We obtained upper and lower AECs from 145 people from two independent cohorts, aged 2-89, Newcastle (n=115), and from Perth (n= 30) Australia. The Newcastle cohort was enriched with people with asthma (n=37) and COPD (n=38). Gene expression for ACE2 and other genes potentially associated with SARS-CoV-2 cell entry were assessed by quantitative PCR, protein expression was confirmed with immunohistochemistry on endobronchial biopsies and cultured AECs. Results: Increased gene expression of ACE2 was associated with older age (p=0.02) and male sex (p=0.03), but not pack-years smoked. When we compared gene expression between adults with asthma, COPD and healthy controls, mean ACE2 expression was lower in asthma (p=0.01). Gene expression of furin, a protease that facilitates viral endocytosis, was also lower in asthma (p=0.02), while ADAM-17, a disintegrin that cleaves ACE2 from the surface was increased (p=0.02). ACE2 protein levels were lower in endobronchial biopsies from asthma patients. Conclusions: Increased ACE2 expression occurs in older people and males. Asthma patients have reduced expression. Altered ACE2 expression in the lower airway may be an important factor in virus tropism and may in part explain susceptibility factors and why asthma patients are not over-represented in those with COVID-19 complications. [**note: maybe this is the reason asthmatics have modest protection from altered ACE2 expression.**]
  https://www.medrxiv.org/content/10.1101/2020.07.26.20162248v1
- Background To be universally applicable in treatment of severe COVID-19, novel therapies, especially those with little toxicity and low cost, are urgently needed. We report here the use of one such therapeutic combination involving two commonly used nutraceuticals, namely resveratrol and copper in patients with this disease. This study was prompted by pre-clinical reports that sepsis-related cytokine storm and fatality in mice can be prevented by oral administration of small quantities of resveratrol and copper. Since cytokine storm and sepsis are major causes of death in severe COVID-19, we retrospectively analyzed outcomes of patients with this condition who had received resveratrol and copper. Methods & Findings Our analysis comprised of 230 patients with severe COVID-19 requiring inhaled oxygen who were admitted in a single tertiary care hospital in Mumbai between April 1 and May 13 2020. Thirty of these patients received, in addition to standard care, resveratrol and copper at doses of 5.6 mg and 560 ng, respectively, orally, once every 6 hours, until discharge or death. These doses were based on our pre-clinical studies, and were nearly 50 times and 2000 times less, respectively, than those recommended as health supplements. A multivariable-adjusted analysis was used to model the outcome of death in these patients and evaluate factors associated with this event. A binary logistic regression analysis was used, with age, sex, presence of comorbidities and receipt of resveratrol-copper as covariates. Data were updated as of May 30 2020. The number of deaths in resveratrol-copper and standard care only groups were 7/30 (23.3%, 95% CI 8.1%-38.4%) and 89/200 (44.5%, 95% CI 37.6%-51.3%), respectively. In multivariable analysis, age >50 years [odds ratio (OR) 2.558, 95% CI 1.454-4.302, P=0.0011] and female sex (OR 1.939, 95% CI 1.079-3.482, P=0.0267) were significantly associated, while presence of co-morbidities was not significantly associated (OR 0.713, 95% CI 0.405-1.256, P=0.2421) with death. There was a trend towards reduction in death in patients receiving resveratrol-copper (OR 0.413, 95% CI 0.164-

1.039, P= 0.0604). Conclusions We provide preliminary results of a novel approach to the treatment of severe COVID-19 using a combination of small amounts of commonly used nutraceuticals, which is non-toxic and inexpensive, and therefore could be widely accessible globally. The nearly two-fold reduction in mortality with resveratrol-copper observed in our study needs to be confirmed in a randomized controlled trial. [**note: from India, a small observational trial on resveratrol and copper. There has been conjecture about resveratrol and I remember a preprint indicating it might have some activity. Of course TIWWDCT (for the uninitiated, This is Why We Do Clinical Trials. For you DIY clinical trialists you can easily purchase both of these compounds. This is not meant to be any kind of medical advice!**] https://www.medrxiv.org/content/10.1101/2020.07.21.20151423v1

- Background Coronavirus disease 2019 (Covid-19) has been declared global emergency with immediate safety, preventative and curative measures to control the spread of virus. Confirmed cases are treated with clinical management as they are diagnosed but so far, there is no effective treatment or vaccine yet for Covid-19. Ayurveda has been recommended by preventative and clinical management guidelines in India and several clinical trials are ongoing. But there is no study to assess impact of Ayurveda on Covid-19. Methods Objective of present study was to evaluate the clinical outcome in Covid-19 confirmed asymptomatic to mild symptomatic patients who had received Ayurveda and compare with control (who has not received Ayurveda or any support therapy). Patients having Ayurveda intervention (Guduchi Ghan Vati-extract of Tinospora cordifolia) were included from Jodhpur Covid Care Centre and non-recipients were taken from Jaipur Covid Care Centre between May 15 to June 15, 2020. Total 91 patients, who were asymptomatic at the time of hospital admission and between 18 - 75 years of age, were included in the study to analyse retrospectively. Results In control group, 11.7% developed mild symptoms after average 1.8 days and none in Ayurveda group reported any symptoms. Significant difference was reported between the group of patients taking Guduchi Ghan Vati (n=40) and patients in standard care (n=51) in terms of virologic clearance at day-7 (97.5% vs 15.6% respectively; p=0.000), at day 14 (100% vs 82.3%) days to stay in hospital (6.4 vs 12.8 respectively; p< 0.0001) . Conclusion Results of the study suggest that Guduchi Ghan Vati, a common and widely used Ayurveda preparation, could benefit treating asymptomatic Covid-19 patients. Larger, randomised controlled Trials are required to confirm the findings. [**note: I linked to a registered clinical trial of this compound the other day. NCT04480398 These are the results**.] https://www.medrxiv.org/content/10.1101/2020.07.23.20160424v1

DRUG DEVELOPMENT

- Antibodies with high affinity against the receptor binding domain (RBD) of the SARS-CoV-2 S1 ectodomain were identified from screens using the Retained DisplayTM (ReD) platform employing a 1 x 10^11 clone single-chain antibody (scFv) library. Numerous unique scFv clones capable of inhibiting binding of the viral S1 ectodomain to the ACE2 receptor in vitro were characterized. To maximize avidity, selected clones were reformatted as bivalent diabodies and monoclonal antibodies (mAb). The highest affinity mAb completely neutralized live SARS-CoV-2 virus in cell culture for four days at a concentration of 6.7 nM, suggesting potential therapeutic and/or prophylactic use. Furthermore, scFvs were identified that greatly increased the interaction of the viral S1 trimer with the ACE2 receptor, with potential implications for vaccine

development. [**note: this is from an Australian company, Affinity Biosciences**] https://www.biorxiv.org/content/10.1101/2020.07.27.224089v1

- Since the first human case was reported in Wuhan Province, China in December 2019, SARS-CoV-2 has caused millions of human infections in more than 200 countries worldwide with an approximately 4.01% case-fatality rate (as of 27 July, 2020; based on a WHO situation report), and COVID-19 pandemic has paralyzed our global community. Even though a few candidate drugs, such as remdesivir (a broad antiviral prodrug) and hydroxychloroquine, have been investigated in human clinical trials, their therapeutic efficacy needs to be clarified further to be used to treat COVID-19 patients. Here we show that pyronaridine and artesunate, which are the chemical components of anti-malarial drug Pyramax, exhibit antiviral activity against SARS-CoV-2 and influenza viruses. In human lung epithelial (Calu-3) cells, pyronaridine and artesunate were highly effective against SARS-CoV-2 while hydroxychloroquine did not show any effect at concentrations of less than 100 μM. In viral growth kinetics, both pyronaridine and artesunate inhibited the growth of SARS-CoV-2 and seasonal influenza A virus in Calu-3 cells. Taken together, we suggest that artesunate and pyronaridine might be effective drug candidates for use in human patients with COVID-19 and/or influenza, which may co-circulate during this coming winter season. [**note: this must be from the same Korean team that recently registered a clinical trial. The drug was approved by the EMA but AFAIK it has not been submitted to the FDA.**] https://www.biorxiv.org/content/10.1101/2020.07.28.225102v1
- The COVID-19 pathogen, SARS-CoV-2, requires its main protease (SC2MPro) to digest two of its translated polypeptides to form a number of mature proteins that are essential for viral replication and pathogenesis. Inhibition of this vital proteolytic process is effective in preventing the virus from replication in infected cells and therefore provides a potential COVID-19 treatment option. Guided by previous medicinal chemistry studies about SARS-CoV-1 main protease (SC1MPro), we have designed and synthesized a series of SC2MPro inhibitors that contain beta-(S-2-oxopyrrolidin-3-yl)-alaninal (Opal) for the formation of a reversible covalent bond with the SC2MPro active site cysteine C145. All inhibitors display high potency with IC50 values at or below 100 nM. The most potent compound MPI3 has as an IC50 value as 8.5 nM. Crystallographic analyses of SC2MPro bound to 7 inhibitors indicated both formation of a covalent bond with C145 and structural rearrangement from the apoenzyme to accommodate the inhibitors. Virus inhibition assays revealed that several inhibitors have high potency in inhibiting the SARS-CoV-2-induced cytopathogenic effect in both Vero E6 and A549 cells. Two inhibitors MP5 and MPI8 completely prevented the SARS-CoV-2-induced cytopathogenic effect in Vero E6 cells at 2.5-5 uM and A549 cells at 0.16-0.31 uM. Their virus inhibition potency is much higher than some existing molecules that are under preclinical and clinical investigations for the treatment of COVID-19. Our study indicates that there is a large chemical space that needs to be explored for the development of SC2MPro inhibitors with extreme potency. Due to the urgent matter of the COVID-19 pandemic, MPI5 and MPI8 may be quickly advanced to preclinical and clinical tests for COVID-19. [**note: lots of drug discovery papers coming out now. Here are some interesting new compounds that have good inhibitory concentrations. Will a pharma company pick this up for development?**] https://www.biorxiv.org/content/10.1101/2020.07.28.223784v1
- In the absence of a vaccine and other effective prophylactic or therapeutic countermeasures the severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) remains a significant

public health threat. Attachment and entry of coronaviruses including SARS-CoV-2 is mediated by the spike glycoprotein (SGP). Recently, a SARS-CoV-2 Spike Pseudotyped Lentivirus (SSPL) was developed that allows studying spike-mediated cell entry via luciferase reporter activity in a BSL2 environment. Here, we show that iota-carrageenan can inhibit the cell entry of SSPL in a dose dependent manner. SSPL particles were efficiently neutralized with an IC50 value of 2.6 microgram/ml iota-carrageenan. In vitro data on iota-carrageenan against various Rhino- and Coronaviruses showed similar IC50 values and translated readily into clinical effectiveness when a nasal spray containing iota-carrageenan demonstrated a reduction in severity and duration of symptoms of common cold caused by various respiratory viruses. Accordingly, our in vitro data on SSPL suggest that administration of iota-carrageenan may be an effective and safe prophylaxis or treatment for SARS-CoV-2 infections. [**note: paper comes from an Austrian company, Marinomed Biotech. For you DIY medicators, iota-carraneenan is a gelification agent used in cooking and easily obtainable from Amazon. You too can do a clinical trial at home!**] https://www.biorxiv.org/content/10.1101/2020.07.28.224733v1

- On the basis of Covid-19-induced pulmonary pathological and vascular changes, we hypothesized that the anti-VEGF drug bevacizumab might be beneficial for treating Covid-19 patients. We recruited 26 patients from 2-centers (China and Italy) with confirmed severe Covid-19, with respiratory rate ≥30 times/min, oxygen saturation ≤93% with ambient air, or partial arterial oxygen pressure to fraction of inspiration O2 ratio (PaO2/FiO2) >100mmHg and ≤300 mmHg, and diffuse pneumonia confirmed by chest radiological imaging. This trial was conducted from Feb 15 to April 5, 2020, and followed up for 28 days. Relative to comparable control patients with severe Covid-19 admitted in the same centers, bevacizumab showed clinical efficacy by improving oxygenation and shortening oxygen-support duration. Among 26 hospitalized patients with severe Covid-19 (median age, 62 years, 20 [77%] males), bevacizumab plus standard care markedly improved the PaO2/FiO2 ratios at days 1 and 7 (elevated values, day 1, 50.5 [4.0,119.0], p<0.001; day 7, 111.0 [85.0,165.0], p<0.001). By day 28, 24 (92%) patients showed improvement in oxygen-support status, 17 (65%) patients were discharged, and none showed worsen oxygen-support status nor died. Significant reduction of lesion areas and ratios were shown in chest CT or X-ray analysis within 7 days. Of 14 patients with fever, body temperature normalized within 72 hours in 13 (93%) patients. Lymphocyte counts in peripheral blood were significantly increased and CRP levels were markedly decreased as shown in available data. Our findings suggested bevacizumab plus standard care was highly beneficial for treating patients with severe Covid-19. Clinical efficacy of bevacizumab warrants double blind, randomized, placebo-controlled trials. [**note: very small numbers again, but this antibody against VEGF may be clinically useful. There are two trials in China and one in France listed but I don't know the status.**]
  https://www.medrxiv.org/content/10.1101/2020.07.26.20159756v1
- There is an immediate need for therapies related to coronavirus disease 2019 (COVID-19), especially candidate drugs that possess anti-inflammatory and immunomodulatory effects with low toxicity profiles. We hypothesized the application of pleiotropic tetracyclines as potential therapeutic candidates. Here, we present a retrospective multi-institutional cohort study evaluating ventilatory status in patients who had taken a tetracycline antibiotic within a year prior to diagnosis of acute respiratory distress syndrome (ARDS). The primary outcomes were the need for mechanical ventilation and duration of mechanical ventilation. The secondary

outcome was the duration of intensive care unit (ICU) stay. Data was evaluated using logistic regression and treatment effects regression models. Minocycline or doxycycline treatment within a year prior to ARDS diagnosis was associated with a 75% reduced likelihood for mechanical ventilation during hospital stay. Furthermore, tetracycline antibiotic therapy corresponded to significant reductions in duration of mechanical ventilation and ICU stay in ARDS patients. These data suggest tetracyclines may provide prophylactic benefit in reducing ventilatory support for ARDS patients and support further evaluation in a randomized prospective trial. [**note: I am aware of a couple of doxycycline trials that have been registered. Would still like to know what the mechanistic mode of action other than antimicrobial is.**] https://www.medrxiv.org/content/10.1101/2020.07.22.20154542v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Host immune responses play central roles in controlling SARS-CoV2 infection, yet remain incompletely characterized and understood. Here, we present a comprehensive immune response map spanning 454 proteins and 847 metabolites in plasma integrated with single-cell multi-omic assays of PBMCs in which whole transcriptome, 192 surface proteins, and T and B cell receptor sequence were co-analyzed within the context of clinical measures from 50 COVID19 patient samples. Our study reveals novel cellular subpopulations, such as proliferative exhausted CD8+ and CD4+ T cells, and cytotoxic CD4+ T cells, that may be features of severe COVID-19 infection. We condensed over 1 million immune features into a single immune response axis that independently aligns with many clinical features and is also strongly associated with disease severity. Our study represents an important resource towards understanding the heterogeneous immune responses of COVID-19 patients and may provide key information for informing therapeutic development. [**note: very thorough work from Leroy Hood's Institute for Systems Biology in Seattle.**] https://www.biorxiv.org/content/10.1101/2020.07.27.224063v1
- Globally, the COVID-19 pandemic has had extreme consequences for the healthcare system and calls for diagnostic tools to monitor and understand the transmission, pathogenesis and epidemiology, as well as to evaluate future vaccination strategies. Here we have developed novel flexible ELISA-based assays for specific detection of SARS-CoV-2 antibodies against the receptor-binding domain (RBD): An antigen sandwich-ELISA relevant for large population screening and three isotype-specific assays for in-depth diagnostics. Their performance was evaluated in a cohort of 350 convalescent participants with previous COVID-19 infection, ranging from asymptomatic to critical cases. We mapped the antibody responses to different areas on protein N and S and showed that the IgM, A and G antibody responses against RBD are significantly correlated to the disease severity. These assays-and the data generated from them-are highly relevant for diagnostics and prognostics and contribute to the understanding of long-term COVID-19 immunity. [**note: antibody response from Danish investigators**] https://www.medrxiv.org/content/10.1101/2020.07.27.20162321v1

