2020-05-11

I never tire of Schubert. Here is one of his better known songs, 'The Shepherd on the Rock' written for soprano, piano and clarinet. Barbara Bonney does a wonderful job accompanied by Andre Watts and David Shifrin: https://www.youtube.com/watch?v=KEjBGYLohto

There is a very good lay article in today's Washington Post on some of the unravelling mysteries of SARS-CoV-2 infection. If I were going into research these days, it might be virology as a choice.

There have been a number of stories about viral mutation and what it might mean for vaccine and mAb therapy. Derek Lowe discusses some of the aspects. We know that microorganisms are constantly mutating (humans see gene mutations as well but at a much slower rate based on reproduction time; an obvious statement but one worth repeating). Antibiotic and cancer drug resistance are excellent examples of this type of adaptation. Here is the money quote from Derek's article: *It's important to realize, though, that none of these mechanisms are proven. In fact, the very hypothesis that this mutant is more transmissible is as yet unproven – it's not unlikely, but it could be wrong. Here's an important question: what about the patients who are infected with with the D614G form? Turns out that the Sheffield group was able to match up hospitalization data with the sequencing of 453 individual patients, and* they found no correlation *of hospitalization status with the mutant form. Trevor Bedford and the UW group independently checked for this and had the same result: no apparent effect on severity of disease. At most, this mutation may be more transmissible (bad enough, to be sure), but it does* not *appear deadlier once a patient has been infected.* It is another good example of the Rumsfeld Paradigm.

Your dogged correspondent is a long time user of Melitta coffee filters and I noted back on May 5 the advantages of drinking coffee brewed using paper filters (all my newsletters are archived by week if you need to go back and look something up). Here is a cool article about how the company made a fast pivot from making coffee filters to producing masks. Major shout out for thinking outside the box. Speaking of masks, this Chinese group looked at different materials that can be used for homemade masks. DIY projects are always fun whether it's assembling IKEA furniture or building home computers.

I have received several emails in recent days asking for comments on the Judy Mikovits controversy. I don't traffic in conspiracy theories and routinely dismiss these along with all the antivaxxers. Jon Cohen and Martin Eserink discuss the matter in great detail here in Science, where Mikovits published her controversial and later retracted paper.

Some interesting abstract on a short news day. We are still waiting for clinical trial results.

MODELING

- Enclosed societies (i.e. locations that are connected to wider community only by subgroups of their population and that are dominated by within society transmission) have the potential, upon establishment of a respiratory disease, to suffer a large proportion of the population within becoming infected. Care homes are particularly susceptible to COVID19 outbreaks and suffer high mortality due to vulnerable population within. Recent data on the number of new outbreak reports in care homes to Public Health England shows an initial increase then plateau

perhaps associated with an SIS model dynamic. Without change in policy moving forward a high prevalence in such setting is predicted of around 75%. Action is needed to support staff in such settings. **[note: yes, this is an obvious finding but the first one that I've seen that tries to model the situation. Special care has to be take in monitoring staff that work in these facilities as disease transmission can be rampant, once established.**]
https://www.medrxiv.org/content/10.1101/2020.05.07.20089243v1

- Introduction With the pandemic of COVID-19, the number of confirmed cases and related deaths are increasing in the US. We aimed to understand the potential impact of health and demographic factors on the infection and mortality rates of COVID-19 at the population level. Methods We collected total number of confirmed cases and deaths related to COVID-19 at the county level in the US from January 21, 2020 to April 23, 2020. We extracted health and demographic measures for each US county. Multivariable linear mixed effects models were used to investigate potential correlations of health and demographic characteristics with the infection and mortality rates of COVID-19 in US counties. Results Our models showed that several health and demographic factors were positively correlated with the infection rate of COVID-19, such as low education level and percentage of Black. In contrast, several factors, including percentage of smokers and percentage of food insecure, were negatively correlated with the infection rate of COVID-19. While the number of days since first confirmed case and the infection rate of COVID-19 were negatively correlated with the mortality rate of COVID-19, percentage of elders (65 and above) and percentage of rural were positively correlated with the mortality rate of COVID-19. Conclusions At the population level, health and demographic factors could impact the infection and mortality rates of COVID-19 in US counties. [**note: good study down to the county level from Univ of Rochester researchers.**]
https://www.medrxiv.org/content/10.1101/2020.05.06.20093195v1
- As of May 1, 2020, the number of cases of Covid-19 in the US passed 1,062,446, interventions to slow down the spread of Covid-19 curtailed most social activities. Meanwhile, an economic crisis and resistance to the strict intervention measures are rising. Some researchers proposed intermittent social distancing that may drive the outbreak of Covid-19 into 2022. Questions arise about whether we should maintain or relax quarantine measures. We developed novel artificial intelligence and causal inference integrated methods for real-time prediction and control of nonlinear epidemic systems. *We estimated that the peak time of the Covid-19 in the US would be April 24, 2020 and its outbreak in the US will be over by the end of July and reach 1,551,901 cases.* We evaluated the impact of relaxing the current interventions for reopening economy on the spread of Covid-19. We provide tools for balancing the risks of workers and reopening economy. [**note: there are several caveats buried within the paper. My math is not good enough to make any pronouncement on whether this model is good, bad, or indifferent. However, they do state at the end of the paper "A keyto safely reopening the economyis massive virus tests.Relaxing quarantine, self-isolation and business closureis offset by increasingthe number of tests.Question is how many number of tests is needed to ensure the curbing the spread of Covid-19 without intriguing the second wave of the outbreak.**]
https://www.medrxiv.org/content/10.1101/2020.05.04.20091272v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- A single institution, two-centre retrospective analysis following implementation of a Decision Support tool and real-time data dashboard for early detection of patients requiring personalised enhanced care, focussing particularly on respiratory rate, diastolic blood pressure, oxygenation indices, C-reactive protein, D-dimer and ferritin. Protocols differing from conventional practice included high-dose prophylactic anticoagulation for all COVID-19 positive patients and antioxidant prescription. RESULTS: By 22nd April 2020, 923 patients tested COVID-19 positive. 569 patients (61.7%) were male. The majority presented with advanced disease: interquartile ranges were C-reactive protein 44.9-179mg/L, D-dimer 1070-3802ng/L, and ferritin 261-1208µg/L. Completed case fatality rates were 25.1% [95% CI 20.0, 30.0] in females, 40.5% [95% CI 35.9, 45.0] in males. 139 patients were admitted to intensive care where current death rates are 16.2% [95% CI 3.8, 28.7] in females, 38.2% [95% CI 28.6, 47.8] in males with no trends for differences based on ethnicity. A real-time traffic lights dashboard enabled rapid assessment of patients using critical parameters to accelerate adjustments to management protocols. In total 513 (55.6%) of patients were flagged as high risk for thromboembolic disease, exceeding the numbers flagged for respiratory deteriorations (N=391, 42.4%), or cytokine storm (N=68, 7.4%). There was minimal evidence that age was associated with disease severity, but males had higher levels of all dashboard indices, particularly C-reactive protein and ferritin ($p<0.0001$) which displayed no relationship with age. CONCLUSIONS: Survival rates are encouraging. Protocols employed (traffic light-driven personalised care, protocolised early therapeutic anticoagulation based on D-dimer >1,000ng/L and/or CRP>200 mg/L, personalised ventilatory strategies and antioxidants) are recommended to other units. Males are at greater risk of severe disease, most likely as the obligate SARS-CoV-2 receptor is on the X-chromosome, and require especially close, and early attention. [**note: more retrospective data from Britain.**]
  https://www.medrxiv.org/content/10.1101/2020.05.08.20088393v1
- Reliably identifying patients at increased risk for COVID-19 complications could guide clinical decisions, public health policies, and preparedness efforts. The most globally accepted definitions of at-risk patients rely, primarily, on epidemiological characterization of hospitalized COVID-19 patients. However, such characterization overlooks, and fails to correct for, the prevalence of existing conditions in the wider SARS-CoV-2 positive population. Here, we use the complete medical records of 4,353 Israeli SARS-CoV-2 positive individuals, of whom 173 experienced moderate or severe symptoms of COVID-19, to identify the conditions that increase the risk of disease complications, in various age and sex strata. Our analysis suggests that cardiovascular and kidney diseases, obesity, and hypertension are significant risk factors for COVID-19 complications, as previously reported. Interestingly, it also indicates that depression (e.g., odds ratio, OR, for males 65 years or older: 2.94, 95% confidence intervals [1.55, 5.58]; P-value = 0.014) as well cognitive and neurological disorder (e.g., OR for individuals ≥ 65 year old: 2.65 [1.69, 4.17]; P-value < 0.001) are significant risk factors; and that smoking and background of respiratory diseases do not significantly increase the risk of complications. Adjusting existing risk definitions following these observations may improve their accuracy and impact the global pandemic containment efforts. [**note: some more obvious findings but I include it to show how a robust electronic medical record system can be used for rapid research analysis. Perhaps the US will move towards this someday in the future.**]
  https://www.medrxiv.org/content/10.1101/2020.05.07.20091652v1

- Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to spread worldwide and pose a major public health burden. There is increasing evidence that men are more likely to die from SARS-CoV-2 infection than women. However, underlying factors that mediate the observed sex bias in coronavirus disease 2019 (COVID-19) remain unknown. Methods. In this retrospective cohort, we included COVID-19 patients who were admitted to an Intensive Care Unit at the University Hospital Hamburg-Eppendorf, Germany. We obtained demographic data of all patients who were discharged or had died by 29th April 2020. We systematically analyzed sex hormones as well as cytokine and chemokine responses in male and female patients with laboratory-confirmed SARS-CoV-2 infections upon hospital admission. We used uni- and multivariable linear regression methods to identify potential risk factors for disease severity in males and females. Findings. All enrolled patients (n=45; n=35 males and n=10 females) presented comorbidities with hypertension being the most common (45.7% in males; 40% in females), followed by cancer (35% in males; 40% in females), obesity (34.3% in males and 30% in females), type II diabetes (25.7% in males and 20% in females) and chronic heart diseases (8.6% in males and 0% in females). We detected that the vast majority of male COVID-19 patients present low testosterone (68.6%) and low dihydrotestosterone (48.6%) levels. In contrast, most female COVID-19 patients have elevated testosterone levels (60%) without alterations in dihydrotestosterone levels. Both, female and male COVID-19 patients may present elevated estradiol levels (45.7% in males and 40% in females). Disease severity defined by SOFA score correlates with elevated cytokine responses (e.g. IL-6) in males and IL-2 in females. In male COVID-19 patients, testosterone levels negatively correlate with inflammatory IL-2 and IFN-γ, whereas estradiol levels positively correlate with the inflammatory cytokine IL-6. Vice versa, in female COVID-19 patients, testosterone levels positively correlate with inflammatory cytokines (e.g. IL-6). Interpretation. We here show that critically ill male COVID-19 patients suffer from severe testosterone and dihydrotestosterone deficiencies. Both androgens are required to mount antiviral immune responses to combat infection in males. [**note: testosterone patches for all males over 60 years of age!!!**]
  https://www.medrxiv.org/content/10.1101/2020.05.07.20073817v1
- The disease in notably severe in elderly and those with underlying chronic conditions. The molecular mechanism as to why the elderly are vulnerable and why children are resistant is largely unknown. Understanding these differences is critical for safeguarding the vulnerable and guiding effective policy and treatments. Here we show loading cells with cholesterol from blood serum using the cholesterol transport protein apolipoprotein E (apoE) enhances the endocytic entry of pseudotyped SARS-CoV-2. Super resolution imaging of the SARS-CoV-2 entry point with high cholesterol showed markedly increased apparent diameter (~10% to 100 nm) and almost twice the total number of viral entry points. The cholesterol concomitantly traffics angiotensinogen converting enzyme (ACE2) to the viral entry site where SARS-CoV-2 docks to properly exploit entry into the cell. Furthermore, we show cholesterol enhances binding of SARS-CoV-2 to the cell surface which increases association with the endocytic pathway. Decreasing cellular cholesterol has the opposite effect. Based on these findings and known loading of cholesterol into peripheral tissue cholesterol during aging and inflammation, we build a cholesterol dependent model for COVID19 lethality in elderly and the chronically ill. As cholesterol increases with age and inflammation (e.g. smoking and diabetes), the cell surface is coated with viral entry points and optimally assembled viral entry proteins. Importantly our

model suggests high levels of cholesterol is most alarming in the tissue, not the blood. In fact, rapidly dropping cholesterol in the blood may indicate severe loading of cholesterol in peripheral tissue and a dangerous situation for escalated SARS-CoV-2 infectivity. Polyunsaturated fatty acids (PUFAs) oppose the effects of cholesterol and provide a molecular basis for eating healthy diets to avoid severe cases of COVID19. [**note: better throw out the butter and switch to high oleic acid margarine!!! It would assuage my concerns if we had some linkage to normal cholesterol and lipoprotein levels. I've always had good blood markers in this regard. Let's also find out if there is an linkage with statin therapy.**] https://www.biorxiv.org/content/10.1101/2020.05.09.086249v2

DRUG DEVELOPMENT

- Based on analyses of available data, we deduced that the excessive prostaglandins E2 (PGE2) accumulation mediated by cyclooxygenase-2 (COX-2) was the key pathological basis of COVID-19. Methods: The urine PGE2 levels were measured by mass spectrometry. An experimental study about Celebrex to treat COVID-19 was conducted based on routine treatment. A total of 44 confirmed COVID-19 patients were enrolled (Experimental group n=37, Control group n=7). Patients in experimental group were given Celebrex once or twice a day (0.2 g/time) for 7-14 days. The dosage or duration was modified for individuals. Clinical outcomes of Celebrex adjuvant therapy were evaluated by vital signs, laboratory tests, and computed tomography upon the discontinuance of Celebrex. Results: We found that the concentrations of PGE2 in urine samples of COVID-19 patients were significantly higher than that of healthy individuals (mean value is 170 ng/ml vs 18.8 ng/ml, $p<0.01$) and positively correlated with the progression of COVID-19. Among the experimental group (ordinary n=29, severe n=7, critical n=1), 25 cases were treated with full dose and 11 cases with half dose of Celebrex, and 1 case with Ibuprofen. The remission rate were 100%, 82% and 57% in full dose, half dose and control group respectively. Celebrex significantly reduced the PGE2 levels and promoted recovery of ordinary or severe COVID-19. Conclusion: Our study suggests that Celebrex adjuvant treatment may be helpful for the therapy of COVID-19. [**note: I knew they would find a use for celecoxib sooner or later. I never had much luck with it when I had various orthopedic injuries and it is the weakest of all the Cox-2 inhibitors. How cool would it be if this works in SARS-CoV-2. Maybe it's time to bring back rofecoxib which was a far better drug!!! Let's get these clinical trials up and running. Chlorpromazine + rofecoxib will solve the COVID-19 pandemic. You read it first here!**] https://www.medrxiv.org/content/10.1101/2020.05.05.20077610v1