DIAGNOSTIC DEVELOPMENT

- Here we describe a novel immunogenic method to detect COVID-19. The method is a chromogenic magnetic bead-based ELISA which allows inexpensive and quantitative detection of human IgG or IgM antibodies against SARS-CoV-2 in serum or whole blood samples in just 12

minutes. As a proof of concept, we compared the performance of our new method to classical ELISA. Person correlation between optical densities obtained using the two methods was 0.98. The novel magnetic bead-based ELISA performed better than classic ELISA to discriminate one COVID-19 case carrying low antibody titer. The chromogenic magnetic bead-based ELISA method described here can be applied in formats for both point of care and high throughput analysis. The method is readily adaptable to use other protein-based antigens and is readily adaptable to investigate other diseases and other applications. [**note: from Germany another approach to speeding up serology testing.**]
https://www.medrxiv.org/content/10.1101/2020.07.26.20162255v1

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2020-07-30

A lot of years ago when I was still an aspiring boy treble singing at All Soul's, the Episcopal church right around the corner from where we lived we sang classic Rogers and Hammerstein song from Carousel, 'You'll Never Walk Alone' in a choral arrangement. It still gives me hope when I hear it sung. Here is a virtual choir and orchestra from the Brooklyn College Conservatory of Music: https://www.youtube.com/watch?v=FTQr4yU5APE We are all optimists!!! **Contest Alert!!! I desperately want to crown a winner. Here's the question, what sports team has adopted this song as its anthem? Someone out there must know the answer to this one!**

US COVID-19 STATISTICS - **Infection Rate: 1.3%; CFR: 3.4%** (IR unchanged; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

Lots of back to school links for all the teachers, students and parents of both today!!!

The Washington Post has a blistering interview with Richard Horton, editor of The Lancet. He has just written book on the COVID-19 catastrophe and has some very pointed things to say about the US response. This is mandatory reading!! Detroit is experimenting with in class summer school. Let's hope this goes smoothly and they track and trace any outbreaks should one or more occur. This is a reasonable size population to do such field work. Here is the cautionary story of a funeral home owner in a hard hit area of the Rio Grande valley in Texas.

The New York Times discusses why there is no consensus about reopening schools. Lots of confounding questions. Zeke Emanuel and two others offer suggestions on how to open schools safely and here is the illustrated guide! We know that SARS-CoV-2 is in aerosol droplets and here is one of the researchers giving a concise summary of what is known about airborne transmission. Wear a mask and defeat COVID-19 is my mantra. Finally from the Times, some skepticism about whether old vaccines provide protection.

More good news on the vaccine front! Johnson & Johnson (disclosure: I am a shareholder) published animal results. A single shot of their adenovirus vector vaccine prevented SARS-CoV-2 infection in rhesus monkeys. Clinical trials in humans started last week in Europe and will begin this week in the US. While on the vaccine front, STAT have a nice article on how we made so much progress on a COVID-19 vaccine

STAT has an opinion piece by Melinda Gates on sexism and incomplete data related to the world's COVID-19 response; well worth reading. A news piece from The Lancet discusses the indirect impact on women.

FDA have issued guidance for the development of in-home diagnostic tests for COVID-19. The story also notes that XPRIZE has a competition for developers who can produce a test that delivers results in as little as 15 minutes and costs less than $15. Let us hope there is a winner(s).

The New England Journal of Medicine has wise words from three top researchers about opening primary schools for in class instruction. They note that opening up middle and high schools pose more challenging dynamics. There is also a perspective on CDC's Pledge to the American People.

JAMA have a research paper on the closing of schools between March 9 and May 7. Closure was temporally associated with decreased COVID-19 incidence and mortality; states that closed schools earlier, when cumulative incidence of COVID-19 was low, had the largest relative reduction in incidence and mortality. However, it remains possible that some of the reduction may have been related to other concurrent nonpharmaceutical interventions. There is also an accompanying editorial. The school beat keeps rocking on with a viewpoint on the recent report from the National Academies of Sciences, Engineering and Medicine by some of those involved in the study. There is also a short news article on the report. I am glad I am not a school administrator or member of a school board! The final school paper comes from The Netherlands where mood homeostasis before and during the lockdown there was studied. Thanks to a loyal reader who pointed me to this video discussion: https://www.youtube.com/watch?v=gf-dGFbryho

Lots of stuff to read today!!!! If any one has tried KELEA activated water (see newly registered clinical trials), let me know!!!

MODELING

- The objective of this paper is to examine the influence that various contextual variables have upon the number of deaths due to covid-19, across the world. Setting Level: This study utilizes data for 125 countries for contextual variables from 1st January 2020 until the 15th June 2020. Participants: This study considers deaths from covid-19. Primary and secondary outcome measures: The contextual variables considered in this study are stringency index, stringency variability, lockdown date, population density, level of airline passengers and country health security index. Results: It is shown there is a very strong association between the level of airline passengers and covid-19 deaths. The results from regression analysis conducted in this study show significant positive relationships at the 5% level of statistical significance between Deaths from covid-19 and airline passenger levels and stringency variability; significant negative relationships are revealed for stringency index and lockdown date supporting the notion that lock down and social distancing measures mattered and were effective. The Global health security index and population density did not significantly affect deaths. Conclusion: *This study highlights the strong link between a country's airline passengers and covid-19 deaths and found that the lockdown date and stringency measures had a significant effect upon deaths. The implications of the research is that lockdown and stringency measures implemented by governments around the world worked and mattered.* Further, the fact that global health security did not affect deaths may indicate better preparedness required to confront future

pandemics. [**note: this is from Malaysia and it may be first one from that country! Lockdowns and stringency measures worked and mattered.**] https://www.medrxiv.org/content/10.1101/2020.07.28.20163394v1

- How respiratory physiology and airflow therein proceed to impact SARS-CoV-2 transmission, leading to the initial infection, is an open question. An answer can help determine the susceptibility of an individual on exposure to a COVID-2019 carrier and can also quantify the still-unknown infectious dose for the disease. Combining computational fluid mechanics-based tracking of respiratory transport in anatomic domains with sputum assessment data from hospitalized patients and earlier measurements of ejecta size distribution during regular speech - this study shows that targeted deposition at the initial nasopharyngeal infection sites peaks over the droplet size range of 2.5 - 19 μ, and reveals that the number of virions that can establish the infection is at most of O(100). [**note: everyone is interested in what the infectious dose of SARS-CoV-2 is. This researcher has an answer and it's pretty scary if true: 100 virions. This strikes me as incorrect as we would see huge numbers of infections which we don't. It's a good paper to scan as there are some good pictures nasopharynx and the calculations are meticulous.**] https://www.medrxiv.org/content/10.1101/2020.07.27.20162362v1
- Introduction Contact tracing is a key pillar of COVID-19 control. In response to the COVID-19 epidemic in the Autonomous Province of Trento (Italy) a software was developed to standardize data collection and facilitate surveillance of contacts and outbreaks and map the links between bases and contacts. In this paper, we present the results of contact tracing efforts during Phase I of the epidemic (March-April, 2020, mostly under lockdown), including sociodemographic characteristics of contacts who became cases and of the cases who infected one or more contact. Methods A contact tracing website was developed that included components for geolocation and linking of cases and contacts using open source software. Information on community-based confirmed and probable cases and their contacts was centralized on the website. Information on cases came directly from the central case database, information on contacts was collected by telephone interviews following a standard questionnaire. Contacts were followed via telephone, emails, or an app. Results The 2,812 laboratory-diagnosed community cases of COVID-19 had 6,690 community contacts, of whom 890 (13.3%) developed symptoms. Risk of developing symptomatic disease increased with age and was higher in workplace contacts than cohabitants or non-cohabiting family or friends. The greatest risk of transmission to contacts was found for the 14 cases <15 years of age (22.4%); 8 of the 14, who ranged in age from <1 to 11 years) infected 11 of 49 contacts. Overall, 606 outbreaks were identified, 74% of which consisted of only two cases. Discussion The open-source software program permitted the centralized tracking of contacts and rapid identification of links between cases. Workplace contacts were at higher risk of developing symptoms. Although childhood contacts were less likely to become cases, children were more likely to infect household members, perhaps because of the difficulty of successfully isolating children in household settings. [**note: this is a good study of how to implement contact tracing and the findings. Childhood contacts are less likely to become cases but more likely to infect household members.**] https://www.medrxiv.org/content/10.1101/2020.07.16.20127357v1
- Background: Understanding of the true asymptomatic rate of infection of SARS-CoV-2 is currently limited, as is understanding of the population-based seroprevalence after the first wave of COVID-19 within the UK. The majority of data thus far come from hospitalised patients,

with little focus on general population cases, or their symptoms. Methods: We undertook enzyme linked immunosorbent assay characterisation of IgM and IgG responses against SARS-CoV-2 spike glycoprotein and nucleocapsid protein of 431 unselected general-population participants of the TwinsUK cohort from South-East England, aged 19-86 (median age 48; 85% female). 382 participants completed prospective logging of 14 COVID-19 related symptoms via the COVID Symptom Study App, allowing consideration of serology alongside individual symptoms, and a predictive algorithm for estimated COVID-19 previously modelled on PCR positive individuals from a dataset of over 2 million. Findings: We demonstrated a seroprevalence of 12% (51participants of 431). Of 48 seropositive individuals with full symptom data, nine (19%) were fully asymptomatic, and 16 (27%) were asymptomatic for core COVID-19 symptoms: fever, cough or anosmia. Specificity of anosmia for seropositivity was 95%, compared to 88% for fever cough and anosmia combined. 34 individuals in the cohort were predicted to be Covid-19 positive using the App algorithm, and of those, 18 (52%) were seropositive. Interpretation: Seroprevalence amongst adults from London and South-East England was 12%, and 19% of seropositive individuals with prospective symptom logging were fully asymptomatic throughout the study. Anosmia demonstrated the highest symptom specificity for SARS-CoV-2 antibody response. [**note: here is a serology study from southeast England. Interesting that anosmia was the major symptom. I really do need to get my patented COVID-19 Scent Strips developed.**]
https://www.medrxiv.org/content/10.1101/2020.07.29.20162701v1

- After several weeks of "lockdown" as the sole answer to the COVID-19 pandemic, many countries are restarting their economic and social activities. However, balancing the re-opening of society against the implementation of non-pharmaceutical measures needed for minimizing interpersonal contacts requires a careful assessment of the risks of infection as a function of the confinement relaxation strategies. Here, we present a stochastic coarse grained model that examines this problem. In our model, people are allowed to move between discrete positions on a one-dimensional grid with viral infection possible when two people are collocated at the same site. Our model features three sets of adjustable parameters, which characterize (i) viral transmission, (ii) viral detection, and (iii) degree of personal mobility, and as such, it is able to provide a qualitative assessment of the potential for second-wave infection outbreaks based on the timing, extent, and pattern of the lockdown relaxation strategy. In line with general expectations, our model predicts that a full lockdown yields the best results, namely, the lowest number of total infections. A less anticipated result was that when personal mobility is increased beyond a critical level, the risk of infection rapidly reaches a constant value, which depends solely on the population density. *Furthermore, according to our model, confinement alone is not effective if it is not accompanied by a detection capacity (coupled with quarantine) that surpasses 40% of the patients during their symptomatic phase. The results of our simulation also showed that keeping the virus transmission probability to less than 0.4, which can be achieved in real life by respecting social distancing or wearing masks, is as effective as imposing a mild lockdown. Finally, we note that detection and quarantine of pre-symptomatic patients, even with a probability as low as 0.2, would reduce the final numbers of infections by a factor of ten or more*. [**note: I continue to be amazed at all the interesting models coming out from scientists all over the world. Here is a Japanese group that use a stochastic model that may**

inform us about the impact of personal mobility. Common sense conclusions arise from this study!] https://www.medrxiv.org/content/10.1101/2020.07.28.20163980v1

- Introduction: How long individuals may transmit virus after infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is unclear. Understanding the communicability period of SARS-CoV-2 is important to inform the period of isolation required to prevent nosocomial and community spread. The objective of this study was to identify the reported communicable period of SARS-CoV-2, based on a rapid review of existing literature. Methods: Studies reporting empirical data on the period of communicability of SARS-CoV-2 through investigations of duration of communicability based on in-person contact ('contact transmission'), isolation and culture of virus ('viral isolation'), and viral shedding by detection of nucleic acids by RT-PCR ('viral shedding') were identified through searches of peer-reviewed and pre-print health sciences literature databases (Ovid MEDLINE, Embase, Google Scholar, medRxiv and arXiv) and the grey literature. Articles were screened for relevance, then data were extracted, analyzed, and synthesized. Results: Out of the 165 studies included for qualitative analysis, one study investigated contact transmission, three investigated viral isolation, 144 investigated viral shedding, and 17 studies focused on both viral shedding and viral isolation. The median length of time until viral clearance across all viral isolation studies was nine days; however, the maximum identified duration was 32 days. Studies with data on both viral isolation and viral shedding showed a prolonged maximum time until viral clearance for viral shedding (9 days vs 24 days). Discussion: Findings from this review support a minimum 10-day period of isolation; however, additional observation should be considered for individuals being released into high-risk settings. [**note: this is the first large scale literature review I've seen about the transmission time after infection. A number of readers have asked me about this and I would refer them to this paper. These authors support a 10 day quarantine period.**] https://www.medrxiv.org/content/10.1101/2020.07.28.20163873v1

NEWLY REGISTERED CLINICAL TRIALS

- This is a randomized, placebo-controlled clinical study to assess the safety and efficacy of EDP1815 in patients hospitalized with COVID-19 infection. The study is designed to evaluate the efficacy of EDP1815 at reducing time to resolution of symptoms, preventing progression of COVID-19 symptoms and preventing COVID-Related Complications (CRC) [**note: sponsor is Evelo Biosciences and I don't know much about the compound.**] NCT04488575
- Several producers of activated water have been in frequent communication with the Principal Investigator (PI) over the last decade or longer. They have fregularly supplied their water products to the PI for laboratory testing, including measuring an activity attributed to the absorption of an environmental force, referred to by the PI as KELEA, an abbreviation for Kinetic Energy Limiting Electrostatic Attraction. KELEA is regarded as the source of cellular energy for the body's alternative cellular energy (ACE) pathway. This pathway can provide a non-immunological defense mechanism against infections, presumably including coronaviruses. The proposed study is to test water products from several suppliers, as well as a naturally available source of KELEA activated water in symptomatic individuals who have tested positive by PCR for Covid-19. The initial mode of administration will be by inhalation using a nebulizer or diffuser. Several deep inhalations will be taken on 5 occasions daily. Prior to the first inhalation and at the end of the second day of inhalation, swabs will be taken for Covid-19 PCR testing. The

severity of symptoms will also be monitored. [**note: Well this one is right up there with homeopathy. I guess anyone can run a trial these days. For those who want to explore this in detail here a core manuscript. Ironically (or maybe not) the paper states 'homeopathy is a misnomer for KELEA activated water. Any way the trial is sponsored by the Institute for Progressive Medicine!**] NCT04490824

- This is a phase I prospective, open-labeled, single-center study to evaluate the safety and immunogenicity of MVC-COV1901. [**note: wait for it……..another vaccine trial!! This one is from a Taiwan company Medigen Vaccine Biologics using an adjuvant from Dynavax. I don't know much more about the vaccine construct other than it is the Spike protein from NIH.**] NCT04487210
- The objective of this randomized clinical trial is to test whether administration of live attenuated MMR vaccine (measles mumps rubella; Merck) to eligible adults at highest risk for contracting COVID-19 (healthcare workers, first responders), can induce non-specific trained innate immune leukocytes that can prevent/dampen pathological inflammation and sepsis associated with COVID-19-infection, if exposed. [**note: repurposing MMR to see if it offers any protection. This is being done at Louisiana State.**] NCT04475081
- This study is being conducted to evaluate the efficacy of acupressure in promoting health and well-being among healthcare workers during the COVID-19 pandemic. The investigators hypothesize that providing participants with a remote and standardized self-acupressure training program will improve HRQOL and the perception of stress. In the event that the study demonstrates acupressure to be safe and effective for this indication, the training could be scaled up and deployed at low-cost nationally and internationally. [**note: I want to enroll in this UCLA trial!!! I need to improve the quality of my life and reduce stress. Unfortunately, it is only open to health care providers; I wonder if COVID-19 newsletter writers fit that inclusion criteria.**] NCT04472559
- In COVID-19 times, there has been a large increase in number of people working from home; with limited places to go, an abrupt change to people's lives and lack of knowledge about the dangers of sedentary behaviour (SB), it is important to help workers develop and effortlessly incorporate healthy movement routines to optimize daily productivity and health. The combined lack of knowledge on literature on SB profiles of full time, home-based workers, effects of framing of SB reduction strategies, and strategy preference uncertainty makes for a novel study. This will be a 4-week intervention with 3 experimental groups that looks at whether telling a full time, home-based office worker to do pre-selected strategies to break up their sedentary behaviour (SB) (i.e. sitting) will change their SB profiles compared to either letting them select their own strategies (from either a short or long list menu). Investigators are looking to see whether having the choice (or not) to choose strategies in an unfamiliar health related selection (preference uncertainty) will create greater changes in SBs. As well, the researchers are incorporating behavioural economics' choice overload concept with length of choice menus in relation to behaviour change and program engagement. Workers' work-related SB will be measured by a device at baseline and on the last week of the intervention. Workers will be provided with an SB educational video to increase knowledge and motivation for change. Any SB changes in relation to productivity, mental wellness, behaviour intentions etc. will also be measured. [**note: great trial title 'STAND UP to SARS-CoV-2' Too bad it is a Canadian trial as I**

fit the criteria for this one!! Excuse me while I get up and stretch……..Ah, that is better.] NCT04488796

CLINICAL TRIAL RESULTS

- A global effort is currently undertaken to restrain the COVID-19 pandemic. Host immunity has come out as a determinant for COVID-19 clinical outcome, and several studies investigated the immune profiling of SARS-CoV-2 infected people to properly direct the clinical management of the disease. Thus, lymphopenia, T-cell exhaustion, and the increased levels of inflammatory mediators have been described in COVID-19 patients, in particular in severe cases1. Age represents a key factor in COVID-19 morbidity and mortality2. Understanding age-associated immune signatures of patients is therefore important to identify preventive and therapeutic strategies. In this study, we investigated the immune profile of COVID-19 hospitalized patients identifying a distinctive age-dependent immune signature associated with disease severity. Indeed, defined circulating factors - CXCL8, IL-10, IL-15, IL-27 and TNF-α - positively correlate with older age, longer hospitalization, and a more severe form of the disease and may thus represent the leading signature in critical COVID-19 patients. [**note: from Italy biomarkers for severe COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.07.28.20162735v1
- Severity prediction of COVID-19 remains one of the major clinical challenges for the ongoing pandemic. Here, we have recruited a 144 COVID-19 patient cohort consisting of training, validation, and internal test sets, longitudinally recorded 124 routine clinical and laboratory parameters, and built a machine learning model to predict the disease progression based on measurements from the first 12 days since the disease onset when no patient became severe. A panel of 11 routine clinical factors, including oxygenation index, basophil counts, aspartate aminotransferase, gender, magnesium, gamma glutamyl transpeptidase, platelet counts, activated partial thromboplastin time, oxygen saturation, body temperature and days after symptom onset, constructed a classifier for COVID-19 severity prediction, achieving accuracy of over 94%. Validation of the model in an independent cohort containing 25 patients achieved accuracy of 80%. The overall sensitivity, specificity, PPV and NPV were 0.70, 0.99, 0.93 and 0.93, respectively. Our model captured predictive dynamics of LDH and CK while their levels were in the normal range. This study presents a practical model for timely severity prediction and surveillance for COVID-19, which is freely available at webserver https://guomics.shinyapps.io/covidAI/. [**note: from China, another set of markers for severe COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.07.28.20163022v1
- COVID-19 primarily affects the lungs, but evidence of systemic disease with multi-organ involvement is emerging. Here, we developed a blood test to broadly quantify cell, tissue, and organ specific injury due to COVID-19, using genome-wide methylation profiling of circulating cell-free DNA in plasma. We assessed the utility of this test to identify subjects with severe disease in two independent, longitudinal cohorts of hospitalized patients. Cell-free DNA profiling was performed on 104 plasma samples from 33 COVID-19 patients and compared to samples from patients with other viral infections and healthy controls. We found evidence of injury to the lung and liver and involvement of red blood cell progenitors associated with severe COVID-19. The concentration of cfDNA correlated with the WHO ordinal scale for disease progression and was significantly increased in patients requiring intubation. This study points to the utility of cell-free DNA as an analyte to monitor and study COVID-19. [**note: it looks like it is the day for**

severe COVID-19 markers. In this case it is cell free DNA.]
https://www.medrxiv.org/content/10.1101/2020.07.27.20163188v1

- The rapid detection of COVID-19 uses genotypic testing for the presence of SARS-Cov-2 virus in nasopharyngeal swabs, but it can have a poor sensitivity. A rapid, host-based physiological test that indicated whether the individual was infected or not would be highly desirable. Coagulaopathies are a common accompaniment to COVID-19, especially micro-clots within the lungs. We show here that microclots can be detected in the native plasma of COVID-19 patient, and in particular that such clots are amyloid in nature as judged by a standard fluorogenic stain. This provides a rapid and convenient test ($P<0.0001$), and suggests that the early detection and prevention of such clotting could have an important role in therapy. [**note: good work from these South African researchers in finding microclots in the sera from COVID-19 patients and it's link to possible initiation of anti-clotting therapy.**]
https://www.medrxiv.org/content/10.1101/2020.07.28.20163543v1
- Background: Insufficient evidence of factors predicting the COVID-19 progression from mild to moderate to critical has been established. We retrospectively evaluated risk factors for critical progression in Japanese COVID-19 patients. Method: Seventy-four laboratory-confirmed COVID-19 patients were hospitalized in our hospital between February 20, 2020, and June 10, 2020. We excluded asymptomatic, non-Japanese, and child patients. We divided patients into the stable group (SG) and the progression group (PG) (patients requiring mechanical ventilation). We compared the clinical factors in both groups. We established the cutoff values (COVs) for significantly different factors via receiver operating characteristic (ROC) curve analysis and evaluated risk factors by univariate regression. Results: We enrolled 57 COVID-19 patients (median age 52 years, 56.1% male). The median progression time from symptom onset was eight days. Seven patients developed critical disease (PG: 12.2%), two (3.5%) of whom died; 50 had stable disease. Univariate logistic analysis identified elevated lactate dehydrogenase (LDH) (COV: 309 U/l), decreased estimated glomerular filtration rate (eGFR) (COV: 68 ml/min), lymphocytopenia (COV: 980/μl), and statin use as significantly associated with disease progression. However, in Cox proportional hazards analysis, lymphocytopenia at symptom onset was not significant. Conclusions: We identified three candidate risk factors for adult Japanese patients with mild to moderate COVID-19: statin use, elevated LDH level, and decreased eGFR. [**note: YIKES!!! WHAT DOES THIS STATIN DATA MEAN? Well it is a very small data sample from a single Japanese hospital. Still the fact that statins came up as a risk factor is just weird. I've not seen this in any other reports. Observational studies on small populations can often lead to outliers and let's hope that this is just one of those.**]
https://www.medrxiv.org/content/10.1101/2020.07.29.20159442v1
- Abstract Introduction Several studies have reported an unexpectedly low prevalence of current smoking among hospitalized patients with Covid-19. However, these studies mostly compared observed to expected rates of smoking without direct comparison with individual controls. Objective To examine the association of nicotine-replacement therapy, as a surrogate of smoking, with hospitalization and all-cause mortality during the first wave of SARS-CoV-2 epidemic in France. Methods We conducted a nationwide matched exposed/unexposed cohort study using information from the French national health data system which covers the entire French population. We conducted two separate analyses, the first in individuals exposed to nicotine-replacement therapy without major smoking-related diseases (cancer, cardiovascular

and/or respiratory diseases) and the second in those presenting these conditions. We included all individuals, aged between 18 and 75 years, who had been reimbursed at least one nicotine-replacement therapy between November 15, 2019, and February 15, 2020. For each exposed individual, we randomly selected, from the entire Metropolitan French population, up to two non-exposed individuals (1:2) matched for the following variables: age (same year of birth), sex, department of residence (n=96 in Metropolitan France), and complementary universal health insurance (CMU-C). The three end points were a hospitalization with Covid-19, a death or an intubation in hospitalized patients with Covid-19, and all-cause mortality. We compared outcomes in individuals who were exposed to nicotine-replacement therapy with those in individuals who were not, using a multivariable Cox model with inverse probability weighting according to the propensity score. Results In the first analysis, 297,070 individuals without major smoking-related diseases exposed to nicotine-replacement therapy were matched with 558,228 unexposed individuals without major smoking-related diseases. Individuals were aged on average 45.6 years (standard deviation: 12.7) and 48.8% were male. From February 15, 2020 to June 7, 2020, hospitalization with Covid-19 occurred in 647 patients (151 patients in the nicotine-replacement therapy group and 496 patients in the unexposed group). In the main multivariable analysis, nicotine-replacement therapy was associated with a decreased risk of hospitalization with Covid-19 compared with unexposed individuals (hazard ratio, 0.50; 95% CI, 0.41 to 0.61). Nicotine-replacement therapy exposure was also associated with a decreased risk of intubation or death in hospitalized individuals with Covid-19 (13 vs. 73 patients, hazard ratio, 0.31; 95% CI, 0.17 to 0.57) but with an increased risk of all-cause mortality (251 vs. 231 deaths, hazard ratio, 1.49; 95% CI, 1.24 to 1.80). In the second analysis, 128,768 individuals with major smoking-related diseases exposed to nicotine-replacement therapy were matched with 243,793 unexposed individuals. Individuals were aged on average 55.3 years (standard deviation: 11.4) and 53.3% were male. In the main multivariable analysis, nicotine-replacement therapy exposure was neither associated with risk of hospitalization with Covid-19 (240 patients in the nicotine-replacement therapy group and 398 patients in the unexposed group, hazard ratio, 1.13; 95% CI, 0.94 to 1.38) nor with risk of death or an intubation in hospitalized individuals with Covid-19 (48 vs. 61 patients, hazard ratio, 1.00; 95% CI, 0.65 to 1.54). All-cause mortality was higher in the nicotine-replacement therapy group (1040 vs. 366 deaths, hazard ratio, 3.83; 95% CI, 3.41 to 4.31). Conclusions *This large-scale observational study suggests that smoking, measured by exposure to nicotine-replacement therapy, was associated with an increased risk of overall mortality during the first wave of SARS-CoV-2 epidemic in France, although it was associated with a lower risk of severe Covid-19 in individuals without major related-smoking diseases. Experimental and clinical studies are needed to disentangle the potential mechanisms of nicotine and/or smoking in Covid-19 risk. Whatever the nature of these associations, the global impact of smoking is harmful for health even over a short epidemic period*. [**note: the conclusions speak for themselves – don't smoke, vape or take nicotine supplements.**] https://www.medrxiv.org/content/10.1101/2020.07.28.20160630v1

- To determine the effect of COVID-19 convalescent plasma on mortality, we aggregated patient outcome data from randomized clinical trials, matched control, and case-series studies. Fixed-effects analyses demontrated that hospitalized COVID-19 patients transfused with convalescent plasma exhibited a ã57% reduction in mortality rate (13%) compared to matched-patients receiving standard treatments (25%; OR: 0.43, P < 0.001). These data provide evidence favouring

the efficacy of human convalescent plasma as a therapeutic agent in hospitalized COVID-19 patients. [**note: sera from convalescent patients appears to lower mortality compared to SOC. Let's hope the mAb trials show the same!**]
https://www.medrxiv.org/content/10.1101/2020.07.29.20162917v1