DIAGNOSTIC DEVELOPMENT

- We describe a quantitative droplet digital PCR (ddPCR) assay for detection of SARS-CoV-2 viral ribonucleic acid (RNA) in total RNA extracted from human sputum. This method was validated using the guidance of the United States Food and Drug Administration Accelerated Emergency Use Authorization (EUA) Template for SARS-CoV-2 that Causes Coronavirus Disease (COVID-19) Molecular Testing of Respiratory Speciment in CLIA Certified High-Complexity Laboratories. Though our laboratory is not CLIA certified, this method met all criteria specified by the guidance document with a Limit of Detection (LOD) of 0.25 copies/microliter in the final ddPCR (at least 19/20 replicates reactive), which we consider to be a Lower Limit of Quantification (LLOQ); inclusivity of all known annotated SARS-CoV-2 genomes; no cross-reactivity with other

respiratory pathogens; and reactivity of all contrived positives at or above the LOD. [**note: sound science behind this approach to sputum testing.**]
https://www.medrxiv.org/content/10.1101/2020.05.06.20090449v1

- As the current SARS-CoV-2 pandemic continues, serological assays are urgently needed for rapid diagnosis, contact tracing and for epidemiological studies. So far, there is little data on how commercially available tests perform with real patient samples and if detected IgG antibodies provide protective immunity. Focusing on IgG antibodies, we demonstrate the performance of two ELISA assays (Euroimmun SARS-CoV-2 IgG & Vircell COVID-19 ELISA IgG) in comparison to one lateral flow assay ((LFA) FaStep COVID-19 IgG/IgM Rapid Test Device) and two in-house developed assays (immunofluorescence assay (IFA) and plaque reduction neutralization test (PRNT)). We tested follow up serum/plasma samples of individuals PCR-diagnosed with COVID-19. Most of the SARS-CoV-2 samples were from individuals with moderate to severe clinical course, who required an in-patient hospital stay. For all examined assays, the sensitivity ranged from 58.8 to 76.5% for the early phase of infection (days 5-9) and from 93.8 to 100% for the later period (days 10-18) after PCR-diagnosed with COVID-19. With exception of one sample, all positive tested samples in the analysed cohort, using the commercially available assays examined (including the in-house developed IFA), demonstrated neutralizing (protective) properties in the PRNT, indicating a potential protective immunity to SARS-CoV-2. Regarding specificity, there was evidence that samples of endemic coronavirus (HCoV-OC43, HCoV-229E) and Epstein Barr virus (EBV) infected individuals cross-reacted in the ELISA assays and IFA, in one case generating a false positive result (may giving a false sense of security). This need to be further investigated. [**note: more cross testing of antibody tests.**]
https://www.biorxiv.org/content/10.1101/2020.05.08.085506v1

>>

2020-05-12

Last week (I think it was, but since the days are a blur, I cannot be certain) I had Schubert's 'Death and the Maiden' string quartet. One of the movements of that is a paraphrase of the song by the same name. Here are Dietrich Fischer-Dieskau and his wife, Julia Varady performing the song: https://www.youtube.com/watch?v=DgvM0g4fGBE Fischer-Dieskau is of course well known for the numerous lieder recordings and here he is a more than decent pianist.

Here is a very interesting proposal from an Israeli group on how to reopen the economy. It exploits the virus's latency period so that workers would work four days one week and then a week off with the second group coming in the next week. It's a slow but steady increase in work and best of all it is EASY to understand!!! Money quote: "*The cyclic strategy should be part of a comprehensive exit strategy, including self-quarantine by those with symptoms, contact tracing and isolation, and protection of risk groups. The cyclic strategy can be tested in limited regions for specific trial periods, even a month. If infections rates grow, it can be adjusted to fewer work days. Conversely, if things are going well, additional work days can be added. In certain scenarios, only four or five lockdown days in each two-week cycle could still prevent resurgence*."

Some more news on hydroxychloroquine (HCQ) is in the Clinical Trials Section and there is still another small study showing some efficacy. Small studies really are not worth much in my opinion.

The estimable Derek Lowe weighs in on ivermectin.

An alert reader of the newsletter reminded me that WHO has weighed in on Human Challenge Studies for vaccines.

Here is a good communication on vaccine development from NIH and some others and also from Science an intriguing article on the issues related to digital disease surveillance. Personally, I think the issues discussed in the second paper can be managed and I've posted links in the past about how suitable encryption can be used to accomplish this. We need to use all the possible tools for public health to manage this outbreak and future ones. I always refer people to Thomas Pynchon's Proverbs for Paranoids from his fine novel 'Gravity's Rainbow'. I'm particularly fond of #3, "If they can get you asking the wrong questions, they don't have to worry about answers." [**note to self: maybe it's time to reread this fine book.**]

This doesn't fit in to any of my categories but I guess someone had to do this research. Who better than the Turks to get to the bottom of this morass. Bakcground: **YouTube is an important online source of information.** And its viewing numbers tend to increase exponentially in extraordinary situations. Our aim in this study was to evaluate the contents of the most frequently viewed YouTube videos during the COVID-19 pandemic. Method: In this study, contents of the most frequently viewed Turkish and English videos regarding COVID-19 pandemics are examined and scored with modified DISCERN, MICI and VPI. Results: The mean DISCERN score of Turkish videos is similar to English videos (2.55 and 2.43 respectively). Total MICI score tends to be higher in Turkish videos. 86.9% of all 168 videos and 65.2% of all 23 misleading videos were released by news channels. Average view counts, view ratios, and VPIs of misleading videos are higher than the useful videos. Conclusions: **Since there is not a peer-review system on YouTube, it is very important for the content of videos that are released through news channels to be accurate because the important messages can be spread among people in society through them. Especially some Turkish videos included many different rumors and faulty statements. During the extraordinary situations such as the pandemics, the videos of official health authorities and international institutions should be more visible in YouTube.**

MODELING

- In response to the COVID-19 pandemic, countries have implemented various strategies to reduce and slow the spread of the disease in the general population. For countries that have implemented restrictions on its population in a step-wise manner, monitoring of COVID-19 prevalence is of importance to guide decision on when to impose new, or when to abolish old, restrictions. We are here determining whether measures of odor intensity in a large sample can serve as one such measure. Online measures of how intense common household odors are perceived and symptoms of COVID-19 were collected from 2440 Swedes. Average odor intensity ratings were then compared to predicted COVID-19 population prevalence over time in the Swedish population and were found to closely track each other (r=-0.83). Moreover, we found that there was a large difference in rated intensity between individuals with and without COVID-19 symptoms and number of symptoms was related to odor intensity ratings. Finally, we found that individuals progressing from reporting no symptoms to subsequently reporting COVID-19

symptoms demonstrated a large drop in olfactory performance. These data suggest that measures of odor intensity, if obtained in a large and representative sample, can be used as an indicator of COVID-19 disease in the general population. Importantly, this simple measure could easily be implemented in countries without widespread access to COVID-19 testing or implemented as a fast early response before wide-spread testing can be facilitated. [**note: I have just developed a scratch and sniff card (patent pending) that will aid in the pre-screening diagnosis of SARS-CoV-2. I am now seeking venture funding for this effort which should make all my donors lots of money!! Joking aside, this is an interesting concept and I wonder what fragrance can be use. In the past I could have collected all the cologne and perfume adverts that came in the mail but those have largely stopped. Perhaps, there is a role for those types of sniff cards. You read it here first!!!!**]
https://www.medrxiv.org/content/10.1101/2020.05.07.20094516v1