- INTRODUCTION Hypoxemia in Severe Acute Respiratory Syndrome due to Novel Coronavirus of 2019 (SARS-CoV-2) is mediated by severe inflammation that may be mitigated by corticosteroids. We evaluated pattern and effects of corticosteroid use in these patients during an early surge of the pandemic. METHODS Observational study of 136 SARS-CoV-2 patients admitted to the Intensive care Unit between March 1 and April 27, 2020 at a tertiary care hospital in Indianapolis, USA. Statistical comparison between cohorts and dosing pattern analysis was done. Outcome measures included number of patients requiring intubation, duration of mechanical ventilation, length of ICU stay and inpatient mortality. RESULTS: Of 136 patients, 72 (53%) received corticosteroids. Groups demographics: Age (60.5 vs. 65; p .083), sex (47% male vs. 39% female; p .338) and comorbidities were similar. Corticosteroid group had increased severity of illness: PaO2/FiO2 (113 vs. 130; p .014) and SOFA (8 vs. 5.5; p < .001). Overall mortality (21% vs. 30%; p .234) or proportion of patients intubated (78 vs. 64%; p .078) was similar. Mortality was similar among mechanically ventilated (27% vs. 15%; p .151) however there were no deaths among patients who were not mechanically ventilated and received corticosteroids (0% vs. 57%; p <.001). Early administration (within 48 hours) showed decrease in proportion of intubation (66% vs. 87 vs. 100%; p.045), ICU days (6 vs., 16 vs. 18; p <.001), and ventilator days (3 vs. 12 & 14; p <.001). 45% received methylprednisolone. CONCLUSION: Corticosteroids were used more frequently in SARS CoV-2 patients with higher severity of illness. Early administration of corticosteroids improved survival in non-mechanically ventilated patients; decreased ICU stay and may have prevented intubation. [**note: more good data on the use of corticosteroids. I think that this part of the story has come to a conclusion. These drugs do work and need to be incorporated into SOC. I probably will not be reporting out any further preprints on this topic.**]
https://www.medrxiv.org/content/10.1101/2020.07.29.20164277v1
- Background: Hyperinflammation is a key feature of the pathogenesis of COVID-19 with a central role of the interleukin-6 pathway. We aimed to study the impact of the IL-6 receptor antagonist tocilizumab on the outcome of patients admitted to the intensive care unit (ICU) with acute respiratory distress syndrome (ARDS) related to COVID-19. Methods: Eighty-seven patients with confirmed SARS-CoV-2 infection and moderate to severe ARDS were included (n tocilizumab = 29, n controls = 58). A matched cohort was created using a propensity score. The primary endpoint was 30-day all-cause mortality, secondary endpoints included ventilation-free days and length of stay. Results: No difference was found in 30-day all-cause mortality in patients treated with tocilizumab compared to controls (17.2% vs. 32.8%, p = 0.2; HR = 0.52 [0.19 - 1.39], p = 0.19). Ventilator-free days were 19.0 (IQR 12.5 - 20.0) versus 9 (IQR 0.0 - 18.5; p = 0.04), respectively. A higher rate of freedom from mechanical ventilation at 30 days was achieved in patients receiving tocilizumab (HR 2.83 [1.48 - 5.40], p < 0.002). Median length of stay in ICU and total length of stay were reduced by 8 and 9.5 days in patients treated with tocilizumab. Similar results were obtained in the analysis of the propensity score matched cohort. Conclusions: Treatment of critically ill patients with ARDS due to COVID-19 with tocilizumab was not associated with reduced 30-day all-cause mortality, but shorter duration on ventilatory support

as well as shorter overall length of stay in hospital and in ICU. [**note: more data on tocilizumab. This is a Swedish small study looking at 30 day all-cause mortality. Mortality was not reduced but there was shorter ICU stay so a benefit is there. We need to see the clinical trial data ASAP.**] https://www.medrxiv.org/content/10.1101/2020.07.29.20164160v1

- Fatigue is a common symptom in those presenting with symptomatic COVID-19 infection. However, it is unknown if COVID-19 results in persistent fatigue in those recovered from acute infection. We examined the prevalence of fatigue in individuals recovered from the acute phase of COVID-19 illness using the Chalder Fatigue Score (CFQ-11). We further examined potential predictors of fatigue following COVID-19 infection, evaluating indicators of COVID-19 severity, markers of peripheral immune activation and circulating pro-inflammatory cytokines. Of 128 participants (49.5 ± 15 years; 54% female), more than half reported persistent fatigue (52.3%; 45/128) at 10 weeks (median) after initial COVID-19 symptoms. There was no association between COVID-19 severity (need for inpatient admission, supplemental oxygen or critical care) and fatigue following COVID-19. Additionally, there was no association between routine laboratory markers of inflammation and cell turnover (leukocyte, neutrophil or lymphocyte counts, neutrophil-to-lymphocyte ratio, lactate dehydrogenase, C-reactive protein) or pro-inflammatory molecules (IL-6 or sCD25) and fatigue post COVID-19. Female gender and those with a pre-existing diagnosis of depression/anxiety were over-represented in those with fatigue. *Our findings demonstrate a significant burden of post-viral fatigue in individuals with previous SARS-CoV-2 infection after the acute phase of COVID-19 illness. This study highlights the importance of assessing those recovering from COVID-19 for symptoms of severe fatigue, irrespective of severity of initial illness, and may identify a group worthy of further study and early intervention*. [**note: persistent fatigue 10 weeks post infection in this patient cohort. This appeared not to be linked to various biomarkers.**]
https://www.medrxiv.org/content/10.1101/2020.07.29.20164293v1

DRUG DEVELOPMENT

- Coronavirus disease 2019 (COVID-19), caused by the SARS-CoV-2 virus, has had a dramatic global impact on public health, social, and economic infrastructures. Here, we assess immunogenicity and anamnestic protective efficacy in rhesus macaques of the intradermal (ID)-delivered SARS-CoV-2 spike DNA vaccine, INO-4800. INO-4800 is an ID-delivered DNA vaccine currently being evaluated in clinical trials. Vaccination with INO-4800 induced T cell responses and neutralizing antibody responses against both the D614 and G614 SARS-CoV-2 spike proteins. Several months after vaccination, animals were challenged with SARS-CoV-2 resulting in rapid recall of anti-SARS-CoV-2 spike protein T and B cell responses. These responses were associated with lower viral loads in the lung and with faster nasal clearance of virus. These studies support the immune impact of INO-4800 for inducing both humoral and cellular arms of the adaptive immune system which are likely important for providing durable protection against COVID-19 disease. [**note: I believe this is the first animal data for the Inovio vaccine that is in trials.**]
https://www.biorxiv.org/content/10.1101/2020.07.28.225649v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Surprisingly nothing today!!!

DIAGNOSTIC DEVELOPMENT

- We have developed a novel multiplexed flow cytometric bead array (C19BA) for the detection of SARS-CoV-2 seroconversion that allows sensitive identification of IgG and IgM antibodies against three immunogenic proteins: the spike receptor-binding domain (RBD), the spike protein subunit 1 (S1) and the nucleoprotein (N) simultaneously. This assay is more sensitive than ELISA, and the combination of three antigens allows for the interrogation of full seroconversion. Importantly, we have detected N-reactive antibodies in COVID-19-negative individuals. [**note: another new multiplex assay method. What is interesting is the finding of N-reactive antibodies in COVID-19 negative indivdiuals. They theorize this may be a result of an endemic coronavirus infection.]** https://www.medrxiv.org/content/10.1101/2020.07.28.20162941v1
- Recent advances in CRISPR-based diagnostics suggest that DETECTR, a combination of isothermal reverse transcriptase loop mediated amplification (RT-LAMP) and subsequent Cas12 bystander nuclease activation by amplicon targeting ribonucleoprotein complexes, could be a faster and cheaper alternative to qRT-PCR without sacrificing sensitivity/specificity. Here we compare qRT-PCR with DETECTR to diagnose COVID-19 on 378 patient samples and report a 95% reproducibility. Patient sample dilution assays suggest a higher analytical sensitivity of DETECTR compared to qRT-PCR, however, this was not confirmed in a large patient cohort. The data showed that both techniques are equally sensitive in detecting SARS-CoV-2 providing an added value of DETECTR to the currently used qRT-PCR platforms. For DETECTR, different gRNAs can be used simultaneously to obviate negative results due to mutations in N-gene. Lateral flow strips, suitable as a point of care test (POCT), showed a 100% correlation to the high-throughput DETECTR assay. Importantly, DETECTR was 100% specific for SARS-CoV-2 and did not detect other human coronaviruses. As there is no need for specialized equipment, DETECTR could be rapidly implemented as a complementary technically independent approach to qRT-PCR thereby increasing the testing capacity of medical microbiological laboratories and relieving the existent PCR-platforms for routine non- SARS-CoV-2 diagnostic testing. [**note: this Dutch group has another approach using a CRISPR-based diagnostic. We need more validation on all these differing approaches.**] https://www.medrxiv.org/content/10.1101/2020.07.27.20147249v1
- During COVID19 and other viral pandemics, rapid generation of host and pathogen genomic data is critical to tracking infection and informing therapies. There is an urgent need for efficient approaches to this data generation at scale. We have developed a scalable, high throughput approach to generate high fidelity low pass whole genome and HLA sequencing, viral genomes, and representation of human transcriptome from single nasopharyngeal swabs of COVID19 patients. [**note: from Stanford a short and to the point abstract regarding a scalable approach to whole genome sequencing and more from a single nasal swab.**] https://www.medrxiv.org/content/10.1101/2020.07.27.20163147v1
- Sars-CoV-2 is a human pathogen and is the main cause of COVID-19 disease. COVID-19 is announced as a global pandemic by World Health Organization. COVID-19 is characterized by severe conditions and early diagnosis can make dramatic changes both for personal and public health. In order to increase the reach for low cost equipment which requires a very limited technical knowledge can be beneficial to diagnose the viral infection. Such diagnostic capabilities can have a very critical role to control the transmission of the disease. Here we are reporting a state-of-the-art diagnostic tool developed by using an in vitro synthetic biology approach by employing engineered de novo riboregulators. Our design coupled with a home-

made point-of-care device setting can detect and report presence of Sars-CoV-2 specific genes. The presence of Sars-CoV-2 related genes triggers translation of sfGFP mRNAs, resulting in green fluorescence output. *The approach proposed here has the potential of being a game changer in Sars-COV-2 diagnostics by providing an easy-to-run, low-cost-demanding diagnostic capability*. [**note: I don't think technical papers should use the term 'game changer'. This is an interesting paper and I believe the first one from Turkey!! It is interesting new technology for a POC test. The test takes 40 minutes to run and I look forward to further development and validation of this technology.**] https://www.medrxiv.org/content/10.1101/2020.07.28.20164004v1

- SARS-CoV-2 control in the United States is hampered by limited testing capacity. We evaluated a simple, outdoor, mobile RT-LAMP assay workflow where self-collected saliva is tested for SARS-CoV-2 RNA. 195 volunteers were tested over 8 days. Testing results were 100% concordant with qRT-PCR, with all but one person testing SARS-CoV-2-negative. [**note: from Univ of Wisconsin and they received funding from the new NIH diagnostics accelerator program. Let's get these tests validated and deployed!**] https://www.medrxiv.org/content/10.1101/2020.07.28.20164038v1
- Abstract Serological surveillance is necessary to the reestablishment of school activities in safe conditions and to avoid school-related outbreaks. In this study, DBS (Dried Blood Spots) have proven to be a simple, rapid and reliable sample collection tool for detecting antibodies against SARS-CoV-2 by ELISA test compared to matched serum samples from venous sampling (R2=0.9553; Pearson's coefficient=0.98; Cohen's unweighted k=0.93; overall agreement=96.2%). This approach may facilitate sample collection from schoolchildren for serological surveys useful to an adequate risk-assessment. [**note: these Milan investigators show that dried blood spot collection works well compared to serum. They are planning to use this for testing children as they return to school.]** https://www.medrxiv.org/content/10.1101/2020.07.29.20164186v1

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2020-07-31

Baseball season is here except for the Miami Marlins who have 14 players sidelined with COVID-19 and maybe the Phillies who had a couple of players test positive yesterday. There are a number of topflight players who have decided to sit this abbreviated season out because of either personal or family health concerns. For those and the active players stay safe as possible. Here is a delightful choral arrangement of 'Take Me Out to the Ballgame' by the same arranger who did yesterday's 'You'll Never Walk Alone' and several ball players participate in this one! Play Ball: https://www.youtube.com/watch?v=MlwII9fQlwU Reminder – pick up some Cracker Jacks next time I am out running errands.

Alas, we did not have a winner in yesterday's contest. The correct answer was Liverpool Football Club whose supporters sing a rousing 'You'll Never Walk Alone' at every home match.

According to The Washington Post, the US government is planning to purchase 100M doses of the SARS-C0V-2 vaccine being developed by Sanofi/GSK. GSK is providing the adjuvant and Sanofi the vaccine platform that is based on their influenza work.

The New York Times explains why contact track and tracing is failing in the state. Yes, it's all a result of testing inadequacies. According to these Yale immunologists there is no reason to be afraid that COVID-19 immunity won't last. Here is more on the question of how much virus load is present in children. Here is the JAMA article on the Chicago study. It remains an open question whether they are key transmitters of viral infections to others. A computer analysis of the Diamond Princess outbreak suggests how aerosol transmission spreads the virus. Here is the preprint.

STAT have an opinion piece on one scientists concerns about the forthcoming COVID-19 vaccine.

The Lancet have a commentary on the conundrum of using inhaled corticosteroids in virus pandemics. Confounding information is difficult to tease apart and well-designed clinical trials offer the best solution. It looks like atypical thyroiditis can be added to the list of not so good side effects of SARS-CoV-2 infection according to these Italian researchers. The virus is indeed nefarious!

Annals of Internal Medicine has a very clear update alert on practice points: 1) Do not use chloroquine or hydroxychloroquine alone or in combination with azithromycin as prophylaxis against COVID-19; 2) do not use chloroquine or hydroxychloroquine alone or in combination with azithromycin as a treatment of patients with COVID-19; and 3) clinicians may choose to treat hospitalized COVID-19–positive patients with chloroquine or hydroxychloroquine alone or in combination with azithromycin in the context of a clinical trial, using shared and informed decision making with patients (and their families). *Bring me the stake Igor*! Annals also have an interesting cohort study from the Columbia Univ hospital system. In looking at the correlation between obesity and severe COVID-19 they studied 2466 adults hospitalized over a 45 day period. The interesting finding is that obesity association with intubation or death was primarily observed among patients younger than 65 years and not older patients. The important takeaway is maintain a healthy weight.

I didn't see this one coming, Derek Lowe has report on tocilizumab and apilimod. The first is the Roche IL-6 blocker and the company announced that it did not meet endpoint. Apilimod has some interesting properties that Derek discusses and there is a clinical trial registered for this compound (NCT04446377). Derek also discusses animal challenge studies of the major vaccine candidates.

A thankfully slow reading day!!

MODELING

- The 2020 SARS-CoV-2 pandemic is caused by a zoonotic coronavirus transmitted to humans, similar to earlier events. Whether the other, seasonally circulating coronaviruses induce cross-reactive, potentially even cross-neutralizing antibodies to the new species in humans is unclear. The question is of particular relevance for people with immune deficiencies, as their health depends on treatment with immunoglobulin preparations that need to contain neutralizing antibodies against the pathogens in their environment. Testing 54 IVIG preparations, produced from plasma collected in Europe and the US, highly potent neutralization of a seasonal coronavirus was confirmed, yet no cross-neutralization of the new SARS-CoV-2 was seen. [**note: not modeling but I don't know where it quite fits in. this comes from Baxter, a major blood products processor and shows that pre-pandemic plasma preparations do not have any cross-**

neutralization with SARS-CoV-2 despite having neutralization properties against a seasonal coronavitus.] https://www.biorxiv.org/content/10.1101/2020.07.30.228213v1

NEWLY REGISTERED CLINICAL TRIALS

- Did not check today.

CLINICAL TRIAL RESULTS

- The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has caused a recent outbreak of Coronavirus Disease (COVID-19). In Cuba, the first case of COVID-19 was reported on March 11. Elderly with multiple comorbidities are particularly susceptible to adverse clinical outcomes in the course of SARS CoV-2 infection. During the outbreak, a local transmission event took place in a nursing home in Villa Clara province, Cuba, in which nineteen elderly residents were positive for SARS-CoV-2. Methods: Based on the increased susceptibility to viral-induced cytokine release syndrome inducing respiratory and systemic complications in this population, the patients were included in an expanded access clinical trial to receive itolizumab, an anti-CD6 monoclonal antibody. Results: All the patients had underlying medical conditions. The product was well tolerated. After the first dose, the course of the disease was favorable and 18 out of 19 (94.7%) patients were discharged clinically recovered with negative RT-PCR at 13 days (median). One dose of itolizumab, circulating IL-6 decreased in the first 24-48 hours in patients with high baseline values, whereas in patients with low levels, this concentration remained over low values. To preliminary assess the effect of itolizumab, a control group was selected among the Cuban COVID-19 patients, which did not receive immunomodulatory therapy. Control subjects were well-matched regarding age, comorbidities and severity of the disease. Every three moderately ill patients treated with itolizumab, one admission in intensive care unit (ICU) was prevented. Discussion/Conclusion: Itolizumab was well tolerated. Its effect is associated with a reduction and controlling IL-6 serum levels. Moreover, treated patients had a favorable clinical outcome, considering their poor prognosis. This treatment is associated significantly with a decrease the risk to be admitted in ICU and reduced 10 times the risk of death. This study corroborates that the timely use of itolizumab, in combination with other antiviral and anticoagulant therapies, is associated with a reduction the COVID-19 disease worsening and mortality. The humanized antibody itolizumab emerges as a therapeutic alternative for patients with COVID-19 and suggests its possible use in patients with cytokine release syndrome from other pathologies. [**note: I think this is the first paper I've seen from Cuba! We are all in this together. Itolizumab is an IL-6 blocking monoclonal manufactured in Cuba. As with tocilizumab it shows activity in terms of reducing cytokine storm associated with severe COVID-19**] https://www.medrxiv.org/content/10.1101/2020.07.24.20153833v1
- Mitochondrial DNA (MT-DNA) are intrinsically inflammatory nucleic acids released by damaged solid organs. Whether circulating MT-DNA levels could be used to predict the development of poor COVID-19 outcomes remains undetermined. Here, we measured circulating MT-DNA levels in prospectively collected, cell-free plasma samples from 97 subjects with COVID-19 at the time of hospital presentation. Circulating MT-DNA were sharply elevated in patients who eventually died, required ICU admission or intubation. Multivariate regression analysis demonstrated MT-DNA levels as an independent risk factor for all of these outcomes after adjusting for age, sex and comorbidities. Importantly, we found MT-DNA levels had a similar or superior area-under-

the curve when compared to clinically established inflammatory indicators, as well as emerging markers currently of interest as targets for investigational therapies. These results show that high circulating MT-DNA levels are a potential indicator of poor COVID-19 outcomes. [**note: add circulating mitochondrial DNA to the list of markers for poor COVID-19 outcomes**] https://www.biorxiv.org/content/10.1101/2020.07.30.227553v1

DRUG DEVELOPMENT

- Development of effective preventative interventions against SARS-CoV-2, the etiologic agent of COVID-19 is urgently needed. The viral surface spike (S) protein of SARS-CoV-2 is a key target for prophylactic measures as it is critical for the viral replication cycle and the primary target of neutralizing antibodies. We evaluated design elements previously shown for other coronavirus S protein-based vaccines to be successful, e.g. prefusion-stabilizing substitutions and heterologous signal peptides, for selection of a S-based SARS-CoV-2 vaccine candidate. In vitro characterization demonstrated that the introduction of stabilizing substitutions (i.e., furin cleavage site mutations and two consecutive prolines in the hinge region of S1) increased the ratio of neutralizing versus non-neutralizing antibody binding, suggestive for a prefusion conformation of the S protein. Furthermore, the wild type signal peptide was best suited for the correct cleavage needed for a natively-folded protein. These observations translated into superior immunogenicity in mice where the Ad26 vector encoding for a membrane-bound stabilized S protein with a wild type signal peptide elicited potent neutralizing humoral immunity and cellular immunity that was polarized towards Th1 IFN-γ. This optimized Ad26 vector-based vaccine for SARS-CoV-2, termed Ad26.COV2.S, is currently being evaluated in a phase I clinical trial (ClinicalTrials.gov Identifier: NCT04436276). [**note: this is a preprint of the Johnson & Johnson vaccine candidate data. Derek Lowe discussed this in the article above the fold.**] https://www.biorxiv.org/content/10.1101/2020.07.30.227470v1
- In response to the health crisis presented by the COVID-19 pandemic, rapid development of safe and effective vaccines that elicit durable immune responses is imperative. Recent reports have raised the concern that antibodies in COVID-19 convalescent patients may not be long lasting and thus even these individuals may require vaccination. Vaccine candidates currently in clinical testing have focused on the SARS-CoV-2 wildtype spike (S) protein (S-WT) as the major antigen of choice and while pre-clinical and early clinical testing have shown that S elicits an antibody response, we believe the optimal vaccine candidate should be capable of inducing robust, durable T-cell responses as well as humoral responses. We report here on a next generation bivalent human adenovirus serotype 5 (hAd5) vaccine capable of inducing immunity in patients with pre-existing adenovirus immunity, comprising both an S sequence optimized for cell surface expression (S-Fusion) and a conserved nucleocapsid (N) antigen designed to be transported to the endosomal subcellular compartment, with the potential to generate durable immune protection. Our studies suggest that this next generation bivalent vaccine is optimized for immunogenicity as evidenced by the following findings: (i) The optimized S-Fusion displayed improved S receptor binding domain (RBD) cell surface expression compared to S-WT where little surface expression was detected; (ii) the expressed RBD from S-Fusion retained conformational integrity and recognition by ACE2-Fc; (iii) the viral N protein modified with an enhanced T-cell stimulation domain (ETSD) localized to endosomal/lysosomal subcellular compartments for MHC I/II presentation; and (iv) these optimizations to S and N (S-Fusion and

N-ETSD) generated enhanced de novo antigen-specific B cell and CD4+ and CD8+ T-cell responses in antigen-naive pre-clinical models. Both the T-cell and antibody immune responses to S and N demonstrated a T-helper 1 (Th1) bias. The antibody responses were neutralizing as demonstrated by two independent SARS-CoV-2 neutralization assays. Based on these findings, we are advancing this next generation bivalent hAd5 S-Fusion + N-ETSD vaccine as our lead clinical candidate to test for its ability to provide robust, durable cell-mediated and humoral immunity against SARS-CoV-2 infection. *Further studies are ongoing to explore utilizing this vaccine construct in oral, intranasal, and sublingual formulations to induce mucosal immunity in addition to cell-mediated and humoral immunity. The ultimate goal of an ideal COVID-19 vaccine is to generate long-term T and B cell memory*. [**note: what would Friday be without a new vaccine candidate? This one comes from ImmunityBio and is built on an adenovirus platform.**] https://www.biorxiv.org/content/10.1101/2020.07.29.227595v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- There is currently a lack of biological tools to study the replication cycle and pathogenesis of SARS-CoV-2, the etiological agent of COVID-19. Repurposing the existing tools, including antibodies of SARS-CoV, is an effective way to accelerate the development of therapeutics for COVID-19. Here, we extensively characterized antibodies of the SARS-CoV structural proteins for their cross-reactivity, experimental utility, and neutralization of SARS-CoV-2. We assessed a total of 10 antibodies (six for Spike, two for Membrane, and one for Nucleocapsid and Envelope viral protein). We evaluated the utility of these antibodies against SARS-CoV-2 in a variety of assays, including immunofluorescence, ELISA, biolayer interferometry, western blots, and micro-neutralization. Remarkably, a high proportion of the antibodies we tested showed cross-reactivity, indicating a potentially generalizable theme of cross-reactivity between SARS-CoV and SARS-CoV-2 antibodies. These antibodies should help facilitate further research into SARS-CoV-2 basic biology. Moreover, our study provides critical information about the propensity of SARS-CoV antibodies to cross-react with SARS-CoV-2 and highlights its relevance in defining the clinical significance of such antibodies to improve testing and guide the development of novel vaccines and therapeutics. [**note: more work on cross reactivity of antibodies between the original SARS virus and the CoV-2 version. Looks like some epitopes are preserved.**] https://www.biorxiv.org/content/10.1101/2020.07.30.229377v1
- The ongoing COVID-19 pandemic exemplifies the general need to better understand viral infections. The positive single strand RNA genome of its causative agent, the SARS coronavirus 2 (SARS-CoV-2) encodes all viral enzymes. In this work, we focus on one particular methyltransferase (MTase), nsp16, which in complex with nsp10 is capable of methylating the first nucleotide of a capped RNA strand at the 2'-O position. This process is part of a viral capping system and is crucial for viral evasion of the innate immune reaction. In light of recently discovered non-canonical RNA caps, we tested various dinucleoside polyphosphate-capped RNAs as substrates for nsp10-nsp16 MTase. We developed an LC-MS-based method and discovered five types of capped RNA (m7Gp3A(G)-, Gp3A(G)- and Gp4A-RNA) that are substrates of the nsp10-nsp16 MTase. Our technique is an alternative to the classical isotope labelling approach for measurement of 2'-O-MTase activity. Further, we determined the IC50 value of sinefungin (286 +/- 66 nM) to illustrate the value of our approach for inhibitor screening. In the future, this approach can be used for screening inhibitors of any type of 2'-O-MTase. [**note: it is**

amazing how many functional enzymes this virus has. I need to find a good review that discusses all the modes of action. I don't know much about sinefungin other than it comes from some Streptomyces species and has antifungal, antiviral and antiparasitic activity. Here is some more reading on this compound and some derivatives.]
https://www.biorxiv.org/content/10.1101/2020.07.30.228478v1

- The densely glycosylated spike (S) proteins that are highly exposed on the surface of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) facilitate viral attachment, entry, and membrane fusion. We have previously reported all the 22 N-glycosites and site-specific N-glycans in the S protein protomer. Herein, we report the comprehensive and precise site-specific O-glycosylation landscapes of SARS-CoV-2 S proteins, which were characterized using high-resolution mass spectrometry. Following digestion using trypsin and trypsin/Glu-C, and de-N-glycosylation using PNGase F, we determined the mucin-type (GalNAc-type) O-glycosylation pattern of S proteins, including unambiguous O-glycosites and the 6 most common O-glycans occupying them, via Byonic identification and manual validation. Finally, 43 O-glycosites were identified in the insect cell-expressed S protein. Most glycosites were modified by non-sialylated O-glycans such as HexNAc(1) and HexNAc(1)Hex(1). In contrast, 30 O-glycosites were identified in the human cell-expressed S protein S1 subunit. Most glycosites were modified by sialylated O-glycans such as HexNAc(1)Hex(1)NeuAc(1) and HexNAc(1)Hex(1)NeuAc(2). Our results are the first to reveal that the SARS-CoV-2 S protein is a mucin-type glycoprotein; clustered O-glycans often occur in the N- and the C-termini of the S protein, and the O-glycosite and O-glycan compositions vary with the host cell type. These site-specific O-glycosylation landscapes of the SARS-CoV-2 S protein are expected to provide novel insights into the viral binding mechanism and present a strategy for the development of vaccines and targeted drugs. [**note: more on the glycosylation of the Spike protein.**]
https://www.biorxiv.org/content/10.1101/2020.07.29.227785v1
- The spike (S) glycoprotein in the envelope of SARS-CoV-2 is densely glycosylated but the functions of its glycosylation are unknown. Here we demonstrate that S is recognized in a glycan-dependent manner by multiple innate immune receptors including the mannose receptor MR/CD206, DC-SIGN/CD209, L-SIGN/CD209L, and MGL/CLEC10A/CD301. Single-cell RNA sequencing analyses indicate that such receptors are highly expressed in innate immune cells in tissues susceptible to SARS-CoV-2 infection. Binding of the above receptors to S is characterized by affinities in the picomolar range and consistent with S glycosylation analysis demonstrating a variety of N- and O-glycans as receptor ligands. These results indicate multiple routes for SARS-CoV-2 to interact with human cells and suggest alternative strategies for therapeutic intervention. [**note: building on the previous abstract, here is information on various interactions that are glycan dependent. Maybe there is a therapeutic approach here but I am skeptical.**] https://www.biorxiv.org/content/10.1101/2020.07.29.227462v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

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2020-08-01

Let's close out this week's pandemic choral selections with this collection of New York City singers and musicians. 'How Can I Keep from Singing': https://www.youtube.com/watch?v=VLPP3XmYxXg This captures the spirit quite well. I'll add a second song video from a Canadian singer who pretty much does only covers, Brigitte Wickens. I don't know much about her at all but I found the clips of all the COVID-19 health care workers quite moving: https://www.youtube.com/watch?v=Osdpf55Y4Fg

US COVID-19 STATISTICS - **Infection Rate: 1.4%; CFR: 3.3%** (IR up 0.1%; CFR down 0.1%; **note:** the CFR for this current outbreak continues to hover at 2%)

Here is a good article from a Wayne State physician on why HCQ is so hard to kill or as I note, a Zombie drug. America's failure on the testing front is covered by Vanity Fair and The Guardian.

The New York Times has a nice story on expected infections of SARS-CoV-2 might be expected in schools were to open now. Maybe the US should be more like Italy. More on the vaccine effort in India; I did not know this company is the largest vaccine manufacturer in the world. An Indiana school reopens only to be quickly confronted with a student ill with COVID-19. Here is a Silicon Valley group house cautionary tale. Alas, contact tracing in the US is failing.

This summer camp report is not encouraging as we near school reopenings. According to the WaPo, counselors and kids were supposed to have been virus free but the kids did not have to wear masks. Will college football players in the South balk at playing ball this fall? Meanwhile, the NFL pushes forward to holding their season on schedule; we'll see how that one plays out. Here is a feel good story about how a Mumbai slum quashed a SARS-CoV-2 outbreak; maybe this provides a way forward for the US. On the topic of Mumbai slums, Katherine Boo's "*Behind the Beautiful Forevers*: Life, Death, and Hope in a Mumbai Undercity" is an excellent chronicle of one such area.

Science has a good overview of how SARS-CoV-2 causes COVID-19 along with and editorial expressing cautious optimism. I would be more optimistic if people would mask up when going out. Jon Cohen has an exceptionally good interview with Wuhan coronavirus hunter Shi Zhengli.

The Lancet publishes a study of the risk of front line heath care workers and the general community of contracting COVID-19. Data was obtained using the COVID Symptom Study smartphone app: http://clinicaltrials.gov/show/NCT04331509 *Among 2 035 395 community individuals and 99 795 front-line health-care workers, we recorded 5545 incident reports of a positive COVID-19 test over 34 435 272 person-days. Compared with the general community, front-line health-care workers were at increased risk for reporting a positive COVID-19 test (adjusted HR 11·61, 95% CI 10·93–12·33). To account for differences in testing frequency between front-line health-care workers and the general community and possible selection bias, an inverse probability-weighted model was used to adjust for the likelihood of receiving a COVID-19 test (adjusted HR 3·40, 95% CI 3·37–3·43). Secondary and post-hoc analyses suggested adequacy of PPE, clinical setting, and ethnic background were also important factors*.

JAMA have a viewpoint piece on the benefits and risks of proliferating observational treatment assessments. This is a particularly good article and in accord with my thinking. Rob Califf and Martin Landray are two of the three authors.

Here is an intriguing preprint on how to use a microwave oven to decontaminate N-95 respirators. I'm always supportive of DIY projects! Speaking of DIY, do scroll down to the Diagnostics section to read a nice project undertaken by Georgia Tech to develop their own PCR test kits.

MODELING

- Nothing to Report!