- In anticipation of subsequent waves, reliable prediction of disease severity is essential for critical care capacity management and may enable earlier targeted interventions to improve patient outcomes. The purpose of this study is to develop and externally validate a prognostic model/clinical tool for predicting COVID-19 critical disease at presentation to medical care. Methods: This is a retrospective study of a prognostic model for the prediction of COVID-19 critical disease where critical disease was defined as ICU admission, ventilation, and/or death. The derivation cohort was used to develop a multivariable logistic regression model. Covariates included patient comorbidities, presenting vital signs, and laboratory values. Model performance was assessed on the validation cohort by concordance statistics. The model was developed with consecutive patients with COVID-19 who presented to University of California Irvine Medical Center in Orange County, California. External validation was performed with a random sample of patients with COVID-19 at Emory Healthcare in Atlanta, Georgia. Results: Of a total 3208 patients tested in the derivation cohort, 9% (299/3028) were positive for COVID-19. Clinical data including past medical history and presenting laboratory values were available for 29% (87/299) of patients (median age, 48 years [range, 21-88 years]; 64% [36/55] male). The most common comorbidities included obesity (37%, 31/87), hypertension (37%, 32/87), and diabetes (24%, 24/87). Critical disease was present in 24% (21/87). After backward stepwise selection, the following factors were associated with greatest increased risk of critical disease: number of comorbidities, body mass index, respiratory rate, white blood cell count, % lymphocytes, serum creatinine, lactate dehydrogenase, high sensitivity troponin I, ferritin, procalcitonin, and C-reactive protein. Of a total of 40 patients in the validation cohort (median age, 60 years [range, 27-88 years]; 55% [22/40] male), critical disease was present in 65% (26/40). Model discrimination in the validation cohort was high (concordance statistic: 0.94, 95% confidence interval 0.87-1.01). A web-based tool was developed to enable clinicians to input patient data and view likelihood of critical disease. Conclusions and Relevance: We present a model which accurately predicted COVID-19 critical disease risk using comorbidities and presenting vital signs and laboratory values, on derivation and validation cohorts from two different institutions. If further validated on additional cohorts of patients, this model/clinical tool may provide useful prognostication of critical care needs. [**note: this is a good idea. Try to collect as much information on past progressions to poor outcomes and develop a model that can be easily used to predict hospital needs. The chief worry is that prediction of needs can**

be overwhelmed if infection rates are very high in the community.]
https://www.medrxiv.org/content/10.1101/2020.05.06.20093435v1

- Background: Some key gaps in the understanding of SARS-CoV-2 infection remain. One of them is the contribution to transmission from individuals experiencing asymptomatic infections. We aimed to characterise the proportion and infectiousness of asymptomatic infections using data from the outbreak on the Diamond Princess cruise ship. Methods: We used a transmission model of COVID-19 with asymptomatic and presymptomatic states calibrated to outbreak data from the Diamond Princess, to quantify the contribution of asymptomatic infections to transmission. Data available included the date of symptom onset for symptomatic disease for passengers and crew, the number of symptom agnostic tests done each day, and date of positive test for asymptomatic and presymptomatic individuals. Findings: On the Diamond Princess 74% (70-78%) of infections proceeded asymptomatically, i.e. a 1:3.8 case-to-infection ratio. Despite the intense testing 53%, (51-56%) of infections remained undetected, most of them asymptomatic. Asymptomatic individuals were the source for 69% (20-85%) of all infections. While the data did not allow identification of the infectiousness of asymptomatic infections, assuming no or low infectiousness resulted in posterior estimates for the net reproduction number of an individual progressing through presymptomatic and symptomatic stages in excess of 15. Interpretation: *Asymptomatic SARS-CoV-2 infections may contribute substantially to transmission. This is essential to consider for countries when assessing the potential effectiveness of ongoing control measures to contain COVID-19*. [**note: any abstract with 'Diamond Princess' in it automatically gets published in this newsletter!!!**]
https://www.medrxiv.org/content/10.1101/2020.05.07.20093849v1

NEWLY REGISTERED CLINICAL TRIALS

- This is just a phase 1 study for plitidepsin, a drug I have never heard of. It's been approved in Australia for multiple myeloma. NCT04382066
- I think this may be the second trial for atorvastatin but any port in a storm. NCT04380402
- A Turkish trial proposed to look at the flavonoid, quercetin (regulated as a dietary supplement). NCT04377789
- I'm not sure this trial will go anywhere but it is the first one we have seen from Sudan so let's cite it. To study the efficacy of Gum Arabic as an immuno modulator and anti-inflammatory agent among COVID 19 seropostive patients..Half of participants will receive Gum Arabic and the other half will receive placebo NCT04381871

CLINICAL TRIAL RESULTS

- Oh, Oh, the HCQ fans are going to be very disappointed today. A large retrospective study was published in JAMA yesterday. In a retrospective cohort study of 1438 patients hospitalized in metropolitan New York, compared with treatment with neither drug, the adjusted hazard ratio for in-hospital mortality for treatment with hydroxychloroquine alone was 1.08, for azithromycin alone was 0.56, and for combined hydroxychloroquine and azithromycin was 1.35. None of these hazard ratios were statistically significant. *Among patients hospitalized with COVID-19, treatment with hydroxychloroquine, azithromycin, or both was not associated with significantly lower in-hospital mortality*. [**note: on to Plan B -> wait, you mean there is no plan B? Clearly we need to wait for the controlled trial results but the mounting observational**

evidence is not good. We don't even know if it has any prophylactic value and as I've been tirelessly saying there are likely some drugs out there that will never enter trials because of this over emphasis.] https://jamanetwork.com/journals/jama/fullarticle/2766117 and even the Saudis do not find HCQ to be efficacious in a small study https://www.medrxiv.org/content/10.1101/2020.05.08.20095679v1 but wait, there's more!! This small French hospital study suggests that HCQ+azithromycin does work https://www.medrxiv.org/content/10.1101/2020.05.05.20088757v1.full.pdf [**note: the French authors do list a number of qualifications for this study.**]

- This is curious and perhaps good news. This was a multicentre, prospective, open-label, randomised, phase 2 trial in adults with COVID-19 who were admitted to six hospitals in Hong Kong. Patients were randomly assigned (2:1) to a 14-day combination of lopinavir 400 mg and ritonavir 100 mg every 12 h, ribavirin 400 mg every 12 h, and three doses of 8 million international units of interferon beta-1b on alternate days (combination group) or to 14 days of lopinavir 400 mg and ritonavir 100 mg every 12 h (control group). The primary endpoint was the time to providing a nasopharyngeal swab negative for severe acute respiratory syndrome coronavirus 2 RT-PCR, and was done in the intention-to-treat population. The study is registered with ClinicalTrials.gov, NCT04276688. Between Feb 10 and March 20, 2020, 127 patients were recruited; 86 were randomly assigned to the combination group and 41 were assigned to the control group. The median number of days from symptom onset to start of study treatment was 5 days (IQR 3–7). The combination group had a significantly shorter median time from start of study treatment to negative nasopharyngeal swab (7 days [IQR 5–11]) than the control group (12 days [8–15]; hazard ratio 4·37 [95% CI 1·86–10·24], p=0·0010). Adverse events included self-limited nausea and diarrhoea with no difference between the two groups. One patient in the control group discontinued lopinavir–ritonavir because of biochemical hepatitis. No patients died during the study. [**note: it's a small number of patients and runs counter to the already published study that lopinavir/ritonavir did not work alone. I've been saying for some time that a combination therapy is best and maybe this is one step along the way to that confirmation. Is this better than remdesivir? Is it better than HCQ±azithromycin? Your guess is as good as mine.**] https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)31042-4/fulltext
- One of the features distinguishing SARS-CoV-2 from its more pathogenic counterpart SARS-CoV is the presence of premature stop codons in its ORF3b gene. Here, we show that SARS-CoV-2 ORF3b is a potent interferon antagonist, suppressing the induction of type I interferon more efficiently than its SARS-CoV ortholog. Phylogenetic analyses and functional assays revealed that SARS-CoV-2-related viruses from bats and pangolins also encode truncated ORF3b gene products with strong anti-interferon activity. Furthermore, analyses of more than 15,000 SARS-CoV-2 sequences identified a natural variant, in which a longer ORF3b reading frame was reconstituted. This variant was isolated from two patients with severe disease and further increased the ability of ORF3b to suppress interferon induction. Thus, our findings not only help to explain the poor interferon response in COVID-19 patients, but also describe a possibility of the emergence of natural SARS-CoV-2 quasispecies with extended ORF3b that may exacerbate COVID-19 symptoms. [**note: whether this variant has any survival advantage remains to be seen. We know that viral genes are subject to mutation (otherwise we wouldn't have**