NEWLY REGISTERED CLINICAL TRIALS

- Did not read today

CLINICAL TRIAL RESULTS

- Coronavirus disease-2019 (COVID-19) is a growing pandemic with an increasing death toll that has been linked to various comorbidities as well as racial disparity. However, the specific characteristics of these at-risk populations are still not known and approaches to lower mortality are lacking. METHODS: We conducted a retrospective electronic health record data analysis of 25,326 subjects tested for COVID-19 between 2/25/20 and 6/22/20 at the University of Alabama at Birmingham Hospital, a tertiary health care center in the racially diverse Southern U.S. The primary outcome was mortality in COVID-19-positive subjects and the association with subject characteristics and comorbidities was analyzed using simple and multiple linear logistic regression. RESULTS: The odds ratio of contracting COVID-19 was disproportionately high in Blacks/African-Americans (OR 2.6; 95%CI 2.19-3.10; p<0.0001) and in subjects with obesity (OR 1.93; 95%CI 1.64-2.28; p<0.0001), hypertension (OR 2.46; 95%CI 2.07-2.93; p<0.0001), and diabetes (OR 2.11; 95%CI 1.78-2.48; p<0.0001). Diabetes was also associated with a dramatic increase in mortality (OR 3.62; 95%CI 2.11-6.2; p<0.0001) and emerged as an independent risk factor in this diverse population even after correcting for age, race, sex, obesity and hypertension. Interestingly, we found that metformin treatment was independently associated with a significant reduction in mortality in subjects with diabetes and COVID-19 (OR 0.33; 95%CI 0.13-0.84; p=0.0210). CONCLUSION: Thus, these results suggest that while diabetes is an independent risk factor for COVID-19-related mortality, this risk is dramatically reduced in subjects taking metformin, raising the possibility that metformin may provide a protective approach in this high risk population. [**note: let's put metformin in the water supply!! Here is an observational study on 25K patients and metformin seems to be somewhat protective. Hey, it likely better than HCQ!**]
  https://www.medrxiv.org/content/10.1101/2020.07.29.20164020v1
- There is ongoing debate as to whether angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin-receptor blockers (ARBs) use is associated with poor prognosis of coronavirus disease-2019 (COVID-19). We sought to investigate the association between ACEI/ARB use and risk of poor clinical outcomes from COVID-19. We identified 1,290 patients with hypertension, of which 682 had recorded ACEI/ARB use and 608 without the use during 30 days preceding the date of COVID-19 diagnosis in completely enumerated COVID-19 cohort in South Korea. Our primary endpoint was the clinical outcomes comprised of all-cause mortality, use of mechanical ventilation, intensive care unit (ICU) admission, and sepsis. We used inverse probability of

treatment weighting (IPTW) to mitigate selection bias, and Poisson regression model to estimate the relative risks (RR) and 95% confidence intervals (CI) to compare outcomes in ACEI/ARB users with non-users. Compared to non-use, ACEI/ARB use was associated with lower clinical outcomes (IPTW adjusted RR, 0.60; 95% CI, 0.42-0.85; p=0.0046). When assessed by individual outcomes, ACEI/ARB use was not associated with all-cause mortality (IPTW adjusted RR, 0.62; 95% CI, 0.35-1.09; p=0.0973) and respiratory events (IPTW adjusted RR, 0.99; 95% CI, 0.84-1.17; p=0.9043). Subgroup analysis showed a trend toward protective role of ACEIs and ARBs against overall outcomes in men (IPTW adjusted RR, 0.84; 95% CI, 0.69-1.03; p-for-interaction=0.008) and with pre-existing respiratory disease (IPTW adjusted RR, 0.74; 95% CI, 0.60-0.92; p-for-interaction=0.0023). We present clinical evidence to support continuing ACE/ARB use in completely enumerated hypertensive COVID-19 cohort in South Korea. [**note: this is a large cohort study of clinical outcomes among patients being treated for hypertension in South Korea. Note the subgroup analysis showing some protection in men with pre-existing respiratory disease.**] https://www.medrxiv.org/content/10.1101/2020.07.29.20164822v1

DRUG DEVELOPMENT

- It has been known that SARS-CoV-2 which is considered similar to SARS-CoV invades human respiratory epithelial cells through interaction with the human angiotensin converting enzyme II (ACE2). In this work, SARS-CoV-2S-RBD and its cell receptor ACE2 were used to investigate the blocking effect and mechanism of β-chitosan to the binding of them. Besides, inhibitory effect of β-chitosan on inflammation induced by SARS-CoV-2S-RBD was also studied. Firstly, Native-PAGE results showed that β-chitosan could bind with ACE2 or SARS-CoV-2S-RBD and the conjugate of β-chitosan and ACE2 could no longer bind with SARS-CoV-2S-RBD. HPLC analyses suggested that was found the conjugate of β-chitosan and SARS-CoV-2S-RBD displayed high binding affinity under the condition of high pressure (40 MPa) compared with that of ACE2 and SARS-CoV-2S-RBD. Furthermore, immunofluorescence detections on Vero E6 cells and hACE2 mice showed that β-chitosan had a significant prevention and treatment effect on SARS-CoV-2S-RBD binding. Meanwhile, SARS-CoV-2S-RBD binding could activate the inflammation signaling pathways of cells and mice, however, β-chitosan could dramatically suppress the inflammations activated by SARS-CoV-2S-RBD. Subsequently, Western blot analyses revealed that the expression levels of ACE2 in experimental groups treated with β-chitosan significantly reduced. However, after the intervention of ADAM17 inhibitor (TAPI), the decreased ACE2 expressions affected by β-chitosan up-regulated correspondingly. The results indicated that β-chitosan has a similar antibody function, which can neutralize SARS-CoV-2S-RBD and effectively block the binding of SARS-CoV-2S-RBD with ACE2. ADAM17 activated by β-chitosan can enhance the cleavage of ACE2 extracellular domain with a catalytic activity of Ang II degradation, and then the extracellular region was released into the extracellular environment. So, β-chitosan could prevent the binding, internalization and degradation of ACE2 with SARS-CoV-2S-RBD and inhibit the activation of inflammatory signaling pathways at the same time. This work provides a valuable reference for the prevention and control of SARS-CoV-2 by β-chitosan. [**note: I put this is the interesting but don't know whether it will lead to anything.]**
https://www.biorxiv.org/content/10.1101/2020.07.31.229781v1
- A successful SARS-CoV-2 vaccine must be not only safe and protective but must also meet the demand on a global scale at low cost. Using the current influenza virus vaccine production

capacity to manufacture an egg-based inactivated Newcastle disease virus (NDV)/SARS-CoV-2 vaccine would meet that challenge. Here, we report pre-clinical evaluations of an inactivated NDV chimera stably expressing the membrane-anchored form of the spike (NDV-S) as a potent COVID-19 vaccine in mice and hamsters. The inactivated NDV-S vaccine was immunogenic, inducing strong binding and/or neutralizing antibodies in both animal models. More importantly, the inactivated NDV-S vaccine protected animals from SARS-CoV-2 infections or significantly attenuated SARS-CoV-2 induced disease. In the presence of an adjuvant, antigen-sparing could be achieved, which would further reduce the cost while maintaining the protective efficacy of the vaccine. [**note: this is an animal study of a vaccine that I reported on several days ago using the Newcastle Virus vector platform.**]
https://www.biorxiv.org/content/10.1101/2020.07.30.229120v1

- IFN-alpha2b and IFN-gamma combination has demonstrated favorable pharmacodynamics for genes underlying antiviral activity which might be involved in the defense of the organism from a SARS-CoV-2 infection. Considering this we conducted a randomized controlled clinical trial for efficacy and safety evaluation of subcutaneous IFN-alpha2b and IFN-gamma administration in patients positive to SARS-CoV-2. Methods: We enrolled 19-82 years-old inpatients at the Military Central Hospital Luis Diaz Soto, Havana, Cuba. They were hospitalized after confirmed diagnosis for SARS-CoV-2 RNA by real-time reverse transcription polymerase chain reaction. Patients were randomly assigned in a 1:1 ratio to receive either, subcutaneous treatment with a co-lyophilized combination of 3.0 MIU IFN-alpha2b and 0.5 MIU IFN-gamma (HeberFERON, CIGB, Havana, Cuba), twice a week for two weeks, or thrice a week intramuscular injection of 3.0 MIU IFN-alpha2b (Heberon Alpha R, CIGB, Havana, Cuba). Additionally, all patients received lopinavir-ritonavir 200/50 mg every 12 h and chloroquine 250 mg every 12 h (standard of care). The primary endpoints were the time to negativization of viral RNA and the time to progression to severe COVID-19, from the start of treatment. The protocol was approved by the Ethics Committee on Clinical Investigation from the Hospital and the Center for the State Control of Medicines, Equipment and Medical Devices in Cuba. Informed consent was obtained from each participant. Results: A total of 79 patients with laboratory-confirmed SARS-CoV-2 infection, including symptomatic or asymptomatic conditions, fulfilled the inclusion criteria and underwent randomization. Thirty-three subjects were assigned to the HeberFERON group, and 33 to the Heberon Alpha R group. Sixty-three patients were analyzed for viral negativization, of them 78.6% in the HeberFERON group negativized the virus after 4 days of treatment versus 40.6% of patients in the Heberon Alpha R groups (p=0.004). Time to reach the negativization of the SARS-CoV-2 measured by RT-PCR in real time was of 3.0 and 5.0 days for the HeberFERON and Heberon Alpha R groups, respectively. A significant improvement in the reduction of time for negativization was attributable to HeberFERON (p=0.0027, Log-rank test) with a Hazard Ratio of 3.2 and 95% CI of 1.529 to 6.948, as compared to Heberon Alpha R treated group. Worsening of respiratory symptoms was detected in two (6.6%) and one (3.3%) patients in HeberFERON and IFN-alpha2b groups, respectively. None of the subjects transit to severe COVID-19 during the study or the epidemiological follow-up for 21 more days. RT-PCR on day 14 after the start of the treatment was negative to SARS-CoV-2 in 100% and 91% of patients of the combination of IFNs and IFN-alpha2b, respectively. Negativization for HeberFERON treated patients was related to a significant increase in lymphocytes counts and an also significant reduction in CRP as early as 7 days after commencing the therapeutic schedule. All the patients in both cohorts recover

by day 14 and were in asymptomatic condition and laboratory parameters return to normal values by day 14 after treatment initiation. Adverse events were identified in 31.5% of patients, 28.5% in the control group, and 34.4% in the HeberFERON group, and the most frequent were headaches (17.4%). Conclusions: In a cohort of 63 hospitalized patients between 19 to 82 years-old with positive SARS-CoV-2, HeberFERON significantly negativized the virus on day 4 of treatment when comparing with IFN-alpha2b. Heberon Alpha R also showed efficacy for the treatment of the viral infection. Both treatments were safe and positively impact on the resolution of the symptoms. None of the patients developed severe COVID-19. Key words: COVID-19, treatment, drug, virus negativization, antiviral, interferon combination, SARS CoV-2. [**note: this trial is from Cuba. HeberFERON is a combination of interferons that was developed in Cuba in 2016 has been investigated there. There are only a small number of patients in the above noted trial. I took a quick look at the paper and it appears that patients were not on any other treatment modalities that might be confounding. It will be important for the Cuban investigators to expand the patient size.**]
https://www.medrxiv.org/content/10.1101/2020.07.29.20164251v1

- Recent studies have provided insights into the autoinflammation triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infection, which is associated with high mortality of coronavirus disease 2019 (COVID-19). Striking similarities has been noted between COVID-19 and anti-melanoma differentiation-associated gene 5 (MDA5) antibody (Ab)-related dermatomyositis (DM), implying a shared autoinflammatory aberrance. However, it is unclear whether anti-MDA5 Ab is present in COVID-19 and correlates with the severity and adverse outcome of COVID-19 patients. Here, we found that the positive rate of anti-MDA5 Ab in patients with COVID-19 was 48.2% and the anti-MDA5 Ab positive patients tended to develop severe disease (88.6% vs 66.9%, P<0.0001). In particular, the titer of anti-MDA5 Ab was increased in the non-survivals (5.95±5.16 vs 8.22±6.64, P=0.030) and the positive rate was also higher than that in the survivals (23.5% vs 12.0%, P=0.012). Regarding to severe COVID-19 patients, we found that high titer of anti-MDA5 Ab (≥10.0 U/mL) was more prevalent in the non-survivals (31.2% vs 14.0%, P=0.006). Moreover, early profiling of anti-MDA5 Ab could distinguish severe patients from those with non-severe ones. Overall, our data reveal that anti-MDA5 Ab is prevalent in the COVID-19 patients and high titer of this antibody is correlated with severe disease and unfavorable outcomes. [**note: from China, another interesting marker for severe COVID-19.** https://www.medrxiv.org/content/10.1101/2020.07.29.20164780v1
- Background: In the absence of evidence-based therapies for Multisystem Inflammatory Syndrome in Children (MIS-C), we aimed to describe the similarities and differences in the evaluation and treatment of MIS-C at hospitals in the United States. Methods: We conducted a cross-sectional survey from June 16 to July 16, 2020 of U.S. pediatric hospitals regarding protocols for patients with MIS-C. Elements included hospital characteristics, clinical definition of MIS-C, evaluation, treatment, and follow-up. We summarized key findings and compared results from centers that had treated >5 patients vs. those that had treated <5 patients. Results: Forty centers of varying size and experience with MIS-C participated. About half (21/40) of centers required only 1 day of fever for MIS-C to be considered. In the evaluation of patients, there was often a tiered approach. Intravenous immunoglobulin was the most widely used medication to treat MIS-C (98% of centers). Corticosteroids were listed in 93% of protocols for primarily the moderate or severe cases. Aspirin was commonly used including for mild cases,

whereas heparin or low molecular weight heparin were used primarily in severe cases. In severe cases, anakinra and vasopressors were frequently recommended. Nearly all centers (39/40) recommended follow up with cardiology. There were similar findings between centers that had treated >5 patients vs. those that had treated <5 patients. A supplement containing hospital protocols is provided. Conclusion: There are many similarities yet some key differences between hospital protocols for MIS-C. These findings can help healthcare providers learn from others regarding options for managing MIS-C patients. [**note: this is a useful study of pediatric care centers in the US and their experience in treating children with multisystem inflammatory syndrome.**] https://www.medrxiv.org/content/10.1101/2020.07.29.20164459v1

- The outbreak and spread of SARS-CoV-2 (Severe Acute Respiratory Syndrome coronavirus 2), the cause of coronavirus disease 2019 (COVID-19), is a current global health emergency and a prophylactic vaccine is needed urgently. The spike glycoprotein of SARS-CoV-2 mediates entry into host cells, and thus is a target for neutralizing antibodies and vaccine design. Here we show that adjuvanted protein immunization with SARS-CoV-2 spike trimers, stabilized in prefusion conformation, results in potent antibody responses in mice and rhesus macaques with neutralizing antibody titers orders of magnitude greater than those typically measured in serum from SARS-CoV-2 seropositive humans. Neutralizing antibody responses were observed after a single dose, with exceptionally high titers achieved after boosting. Furthermore, neutralizing antibody titers elicited by a dose-sparing regimen in mice were similar to those obtained from a high dose regimen. Taken together, these data strongly support the development of adjuvanted SARS-CoV-2 prefusion-stabilized spike protein subunit vaccines. [**note: animal data on vaccine protection from the Karolinska Institute. They worked with a purified Spike protein and adjuvant combination. They note that this approach would be quite challenging to scale up but it is good proof that this combination does provide immunity and are more potent than the three recently reported Phase 1 trials of mRNA or adenovirus-vectored vaccines. I wonder if there is a recombinant DNA production approach to creating this type of subunit vaccine. The dose amount might be low enough to make this possible. Unfortunately, it appears most of the $$$s are being put elsewhere.**]
  https://www.biorxiv.org/content/10.1101/2020.07.31.228486v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- A significant, positive association between selenium status and COVID-19 prognosis has recently been identified. The present study investigated the influence of SARS-CoV-2 on host selenoproteins which mediate many beneficial actions of selenium. We found that SARS-CoV-2 suppressed mRNA expression of selenoproteins associated with ferroptosis (GPX4), ER stress (SELENOF, SELENOK, SELENOM and SELENOS) and DNA synthesis (TXNRD3) in Vero cells, providing a deeper insight into the connection between selenium and SARS-CoV-2. [**note: another curious property of the virus, this time the ability to suppress mRNA associated with several seleno-proteins. Don't know what the implications are here.**]
  https://www.biorxiv.org/content/10.1101/2020.07.31.230243v1
- The dynamic immunological characteristics of COVID-19 patients are essential for clinicians to understand the disease progression. Our data showed that the immune system and function have gradually remodeled and declined with age from 16-91 years old in 25,239 healthy controls. Analyzing the relationship between the number of lymphocytes and age showed that

lymphocytes and subsets tended to decline with age significantly, whereas, the number of natural killer cells tended to increase with age significantly. SARS-CoV-2 specific immunity has declined with age in fatal cases. Furthermore, SARS-CoV-2 specific immunity is associated with survival time in fatal cases. The loss expansion of SARS-CoV-2 specific immunity could be expanded in vitro. A concurrent decline in SARS-CoV-2 specific cellular and humoral immunity and prolonged SARS-CoV-2 exposure predicted fatal outcomes. Our findings have provided a basis for further analysis of SARS-CoV-2 specific immunity and understanding the pathogenesis of fatal COVID-19 patients. [**note: this is from China. They look at age related changes in immunity and the linkage to poor COVID-19 outcomes.**]
https://www.medrxiv.org/content/10.1101/2020.07.29.20164681v1

- Angiotensin-converting enzyme 2 (ACE2) is the main entry point in the airways for SARS-CoV-2. ACE2 binding to SARS-CoV-2 protein Spike triggers viral fusion with the cell membrane, resulting in viral RNA genome delivery into the host. Despite ACE2's critical role in SARS-CoV-2 infection, an understanding of ACE2 expression, including in response to viral infection, remains unclear. Until now ACE2 was thought to encode five transcripts and one 805 amino acid protein. Here we identify a novel short isoform of ACE2. Short ACE2 is expressed in the airway epithelium, the main site of SARS-CoV-2 infection; it is substantially upregulated in response to interferon stimulation and RV infection, but not in response to SARS-CoV-2 infection, and it shows differential regulation in asthma patients. *This short isoform lacks SARS-CoV-2 spike glycoprotein high-affinity binding sites and altogether our data are consistent with a model where short ACE2 may influence host susceptibility to SARS-CoV-2 infection*. [**note: more information on ACE2 and a short isoform that may have an impact for infection.**]
https://www.biorxiv.org/content/10.1101/2020.07.31.230870v1

DIAGNOSTIC DEVELOPMENT

- Widespread testing for the presence novel coronavirus SARS-CoV-2 in patients remains vital for controlling the COVID-19 pandemic prior to the advent of an effective treatment. The early testing shortfall in some parts of the US can be traced to an initial shortage of supplies, expertise and/or instrumentation necessary to detect the virus by quantitative reverse transcription polymerase chain reaction (RT-qPCR). Here we show that academic biochemistry and molecular biology laboratories equipped with appropriate expertise and infrastructure can produce the RT-qPCR assay and backfill pipeline shortages. The Georgia Tech COVID-19 Test Kit Support Group synthesized multiplexed primers and probes and formulated a master mix composed of enzymes and proteins produced in-house. We compare the performance of our in-house kit to a commercial product used for diagnostic testing and describe implementation of environmental testing to monitor surfaces across various campus laboratories for the presence of SARS-CoV-2. [**note: the classic DIY work up. Don't have reagents, make them yourself as this large Georgia Tech group did! Why don't we repurpose all the college labs and have the students make their own COVID-19 test kits? This would keep them both busy and safe. Paging Mitch Daniels at Purdue, this is for you, a good way to reopen your University.**]
https://www.medrxiv.org/content/10.1101/2020.07.29.20163949v1
- SARS-CoV-2 RNA detection with real time PCR is currently the central diagnostic tool to determine ongoing active infection. Nasopharyngeal and oral swabs are the main collection tool of biological material used as the source of viral RNA outside a hospital setting. However,

limitation in swabs availability, trained health professional with proper PPE and potential risk of aerosols may hinder COVID diagnosis. Self-collection with swabs, saliva and throat wash to obtain oropharyngeal wash has been suggested as having comparable performance of regular swab. We performed throat wash (TW) based surveillance with laboratory heath workers and other employees (LHW) at a laboratory research institute. Methods: Consecutive volunteer testing of LWH and external household and close contacts were included. TW self-collection was performed in 5 mL of sterile saline that was returned to original vial after approximate 5 secs of gargle. RNA extraction and rtPCR were performed as part of routine COVID protocols using Allplex (Seegene, Korea). Results: Four hundred and twenty two volunteers, 387 (93%) LHW and 43 (7%) contacts participated in the survey. One or more positive COVID rtPCR was documented in 63 (14.9% CI95 12%-19%) individuals. No correlation was observed between with direct activities with COVID samples to positivity, with infection observed in comparable rates among different laboratory areas, administrative or supportive activities. Among 63 with detected SARS-CoV-2 RNA, 59 with clinical information, 58% reported symptoms at a median of 4 days prior to collection, most with mild disease. Over a third (38%) of asymptomatic cases developed symptoms 1-3 days after collection. Although overall CT values of TW were higher than that of contemporary swab tests from hospitalized cases, TW from symptomatic cases had comparable CTs. Conclusions: The study suggests that TW may be a valid alternative to the detection of SARS-CoV-2 RNA. The proportion of asymptomatic and pre-symptomatic cases is elevated and reinforces the need of universal precautions and frequent surveys to limit the spread of the disease. [**note: this is from the Sao Paolo virology lab in hard hit Brazil. It would be interesting to see if the throat wash collection method is better than saliva.**] https://www.medrxiv.org/content/10.1101/2020.07.29.20163998v1

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2020-08-02

Live music is starting up in Europe under these trying times. Here is a chamber music concert from the Solsberg Festival this past June. There is a sparse audience present. It's a fine performance of works by Beethoven for clarinet, cello and piano: https://www.youtube.com/watch?v=4O1f-X0Fllc I'll be moving away from the pandemic choral works as the novelty is wearing thin (though I continue to applaud all those who are making the effort to perform).

US COVID-19 STATISTICS - **Infection Rate: 1.4%; CFR: 3.3%** (IR unchanged; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

I covered a DIY diagnostic effort by Georgia Tech in yesterday's newsletter. What could possibly top that one? How about a DIY COVID-19 vaccine? Well, one of my frequent correspondents alerted me to such an effort! Check this out: https://radvac.org/ **Yes, you can do it!!!** They say 20 self-experimenters have tried this. I would be interested in seeing antibody titers from those individuals. I've not looked up to see past experiments with this type of delivery system. There was a nasal flu vaccine that I took one year when there was a shortage of the traditional injectable. I don't remember having any side effects and also didn't get the flu. I wonder about the quality control manufacturing of the epitopes. I suspect they are made for lab use only. I will await any reports from my readership on this one. I'm not going to try it. Check out the site. The white paper has a good description of vaccine immunology with some

excellent diagrams. The directions are straightforward though you do need some specialized equipment such as a micro-centrifuge.

It appears that the US is not the only home to COVID-19 deniers. The Washington Post covers this march of 15K in Berlin protesting lockdowns, masks, and vaccines. In the US the outbreak keeps on chugging along. Viruses do not have feelings. I have commented about universities reopening this fall and often referred to Mitch Daniels, president of Purdue and former governor of Indiana. The reason for singling him out was his very early mention that it would be unacceptable not to open in the fall. Since Purdue is a very large land-grant university it may be a good proxy for others who are going through this decision-making process. The latest updates on the opening are HERE. Some sound policies including mandatory flu vaccination. I hope President Daniels succeeds!

The New York Times suggests that riding the subway may be safer than you think. Wow, this COVID-19 patient had a double lung transplant.

Oh dear, Boston Red Sox ace pitcher, Eduardo Rodriguez is out for the season with COVID-related myocarditis. An outbreak within the St. Louis Cardinals forces some more cancelations. We'll see if they can continue the season.

MODELING

- Initial efforts to mitigate transmission of SARS-CoV-2 relied on intensive social distancing measures such as school and workplace closures, shelter-in-place orders, and prohibitions on the gathering of people. Other non-pharmaceutical interventions for suppressing transmission include active case finding, contact tracing, quarantine, immunity or health certification, and a wide range of personal protective measures. Here we investigate the potential effectiveness of these alternative approaches to suppression. We introduce a conceptual framework represented by two mathematical models that differ in strategy. We find both strategies may be effective, although both require extensive testing and work within a relatively narrow range of conditions. Generalized protective measures such as wearing face masks, improved hygiene, and local reductions in density are found to significantly increase the effectiveness of targeted interventions. [**note: this is from Univ of Georgia with the title, "Five approaches to suppression of SARS-CoV-2 without intensive social distancing." It is an interesting paper to read but the key problem that they acknowledge is all the interventions require testing and lots of it. I've been writing about this since mid-March and we still do not have enough testing capacity to achieve these goals. Models are great to provide a conceptual policy framework but worthless without the infrastructure to implement the suggested solutions. I also do not believe that pushing social/physical distancing aside is useful.**]
  https://www.medrxiv.org/content/10.1101/2020.07.30.20165159v1

NEWLY REGISTERED CLINICAL TRIALS

- We hypothesize that sulodexide instituted early in populations at significant risk and symptomatic patients affected with COVID-19 (shortness of breath, fever, weakness, diarrhoea) and risk factors of diabetes, hypertension, COPD, atherosclerosis, chronic kidney disease, will provide improvement in endothelial integrity, decrease inflammatory responses, and improved

clinical outcomes with decreased hospital admission, decrease VTE and arterial complications, morbidity, and mortality. [**note: this is a trial from some Mexican investigators**] NCT04483830

- In a 2x2 factorial design randomized controlled trial, the investigators aim to elaborate the safety and efficacy of two pharmacological regimens on outcomes of critically-ill patients with COVID-19. The first randomization entails open-label assignment to intermediate versus standard dose prophylactic anticoagulation. The investigators hypothesize that intermediate dose compared with standard prophylactic dose anticoagulation will have a superior efficacy with respect to a composite of venous thromboembolism (VTE), requirement for extracorporeal membrane oxygenation (ECMO), or all-cause mortality. The second randomization will be double-blind assignment of the included patients to atorvastatin 20mg daily versus matching placebo. The hypothesis is that statin therapy, compared with placebo, will reduce the composite of VTE, need for ECMO, or all-cause mortality. [**note: this trial is from hard hit Iran. Interesting approach using atorvastatin.**] NCT04486508
- Currently in Côte d'Ivoire, the preferred treatment for COVID-19 is an antiviral: lopinavir/ritonavir (LPV/r), usually directed against the Human Immunodeficiency Virus (HIV). Since the number of viruses (viral load) is high in the respiratory tract during COVID-19 infection, we propose in INTENSE-COV (ICOV) clinical trial to study whether the combination of two drugs is more effective than taking a single drug on reducing the viral load in the respiratory tract but also on reducing inflammation. These drugs include the LPV/r already in use in Côte d'Ivoire as well as an antihypertensive drug - telmisartan, and a drug that lowers blood cholesterol - atorvastatin. All three have been known for a long time and have been shown to be effective against other viruses. In addition, they are generic, inexpensive and readily available in all countries. The objectives of the ICOV study are therefore to improve viral eradication from the patient's body and respiratory tract, to reduce inflammation, to improve more rapidly the patient's state of health and to reduce the risk of transmission of the virus to others. [**note: another combination treatment regimen from the Ivory Coast using an HIV antiviral, atorvastatin and telmisartan. I sure would like to know whether the sartans as a class are preventative.**] NCT04466241
- This multi-center, double-blind, placebo-controlled, randomized Phase 2/3 trial will study the safety, tolerability, and efficacy of bardoxolone methyl in approximately 400-440 patients hospitalized with confirmed COVID-19. The Phase 2 portion of the trial will include approximately 40 patients and is designed to provide an early interim analysis of safety. The Phase 3 portion of the trial will include approximately 360-400 additional patients, and is designed to determine whether bardoxolone methyl increases the probability of recovery at Day 29 when compared with matching placebo. Patients will be randomized using permuted block randomization in a 1:1 fashion to either once-daily administration of bardoxolone methyl (20 mg) or matching placebo and treatment will be administered for the duration of hospitalization (until recovery), with a maximum treatment duration of 29 days. [**note: the sponsor is Reata Pharmaceuticals and the drug has a very mixed history of development with some bad safety issues.**] NCT04494646
- Phase IIa clinical trial in which 75 non-ICU hospital inpatients will be randomized 2:1 to 7 days of Neoral (2.5mg/kg PO BID) + standard of care (SOC) or no CSA + SOC. The primary endpoint is disease severity based on the World Health Organization (WHO) COVID Ordinal Outcomes Scale, on day 14. Secondary endpoints include safety and changes in serum inflammatory markers. [**note: this is a trial with cyclosporine from a Baylor investigator.**] NCT04492891