epidemics) but they have to survive and multiply to cause havoc.]
https://www.biorxiv.org/content/10.1101/2020.05.11.088179v1

- Background The management of healthcare workers (HCWs) exposed to confirmed cases of COVID-19 is still a matter of debate. It is unclear whether these subjects should be tested in the absence of symptoms and if those can guide diagnosis. Methods Occupational and clinical characteristics of all the consecutive HCWs who performed a nasopharyngeal swab for the detection of SARS-CoV-2 in a University Hospital from February 24, 2020, to March 31, 2020, were collected. Frequencies of positive tests were compared according to selected variables. Multivariable logistic regression analyses were then applied. Findings Positive tests were 138 among 1,573 HCWs (8.8%, 95% confidence interval [CI]: 7.4-10.3), with a marked difference between symptomatic (20.2%, 95%CI: 16.7-24.1) and asymptomatic (3.7%, 95%CI: 2.7-5.1) subjects (p<0.001). Physicians were the group with the highest frequency of positive tests (10.6%, 95%CI: 8.3-13.4) whereas clerical workers and technicians displayed the lowest frequency (2.9%, 95%CI: 0.8-7.3). The likelihood of being positive increased with the number of reported symptoms and the strongest predictors of a positive test were taste and smell alterations (odds ratio [OR]= 29.7) and fever (OR = 7.21). The median time from first positive test to a negative test was 23 days (95%CI: 19-24). Interpretation In this Italian group of HCWs exposed to confirmed cases of COVID-19 the presence of symptoms, especially taste and smell alterations and fever, was associated with SARS-CoV-2 infection. The median time to clear the virus from nasopharynx was 23 days. [**note: interesting study of healthcare workers in Lombardy. Viral clearance from the nasopharynx was long.**]
https://www.medrxiv.org/content/10.1101/2020.05.07.20094276v1
- The novel respiratory disease COVID-19 produces varying symptoms, with fever, cough, and shortness of breath being common. In older adults, we found that pre-existing dementia is a major risk factor (OR = 3.07, 95% CI: 1.71 to 5.50) for COVID-19 hospitalization in the UK Biobank (UKB). In another UK study of 16,749 patients hospitalized for COVID-19, dementia was among the common comorbidities and was associated with higher mortality. Additionally, impaired consciousness, including delirium, is common in severe cases. The ApoE e4 genotype is associated with both dementia and delirium, with the e4e4 (homozygous) genotype associated with high risk of dementia. We therefore aimed to test associations between ApoE e4 alleles and COVID-19 severity, using the UKB data. [**note: more interesting information on the genetic predisposition for adverse outcome. The paper is worth reading as it provides more background.**] https://www.medrxiv.org/content/10.1101/2020.05.07.20094409v1

DRUG DEVELOPMENT

- The development of countermeasures to prevent and/or treat COVID-19 is a global health priority. In under 7 weeks, we enrolled a cohort of SARS-CoV-2-recovered participants, developed neutralization assays to interrogate serum and monoclonal antibody responses, adapted our high throughput antibody isolation, production and characterization pipeline to rapidly screen over 1000 antigen-specific antibodies, and established an animal model to test protection. We report multiple highly potent neutralizing antibodies (nAbs) and show that passive transfer of a nAb provides protection against high-dose SARS-CoV-2 challenge in Syrian hamsters. The study suggests a role for nAbs in prophylaxis, and potentially therapy, of COVID-19. The nAbs define protective epitopes to guide vaccine design. [**note: more good work on the**

isolation of neutralizing antibodies from recovered patient's sera. This will help in vaccine design and mAb production. As an aside, I spent to summers working at Scripps Research Institute way back in 1969-70.]
https://www.biorxiv.org/content/10.1101/2020.05.11.088674v1

- ACE2, in concert with the protease TMPRSS2, binds the novel coronavirus SARS-CoV-2 and facilitates its cellular entry. The ACE2 gene is expressed in SARS-CoV-2 target cells, including Type II Pneumocytes (Ziegler, 2020), and is activated by interferons. Viral RNA was also detected in breast milk (Wu et al., 2020), raising the possibility that ACE2 expression is under the control of cytokines through the JAK-STAT pathway. Here we show that Ace2 expression in mammary tissue is induced during pregnancy and lactation, which coincides with the establishment of a candidate enhancer. The prolactin-activated transcription factor STAT5 binds to tandem sites that coincide with activating histone enhancer marks and additional transcription components. The presence of pan JAK-STAT components in mammary alveolar cells and in Type II Pneumocytes combined with the autoregulation of both STAT1 and STAT5 suggests a prominent role of cytokine signaling pathways in cells targeted by SARS-CoV-2. [**note: more on the interesting biology of the ACE2 expression pathway.**]
https://www.biorxiv.org/content/10.1101/2020.05.11.089045v1

DIAGNOSTIC DEVELOPMENT

- Background The antibody response generated following infection with SARS-CoV-2 is expected to decline over time. This may cause individuals with confirmed SARS-CoV-2 infection to test negative according to serological diagnostic tests in the months and years following symptom onset. Methods A multiplex serological assay was developed to measure IgG and IgM antibody responses to four SARS-CoV-2 Spike (S) antigens: spike trimeric ectodomain (Stri), its receptor-binding domain (RBD), spike subunit 1 (S1), and spike subunit 2 (S2). Antibody responses were measured in serum samples from patients in French hospitals with RT-qPCR confirmed infection (n = 259), and negative control serum samples collected before the start of the SARS-CoV-2 epidemic in 2019 (n = 335). The multiplex antibody data was used to train a random forests algorithm for classifying individuals with previous SARS-CoV-2 infection. A mathematical model of antibody kinetics informed by prior information from other coronaviruses was used to estimate time-varying antibody responses and assess the potential sensitivity and classification performance of serological diagnostics during the first year following symptom onset. Results IgG antibody responses to one S antigen identified individuals with previous RT-qPCR confirmed SARS-CoV-2 infection with 90.3% sensitivity (95% confidence interval (CI); 86.1%, 93.4%) and 99.1% specificity (95% CI; 97.4%, 99.7%). Using a serological signature of IgG to four antigens, it was possible to identify infected individuals with 96.1% sensitivity (95% CI; 93.0%, 97.9%) and 99.1% specificity (95% CI; 97.4%, 99.7%). Antibody responses to SARS-CoV-2 increase rapidly 1-2 weeks after symptom onset, with antibody responses predicted to peak within 2-4 weeks. Informed by prior data from other coronaviruses, one year following symptom onset antibody responses are predicted to decay by approximately 60% from the peak response. Depending on the selection of sero-positivity cutoff, we estimate that the sensitivity of serological diagnostics may reduce to 56%-97% after six months, and to 49%-93% after one year. Conclusion Serological signatures based on antibody responses to multiple antigens can provide more accurate and robust serological classification of individuals with previous SARS-CoV-2 infection. Changes in

antibody levels over time may cause reductions in the sensitivity of serological diagnostics leading to an underestimation of sero-prevalence. It is essential that data continue to be collected to evaluate this potential risk. [**note: I'm not sure about how important this is. Most serological markers to viruses tend to go down over time but the immune system is still able to respond to reinfection. Perhaps this is more of a cautionary note. I've long felt that routine SARS-CoV-2 antibody testing should be done as the part of all blood draws to better generate this type of information.**] https://www.medrxiv.org/content/10.1101/2020.05.07.20093963v1

- Rapid, reliable, and widespread testing is required to curtail the ongoing COVID-19 pandemic. Current gold standard diagnostic assays are hampered by supply shortages in critical reagents including nasal swabs, RNA extraction kits, personal protective equipment (PPE), instrumentation, and labor. Here we present an approach to overcome these challenges with the development of a rapid colorimetric assay using reverse-transcription loop-mediated isothermal amplification (RT-LAMP) optimized on human saliva samples without an RNA purification step. We describe our optimizations of the LAMP reaction and saliva pre-treatment protocols that enabled rapid and sensitive detection of < 10^2 viral genomes per reaction in contrived saliva controls. We also observed high performance of this assay on a limited number of clinical saliva samples. While thorough validation on additional clinical samples are needed before such an assay can be widely used, these preliminary results demonstrate a promising approach to overcome the current bottlenecks limiting widespread testing. [**note: the authors have filed for a patent on this! Get it developed and deployed.**] https://www.medrxiv.org/content/10.1101/2020.05.07.20093542v1

>>

2020-05-13

Franz Liszt wrote three songs to poems of Petrarch (Liszt was the rock stat of his generation as those who have had the good fortune to have seen Ken Russell's art house classic, 'Lisztomania' staring Who bandmember Roger Daltrey as the composer). Here is the fine American tenor Lawrence Brownlee singing Sonnet 104, 'Pace non trovo': https://www.youtube.com/watch?v=c8ii2cUAje0

As most of you know one of the symptoms of SARS-CoV-2 is loss of smell. I was serious yesterday when I talked about filing a patent for a quick COVID-19 Scent Strip!! My provisional application was sent in shortly after getting the newsletter out and I'm ready to repurpose all those damn fragrance strips that come in the mail – Oh wait, they are not coming in the mail these days with all the store closures. If any of you see some deceptive claims for COVID-19 sniff tests do let me know, my lawyers stand ready to sue. [I really did file the provisional application and have 365 days to convert it to a full patent. We'll see what happens but I don't have any pretensions of getting rich off of this one.] **But wait, there's more** – this New York Times story discusses development of a smart phone app and how loss of taste and smell is one of the top predictors!! Time to apply for a trademark for the COVID-19 Scent Strip. Is an IPO in the works for this to be established company? *I think I will take the proactive step of issuing each of my loyal readers a share of stock!!*

The Washington Post has a nice story on how the veterinary lab at Oklahoma State made a quick pivot and turned to testing human SARS-CoV-2 samples. I have pointed out before that a large number of labs

can be repurposed to aid in this fight. The CLIA regulations governing lab accreditation need to be altered to streamline this process.

I always love DIY projects. Here is one from University of Wisconsin docs and engineers who crafted a novel box for aerosol and droplet guarding. Some really cool pictures in the paper. Here, we report on the design, construction, and testing of the BADGER (Box for Aerosol and Droplet Guarding and Evacuation in Respiratory Infection), an affordable, scalable device that can contain droplets and aerosol particles. The BADGER has multiple hand-ports for healthcare providers to perform essential tasks on a patient's airway and head. A semi-sealed environment is created inside the BADGER, which maintains at least twelve-air changes per hour using in-wall vacuum suction. Qualitative testing demonstrates that under conditions typical of non-invasive supplemental oxygen treatment, the BADGER can contain nebulized denatonium benzoate and smoke. Quantitative aerosol testing shows more than 90% containment of sub-micrometer aerosolized particles. Overall, the BADGER has the potential to contain large droplets and small airborne particles, and to provide an additional layer of protection for healthcare providers treating COVID-19 patients.
https://www.medrxiv.org/content/10.1101/2020.05.09.20096032v1

Derek Lowe weighs in on traditional Chinese medicine. It's not a pretty picture.

MODELING

- This study quantifies the relationship between school closure timing and COVID-19 deaths and cases in the general population in all U.S. states. COVID-19 has higher symptomatic infection rates among the elderly, suggesting school closures could be unrelated to transmission. However, predicting daily cumulative COVID-19 deaths by state, earlier school closure is related to fewer deaths per capita and slower growth in deaths per capita. Results are similar for COVID-19 cases per capita. [**note: I only include this one as my elder daughter is a special ed schoolteacher and concerned about the transmissibility of SARS-CoV-2. Her school is now closed for this term and she is doing Zoom student meetings and assessments.**]
https://www.medrxiv.org/content/10.1101/2020.05.09.20096594v1
- Background The reported crude case-fatality rates (CFRs) vary widely between countries. The serious limitations of using crude rates for comparisons are sometimes overlooked. In this paper we examined to what extent the age distribution of the cases is responsible for the differences in CFRs between countries. Methods Data on COVID-19 were extracted from the reports of individual countries. Overall and age-specific CFRs were available for six countries. The CFRs by country were adjusted for age using the direct method, using the combined age-specific number of cases of all six countries as the standard population. Findings The age distribution of the cases varied widely between countries. The crude CFRs varied between 1.6% and 11%. The differences in the age-specific CFRs were much smaller and the age-adjusted rates were much closer than the crude rates. The ratio of the crude CFR for the country with the highest to that with the lowest, was reduced substantially from 7.4 to 2.3 for the age-adjusted rates. Conclusions The age structure of the cases dramatically impacts on the differences in the crude CFRs between countries. Adjusting for age substantially reduces this variation. Other factors such as the differences in the definition of the denominators, the definition of a case and the standard of

healthcare are likely to account for much of the residual variation. It is misleading to compare the crude COVID-19 CFRs between countries and should be avoided. Comparisons should be based on age-specific and age-adjusted rates. [**note: another way of looking at the age-related mortality. Using this ratio approach mortalities are similar with less variation.**] https://www.medrxiv.org/content/10.1101/2020.05.09.20096503v1