CLINICAL TRIAL RESULTS

- Background The novel coronavirus disease 2019 (COVID-19) worldwide pandemic has placed a significant burden on hospitals and healthcare providers. The immune response to this disease is thought to lead to a cytokine storm, which contributes to the severity of illness. There is an urgent need to confirm whether the use of tocilizumab provides a benefit in individuals with COVID-19. Methods A single-center propensity-score matched cohort study, including all consecutive COVID-19 patients, admitted to the medical center who were either discharged from the medical center or expired between March 1, 2020, and May 5, 2020, was performed. Patients were stratified according to the receipt of tocilizumab for cytokine storm and matched to controls using propensity scores. The primary outcome was in-hospital mortality. Results A total of 132 patients were included in the matched dataset (tocilizumab=66; standard of care=66). Approximately 73% of the patients were male. Hypertension (55%), diabetes mellitus (31%), and chronic pulmonary disease (15%) were the most common comorbidities present. There were 18 deaths (27.3%) in the tocilizumab group and 18 deaths (27.3%) in the standard of care group (odds ratio, 1.0; 95% confidence interval, 0.465 - 2.151; p=1.00). Advanced age, history of myocardial infarction, dementia, chronic pulmonary disease, heart failure, and malignancy were significantly more common in patients who died. Interpretation The current analysis does not support the use of tocilizumab for the management of cytokine storm in patients with COVID-19. Use of this therapeutic agent should be limited to the context of a clinical trial until more evidence is available. [**note: here is a single center study of tocilizumab on severe COVID-19 patients. It did not work. I have not seen the full Roche trial results but here is the Roche press statement on the trial failure.**]
  https://www.medrxiv.org/content/10.1101/2020.07.30.20114959v1
- The relationship of SARS-CoV-2 lung infection and severity of pulmonary disease is not fully understood. We analyzed autopsy specimens from 24 patients who succumbed to SARS-CoV-2 infection using a combination of different RNA and protein analytical platforms to characterize inter- and intra- patient heterogeneity of pulmonary virus infection. There was a spectrum of high and low virus cases that was associated with duration of disease and activation of interferon pathway genes. *Using a digital spatial profiling platform, the virus corresponded to distinct spatial expression of interferon response genes and immune checkpoint genes demonstrating the intra-pulmonary heterogeneity of SARS-CoV-2 infection*. [**note: post mortem results looking at pulmonary effects of SARS-CoV-2.**]
  https://www.medrxiv.org/content/10.1101/2020.07.30.20165241v1
- Objectives To develop and validate a pragmatic risk score to predict mortality for patients admitted to hospital with covid-19. Design Prospective observational cohort study: ISARIC WHO CCP-UK study (ISARIC Coronavirus Clinical Characterisation Consortium [4C]). Model training was performed on a cohort of patients recruited between 6 February and 20 May 2020, with validation conducted on a second cohort of patients recruited between 21 May and 29 June 2020. Setting 260 hospitals across England, Scotland, and Wales. Participants Adult patients (≥18 years) admitted to hospital with covid-19 admitted at least four weeks before final data extraction. Main outcome measures In-hospital mortality. Results There were 34 692 patients included in the derivation dataset (mortality rate 31.7%) and 22 454 in the validation dataset (mortality 31.5%). The final 4C Mortality Score included eight variables readily available at initial hospital assessment: age, sex, number of comorbidities, respiratory rate, peripheral oxygen

saturation, level of consciousness, urea, and C-reactive protein (score range 0-21 points). The 4C risk stratification score demonstrated high discrimination for mortality (derivation cohort: AUROC 0.79; 95% CI 0.78 - 0.79; validation cohort 0.78, 0.77-0.79) with excellent calibration (slope = 1.0). Patients with a score ≥15 (n = 2310, 17.4%) had a 67% mortality (i.e., positive predictive value 67%) compared with 1.0% mortality for those with a score ≤3 (n = 918, 7%; negative predictive value 99%). Discriminatory performance was higher than 15 pre-existing risk stratification scores (AUROC range 0.60-0.76), with scores developed in other covid-19 cohorts often performing poorly (range 0.63-0.73). Conclusions We have developed and validated an easy-to-use risk stratification score based on commonly available parameters at hospital presentation. This outperformed existing scores, demonstrated utility to directly inform clinical decision making, and can be used to stratify inpatients with covid-19 into different management groups. The 4C Mortality Score may help clinicians identify patients with covid-19 at high risk of dying during current and subsequent waves of the pandemic. [**note: here is a large UK risk stratification study for patients admitted to the hospital with COVID-19. It's useful for placing patients in different treatment groups.**]
https://www.medrxiv.org/content/10.1101/2020.07.30.20165464v1

- Clinical diagnosis of COVID-19 poses an enormous challenge to early detection and prevention of COVID-19, which is of crucial importance for pandemic containment. Cases of COVID-19 may be hard to distinguish clinically from other acute viral diseases, resulting in an overwhelming load of laboratory screening. Sudden onset of taste and smell loss emerge as hallmark of COVID-19. The optimal ways for including these symptoms in the screening of suspected COVID-19 patients should now be established. Methods: We performed a case-control study on patients that were PCR-tested for COVID-19 (112 positive and 112 negative participants), recruited during the first wave (March 2020 - May 2020) of COVID-19 pandemic in Israel. Patients were interviewed by phone regarding their symptoms and medical history and were asked to rate their olfactory and gustatory ability before and during their illness on a 1-10 scale. Prevalence and degrees of symptoms were calculated, and odds ratios were estimated. Symptoms-based logistic-regression classifiers were constructed and evaluated on a hold-out set. Results: Changes in smell and taste occurred in 68% (95% CI 60%-76%) and 72% (64%-80%), of positive patients, with 24 (11-53 range) and 12 (6-23) respective odds ratios. The ability to smell was decreased by 0.5 ± 1.5 in negatives, and by 4.5 ± 3.6 in positives, and to taste by 0.4 ± 1.5 and 4.9 ± 3.8, respectively (mean ± SD). A penalized logistic regression classifier based on 5 symptoms (degree of smell change, muscle ache, lack of appetite, fever, and a negatively contributing sore throat), has 66% sensitivity, 97% specificity and an area under the ROC curve of 0.83 (AUC) on a hold-out set. A classifier based on degree of smell change only is almost as good, with 66% sensitivity, 97% specificity and 0.81 AUC. Under the assumption of 8% positives among those tested, the predictive positive value (PPV) of this classifier is 0.68 and negative predictive value (NPV) is 0.97. Conclusions: Self-reported quantitative olfactory changes, either alone or combined with other symptoms, provide a specific and powerful tool for clinical diagnosis of COVID-19. The applicability of this tool for prioritizing COVID-19 laboratory testing is facilitated by a simple calculator presented here. [**note: from Israel, self-reported olfactory changes are useful in diagnosing COVID-19. Get COVID-19 Scent Strips™ out into the community.**] https://www.medrxiv.org/content/10.1101/2020.07.30.20164327v1

- SARS-CoV-2 causes a wide spectrum of clinical manifestations and significant mortality. Studies investigating underlying immune characteristics are needed to understand disease pathogenesis and inform vaccine design. In this study, we examined immune cell subsets in hospitalized and non-hospitalized individuals. In hospitalized patients, many adaptive and innate immune cells were decreased in frequency compared to healthy and convalescent individuals, with the exception of B lymphocytes which increased. Our findings show increased frequencies of T-cell activation markers (CD69, Ox40, HLA-DR and CD154) in hospitalized patients, with other T-cell activation/exhaustion markers (CD25, PD-L1 and TIGIT) remaining elevated in hospitalized and non-hospitalized individuals. B cells had a similar pattern of activation/exhaustion, with increased frequency of CD69 and CD95 during hospitalization, followed by an increase in PD1 frequencies in non-hospitalized individuals. Interestingly, many of these changes were found to increase over time in non-hospitalized longitudinal samples, suggesting a prolonged period of immune dysregulation following SARS-CoV-2 infection. Changes in T-cell activation/exhaustion in non-hospitalized patients were found to positively correlate with age. Severely infected individuals had increased expression of activation and exhaustion markers. These data suggest a prolonged period of immune dysregulation following SARS-CoV-2 infection highlighting the need for additional studies investigating immune dysregulation in convalescent individuals. [**note: this is not good news. Sustained immune dysfunction in recovering patients. However, it is a small study and the hospitalized patients had a lot of comorbidities and were older than the non-hospitalized patients. Still this is something worthy of a larger study.**]
  https://www.medrxiv.org/content/10.1101/2020.07.30.20165175v1
- Men and older women have been shown to be at higher risk of adverse COVID-19 outcomes. Animal model studies of SARS-CoV and MERS suggest that the age and sex difference in COVID-19 symptom severity may be due to a protective effect of the female sex hormone estrogen. Females have shown an ability to mount a stronger immune response to a variety of viral infections because of more robust humoral and cellular immune responses. Objectives: We sought to determine whether COVID-19 positivity increases in women entering menopause. We also aimed to identify whether premenopausal women taking exogenous hormones in the form of the combined oral contraceptive pill (COCP) and post-menopausal women taking hormone replacement therapy (HRT) have lower predicted rates of COVID-19, using our published symptom-based model. Design: The COVID Symptom Study developed by Kings College London and Zoe Global Limited was launched in the UK on 24th March 2020. It captured self-reported information related to COVID-19 symptoms. Data used for this study included records collected between 7th May - 15th June 2020. Main outcome measures: We investigated links between COVID-19 rates and 1) menopausal status, 2) COCP use and 3) HRT use, using symptom-based predicted COVID-19, tested COVID-19, and disease severity based on requirement for hospital attendance or respiratory support. Participants: Female users of the COVID Symptom Tracker Application in the UK, including 152,637 women for menopause status, 295,689 for COCP use, and 151,193 for HRT use. Analyses were adjusted for age, smoking and BMI. Results: Post-menopausal women aged 40-60 years had a higher rate of predicted COVID (P=0.003) and a corresponding range of symptoms, with consistent, but not significant trends observed for tested COVID-19 and disease severity. Women aged 18-45 years taking COCP had a significantly lower predicted COVID-19 (P=8.03E-05), with a reduction in hospital attendance (P=0.023). Post-menopausal women using HRT or hormonal therapies did not exhibit consistent associations,

including increased rates of predicted COVID-19 (P=2.22E-05) for HRT users alone. Conclusions: Our findings support a protective effect of estrogen on COVID-19, based on positive association between predicted COVID-19 and menopausal status, and a negative association with COCP use. HRT use was positively associated with COVID-19 symptoms; however, the results should be considered with caution due to lack of data on HRT type, route of administration, duration of treatment, and potential comorbidities. [**note: here is a large UK study on the impact of estrogen on COVID-19. It seems to be protective but the investigators have several cautions as noted.**] https://www.medrxiv.org/content/10.1101/2020.07.30.20164921v1

DRUG DEVELOPMENT

- An essential mechanism for SARS-CoV-1 and -2 infection begins with the viral spike protein binding to the human receptor protein angiotensin-converting enzyme II (ACE2). Here we describe a stepwise engineering approach to generate a set of affinity optimized, enzymatically inactivated ACE2 variants that potently block SARS-CoV-2 infection of cells. These optimized receptor traps tightly bind the receptor binding domain (RBD) of the viral spike protein and prevent entry into host cells. We first computationally designed the ACE2-RBD interface using a two-stage flexible protein backbone design process that improved affinity for the RBD by up to 12-fold. These designed receptor variants were affinity matured an additional 14-fold by random mutagenesis and selection using yeast surface display. The highest affinity variant contained seven amino acid changes and bound to the RBD 170-fold more tightly than wild-type ACE2. With the addition of the natural ACE2 collectrin domain and fusion to a human Fc domain for increased stabilization and avidity, the most optimal ACE2 receptor traps neutralized SARS-CoV-2 pseudotyped lentivirus and authentic SARS-CoV-2 virus with half-maximal inhibitory concentrations (IC50) in the tens of ng/ml range. Engineered ACE2 receptor traps offer a promising route to fighting infections by SARS-CoV-2 and other ACE2-utilizing coronaviruses, with the key advantage that viral resistance would also likely impair viral entry. Moreover, such traps can be pre- designed for viruses with known entry receptors for faster therapeutic response without the need for neutralizing antibodies isolated or generated from convalescent patients. [**note: here is an interesting approach to using a genetically modified ACE2 variants that have strong binding to SARS-CoV-2.**]
https://www.biorxiv.org/content/10.1101/2020.07.31.231746v2

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The kinetics of immunoglobulin G (IgG) avidity maturation during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was studied. The IgG avidity assay used a novel label-free immunoassay technology to test IgG against the virus spike protein receptor-binding domain (RBD). The technology, thin-film interferometry (TFI), is able to sense the formation of immune complex on a sensing probe without attaching a reporter (enzyme, fluorophore, etc.). It was found that there was a strong correlation between IgG antibody avidity and days since symptom onset ($p < 0.0001$). In addition, peak readings were significantly higher for specimens from ICU than non-ICU patients for the first month after symptom onset (1-4 weeks) and thereafter ($p<0.0001$). The findings are consistent for what has been reported for SARS-CoV. Given that SARS-CoV-2 specific IgG avidity is strong in ICU patients after 1 month, this suggests that antibody-mediated immune enhancement triggered by suboptimal antibodies may not play

a role in COVID-19 disease progression and severity. [**note: more information and antibody kinetics and severe COVID-19. The technology the use is quite interesting.**]
https://www.medrxiv.org/content/10.1101/2020.07.30.20165522v1

DIAGNOSTIC DEVELOPMENT

- Lateral flow immunoassays for antibody testing have been viewed as a cheap and rapidly deployable method for determining previous infection with SARS-CoV-2; however, these assays have shown unacceptably low sensitivity. We report on nine lateral flow immunoassays currently available and compare their titer sensitivity in serum to a best-practice enzyme-linked immunosorbent assay (ELISA) and viral neutralization assay. For a small group of PCR-positive, we found two lateral flow immunoassay devices with titer sensitivity roughly equal to the ELISA; these devices were positive for all PCR-positive patients harboring SARS-CoV-2 neutralizing antibodies. One of these devices was deployed in Northern Italy to test its sensitivity and specificity in a real-world clinical setting. Using the device with fingerstick blood on a cohort of 27 hospitalized PCR-positive patients and seven hospitalized controls, ROC curve analysis gave AUC values of 0.7646 for IgG. For comparison, this assay was also tested with saliva from the same patient population and showed reduced discrimination between cases and controls with AUC values of 0.6841 for IgG. Furthermore, during viral neutralization testing, one patient was discovered to harbor autoantibodies to ACE2, with implications for how immune responses are profiled. *We show here through a proof-of-concept study that these lateral flow devices can be as analytically sensitive as ELISAs and adopted into hospital protocols; however, additional improvements to these devices remain necessary before their clinical deployment*. [**note: here is a evaluation of point of care lateral flow immunoassays.**]
https://www.medrxiv.org/content/10.1101/2020.07.30.20163824v1
- Serological SARS-CoV-2 assays are needed to support clinical diagnosis and epidemiological investigations. Recently, assays for the large-volume detection of total antibodies (Ab) and immunoglobulin (Ig) G and M against SARS-CoV-2 antigens have been developed, but there are limited data on the diagnostic accuracy of these assays. This study was organized as a Danish national collaboration and included fifteen commercial and one in-house anti-SARS-CoV-2 assays in sixteen laboratories. Sensitivity was evaluated using 150 serum samples from individuals diagnosed with asymptomatic, mild or moderate nonhospitalized (n=129) or hospitalized (n=31) COVID-19, confirmed by nucleic acid amplification tests, collected 13-73 days from symptom onset. Specificity and cross-reactivity were evaluated in samples collected prior to the SARS-CoV-2 epidemic from > 586 blood donors and patients with autoimmune diseases or CMV or EBV infections. Predefined specificity criteria of ≥99% were met by all total-Ab and IgG assays except one (Diasorin/LiaisonXL-IgG 97.2%). The sensitivities in descending order were: Wantai/ELISA total-Ab (96.7%), CUH/NOVO in-house ELISA total-Ab (96.0%), Ortho/Vitros total-Ab (95.3%), YHLO/iFlash-IgG (94.0%), Ortho/Vitros-IgG (93.3%), Siemens/Atellica total-Ab (93.2%), Roche-Elecsys total-Ab (92.7%), Abbott-Architect-IgG (90.0%), Abbott/Alinity-IgG (median 88.0%), Diasorin/LiaisonXL-IgG (84.6%), Siemens/Vista total-Ab (81.0%), Euroimmun/ELISA-IgG (78.0%), and Snibe/Maglumi-IgG (median 78.0%). The IgM results were variable, but one assay (Wantai/ELISA-IgM) had both high sensitivity (82.7%) and specificity (99%). The rate of seropositivity increased with time from symptom onset and symptom severity. In conclusion, predefined sensitivity and specificity acceptance criteria of 90%/99%,

respectively, for diagnostic use were met in five of six total-Ab and three of seven IgG assays. [**note: a large Danish comparison study of serology tests. I'll probably stop posting these links now. Clinical labs know how to do these comparisons and confirm validation.**]
https://www.medrxiv.org/content/10.1101/2020.07.30.20165373v1