- At room temperature, SARS-CoV-2 was stable on environmental surfaces and remained viable up to 7 days on smooth surfaces. This virus could survive for several hours in feces and 3-4 days in urine. [**note: this is a short paper and worth reading. Recoverable viral activity decayed pretty quickly on cotton and paper surfaces (yes, those natural fabrics are best and your loyal fashionista *only* wears the finest cotton shirts. Too bad the researchers didn't look at superfine spun Italian worsted woolen. You won't find me wearing any suit that is not of this fabric. All kidding aside, remember that this and other studies are laboratory based analyses and real world survival is affected by lots of different parameters.**] https://www.medrxiv.org/content/10.1101/2020.05.07.20094805v1
- New emerging infections have no known treatment. Assessing potential drugs for safety and efficacy enables clinicians to make evidence-based treatment decisions, and contributes to overall outbreak control. However, it is difficult to launch clinical trials in the unpredictable environment of an outbreak. We conducted a bibliometric systematic review for the 2009 influenza pandemic to determine the speed, and quality of evidence generation for treatments. This informs approaches to high-quality evidence generation in this and future pandemics. Methods: We searched PubMed for all clinical data (including clinical trial, observational and case series) describing treatment for patients with influenza A(H1N1)pdm09 and ClinicalTrials.gov for research that aimed to enrol patients with the disease. Results: 33869 treatment courses for patients hospitalised with A(H1N1)pdm09 were detailed in 160 publications. Most were retrospective observational studies or case series. 592 patients received treatment (or placebo) as participants in a registered interventional clinical trial with results publicly available. None of these registered trial results were available during the timeframe of the pandemic, and the median date of publication was 213 days after the Public Health Emergency of International Concern ended. Conclusion: Patients were frequently treated for pandemic influenza with drugs not registered for this indication, but rarely under circumstances of high-quality data capture. The result was a reliance on use under compassionate circumstances, resulting in continued uncertainty regarding the potential benefits and harms of anti-viral treatment. Rapid scaling of clinical trials is critical for generating a quality evidence base during pandemics. [**note: the obviousness of this paper cannot be overstressed. High quality data from well-designed trials is always the best approach but coordination to maximize clinical trial capability is also important.**] https://www.medrxiv.org/content/10.1101/2020.05.07.20094839v1
- We report preliminary results from the Vall dHebron prospective cohort study at Vall dHebron University Hospital, in Barcelona (Spain), including all consecutive patients who had a confirmed infection with the severe acute respiratory syndrome coronavirus2 (SARSCoV2) and who were treated with tocilizumab until March 25th. The primary endpoint was mortality at 7 days after tocilizumab administration. Secondary endpoints were admission to the intensive care unit, development of ARDS and respiratory insufficiency among others. Results: 82 patients with COVID19 received at least one dose of tocilizumab. The mean (SD) age was 59.1 (19.8) years,

63% were male, 22% were of non Spanish ancestry, and the median (IQR) ageadjusted Charlson index at baseline was 3 (1 to 4) points. Respiratory failure and ARDS developed in 62 (75.6%) and 45 (54.9%) patients, respectively. Median time from symptom onset to ARDS development was 8 (5 to 11) days. The median time from symptom onset to the first dose of tocilizumab was 9 (7 to 11) days. Mortality at 7 days was 26.8%. Hazard ratio for mortality was 3.3; 95% CI, 1.3 to 8.5 (age adjusted hazard ratio for mortality 2.1; 95% CI, 0.8 to 5.8) if tocilizumab was administered after the onset of ARDS. Conclusion: Time from lung injury onset to tocilizumab administration may be critical to patient recovery. Our preliminary data could inform bedside decisions until more data from clinical trials becomes available. [**note: this paper really does not provide much useful information on tocilizumab. All the patients had co-morbidities and 98% were already on other treatment drugs. While I applaud early publication of results, clinicians have to be careful in adjusting treatment regimens if the data is not supportive.**]
https://www.medrxiv.org/content/10.1101/2020.05.07.20094599v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- Coronavirus disease 2019 (COVID-19) mortality is associated with hypoxaemia, multiorgan failure, and thromboinflammation. However severity of disease varies considerably and understanding physiological changes that may link to poor outcomes is important. Although increased serum ferritin has been observed in COVID-19 patients consistent with inflammation, other iron parameters have not been examined to our knowledge. Because iron is required for immunity and oxygen utilisation, and dysregulated iron homeostasis has been observed in COPD, we investigated serum iron concentrations in 30 patients with COVID-19 requiring ICU admission. All patients had low serum iron but patients with severe hypoxemic respiratory failure had more profound hypoferraemia. The area under the curve for receiver operating characteristic curves for serum iron to identify severe hypoxemia was 0.95; the optimal Youden Index for distinguishing between severe and non-severe hypoxemia was a serum iron concentration of 2.9 micromol/L. By linear regression, serum iron was associated with lymphocyte count and PaO2/FiO2. In conclusion, profound hypoferraemia identifies COVID-19 patients with severe hypoxaemia. Serum iron is a simple biomarker that could be usefully employed to stratify patients and monitor disease. Severe hypoferraemia may plausibly impair critical iron-dependent processes such as lymphocyte responses and hypoxia sensing, contributing to pathology, and is potentially treatable. [**note: serum iron is yet another biomarker to be used for looking disease progression.**]
https://www.medrxiv.org/content/10.1101/2020.05.11.20092114v1
- Importance: Vitamin D treatment has been found to decrease incidence of viral respiratory tract infection, especially in vitamin D deficiency. It is unknown whether COVID-19 incidence is associated with vitamin D deficiency and treatment. Objective: To examine whether vitamin D deficiency and treatment are associated with testing positive for COVID-19. Design: Retrospective cohort study Setting: University of Chicago Medicine Participants: Patients tested for COVID-19 from 3/3/2020-4/10/2020. Vitamin D deficiency was defined by the most recent 25-hydroxycholecalciferol <20ng/ml or 1,25-dihydroxycholecalciferol <18pg/ml within 1 year

before COVID-19 testing. Treatment was defined by the most recent vitamin D type and dose, and treatment changes between the time of the most recent vitamin D level and time of COVID-19 testing. Vitamin D deficiency and treatment changes were combined to categorize vitamin D status at the time of COVID-19 testing as likely deficient(last-level-deficient/treatment-not-increased), likely sufficient(last-level-not-deficient/treatment-not-decreased), or uncertain deficiency(last-level-deficient/treatment-increased or last-level-not-deficient/treatment-decreased). Main Outcomes and Measures: The main outcome was testing positive for COVID-19. Multivariable analysis tested whether the most recent vitamin D level and treatment changes after that level were associated with testing positive for COVID-19 controlling for demographic and comorbidity indicators. Bivariate analyses of associations of treatment with vitamin D deficiency and COVID-19 were performed. Results: Among 4,314 patients tested for COVID-19, 499 had a vitamin D level in the year before testing. Vitamin D status at the time of COVID-19 testing was categorized as likely deficient for 127(25%) patients, likely sufficient for 291(58%) patients, and uncertain for 81(16%) patients. In multivariate analysis, testing positive for COVID-19 was associated with increasing age(RR(age<50)=1.05,p<0.021;RR(age≥50)=1.02,p<0.064)), non-white race(RR=2.54,p<0.01) and being likely vitamin D deficient (deficient/treatment-not-increased:RR=1.77,p<0.02) as compared to likely vitamin D sufficient(not-deficient/treatment-not-decreased), with predicted COVID-19 rates in the vitamin D deficient group of 21.6%(95%CI[14.0%-29.2%]) versus 12.2%(95%CI[8.9%-15.4%]) in the vitamin D sufficient group. Vitamin D deficiency declined with increasing vitamin D dose, especially of vitamin D3. Vitamin D dose was not significantly associated with testing positive for COVID-19. Conclusions and Relevance: Vitamin D deficiency that is not sufficiently treated is associated with COVID-19 risk. Testing and treatment for vitamin D deficiency to address COVID-19 warrant aggressive pursuit and study. [**note: more on Vitamin D deficiency. I wonder what the population prevalence of Vitamin D deficiency is. More on Vitamin D. Do we all need to up our intake of this Vitamin? How much sunshine do we need?**] https://www.medrxiv.org/content/10.1101/2020.05.08.20095893v1

- We aim to decipher SARS-CoV-2 infected cell types, the consequent host immune response and their interplay in the lung of COVID-19 patients. Design: We analyzed single-cell RNA sequencing (scRNA-seq) data of lung samples from 17 subjects (6 severe COVID-19 patients, 3 mild patients who recovered and 8 healthy controls). The expression of SARS-CoV-2 receptors (ACE2 and TMPRSS2) was examined among different cell types in the lung. The immune cells infiltration patterns, their gene expression profiles, and the interplay of immune cells and SARS-CoV-2 target cells were further investigated. Results: Compared to healthy controls, the overall ACE2 (receptor of SARS-CoV-2) expression was significantly higher in lung epithelial cells of COVID-19 patients, in particular in ciliated cell, club cell and basal cell. Comparative transcriptome analysis of these lung epithelial cells of COVID-19 patients and healthy controls identified that SARS-CoV-2 infection activated pro-inflammatory signaling including interferon pathway and cytokine signaling. Moreover, we identified dysregulation of immune response in patients with COVID-19. In severe COVID-19 patients, significantly higher neutrophil, but lower T and NK cells in lung were observed along with markedly increased cytokines (CCL2, CCL3, CCL4, CCL7, CCL3L1 and CCL4L2) compared with healthy controls as well as mild patients who recovered. The cytotoxic phenotypes were shown in lung T and NK cells of severe patients as evidenced by enhanced IFNγ, Granulysin, Granzyme B and Perforin expression. Moreover, SARS-CoV-2 infection altered

the community interplay of lung epithelial cells and immune cells: the interaction between epithelial cells with macrophage, T and NK cell was stronger, but their interaction with neutrophils was lost in COVID-19 patients compared to healthy controls. Conclusions: SARS-CoV-2 infection activates pro-inflammatory signaling in lung epithelial cells expressing ACE2 and causes dysregulation of immune response to release more pro-inflammatory cytokines. Moreover, SARS-CoV-2 infection breaks the interplay of lung epithelial cells and immune cells. [**note: more evidence on the linkage of ACE2 and dysregulation of the immune response.**] https://www.medrxiv.org/content/10.1101/2020.05.08.20096024v1

- Patients (n=25) with severe and/or life-threatening COVID-19 disease were enrolled at the Houston Methodist hospitals from March 28 to April 14, 2020. Patients were transfused with convalescent plasma obtained from donors with confirmed SARS-CoV-2 infection and had been symptom free for 14 days. The primary study outcome was safety, and the secondary outcome was clinical status at day 14 post-transfusion. Clinical improvement was assessed based on a modified World Health Organization 6-point ordinal scale and laboratory parameters. Viral genome sequencing was performed on donor and recipient strains. Results: At baseline, all patients were receiving supportive care, including anti-inflammatory and anti-viral treatments, and all patients were on oxygen support. At day 7 post-transfusion with convalescent plasma, nine patients had at least a 1-point improvement in clinical scale, and seven of those were discharged. By day 14 post-transfusion, 19 (76%) patients had at least a 1-point improvement in clinical status and 11 were discharged. No adverse events as a result of plasma transfusion were observed. The whole genome sequencing data did not identify a strain genotype-disease severity correlation. Conclusions: The data indicate that administration of convalescent plasma is a safe treatment option for those with severe COVID-19 disease. Randomized, controlled trials are needed to determine its efficacy. [**note: this is the first paper on the use of convalescent plasma. Results are encouraging and point to the need for large scale mAb production as a therapeutic modality.**] https://www.medrxiv.org/content/10.1101/2020.05.08.20095471v1
- In this retrospective study, we aimed to evaluate determinants of the prognosis of the disease in 70 patients with COVID-19 severe pneumonia (i.e. requiring at least 3 liters of oxygen) hospitalized between 10 March and 9 April, 2020, in the Centre Hospitalier Alpes Leman, France. The main outcome was oro-tracheal intubation and the exposure of interest was corticotherapy. Since this was not a randomized trial, we used propensity score matching to estimate average treatment effect. Results. There was evidence that corticotherapy lowered the risk of intubation with a risk difference of -47.1% (95% confidence interval -71.8% to -22.5%). Conclusion. Corticosteroid, a well-known, easily available, and cheap treatment, could be an important tool in management of severe COVID-19 patients with respiratory failure. Not only could it provide an individual benefit, but also, in the setting of the COVID-19 ongoing pandemic, lower the burden on our vulnerable healthcare systems. [**note: more data on the utility of corticosteroid therapy and intubation.**] https://www.medrxiv.org/content/10.1101/2020.05.08.20094755v1

DRUG DEVELOPMENT

- Drug repositioning represents an effective way to control the current COVID-19 pandemic. Previously, we identified 24 FDA-approved drugs which exhibited substantial antiviral effect against SARS-CoV-2 in Vero cells. Since antiviral efficacy could be altered in different cell lines, we developed an antiviral screening assay with human lung cells, which is more appropriate

than Vero cell. Comparative analysis of antiviral activities revealed that nafamostat is the most potent drug in human lung cells (IC50 = 0.0022μM). [**note: this is a very useful and short paper. The Korean scientists use a human lung cell line and compare the *in vitro* results to the established Vero cell assays that many researchers have used. The potency of nafamostat is striking and more active than remdesivir. Care to guess how many clinical trials are registered for this drug??? ONE according to the NIH database. This is not the first time nafamostat has come up in a repurposing search.**]
https://www.biorxiv.org/content/10.1101/2020.05.12.090035v1

- SARS-CoV-2 infection occurs through binding and internalization of the viral spike protein to angiotensin converting enzyme 2 (ACE2) on the host cell membrane. Lethal complications are caused by damage and failure of vital organs that express high levels of ACE2, including the lungs, the heart and the kidneys. Here, we established a high-throughput drug screening strategy to identify therapeutic candidates that reduce ACE2 levels in human embryonic stem cell (hESC) derived cardiac cells. Drug target analysis of validated hit compounds, including 5 alpha reductase inhibitors, revealed androgen signaling as a key modulator of ACE2 levels. Treatment with the 5 alpha reductase inhibitor dutasteride reduced ACE2 levels and internalization of recombinant spike receptor binding domain (Spike-RBD) in hESC-derived cardiac cells and human alveolar epithelial cells. Finally, clinical data on coronavirus disease 2019 (COVID-19) patients demonstrated that abnormal androgen states are significantly associated with severe disease complications and cardiac injury as measured by blood troponin T levels. These findings provide important insights on the mechanism of increased disease susceptibility in male COVID-19 patients and identify androgen receptor inhibition as a potential therapeutic strategy. https://www.biorxiv.org/content/10.1101/2020.05.12.091082v1
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other SARS-like-CoVs encode 3 tandem macrodomains within non-structural protein 3 (nsp3). The first of these macrodomains, termed Mac1, is conserved throughout CoVs, binds to mono- and poly-ADP-ribose, and hydrolyzes mono-ADP-ribose (MAR) from target proteins. Mac1 is essential for pathogenesis in multiple animal models of CoV infection, implicating this domain as a prominent virulence factor and potential therapeutic target. Mac1 likely counters host-mediated antiviral ADP-ribosylation, a posttranslational modification that is part of the host response to viral infections. Here we report the crystal structure of SARS-CoV-2 Mac1 in complex with ADP-ribose refined at 2.2 Å resolution. SARS-CoV-2, SARS-CoV and MERS-CoV Mac1 exhibit similar structural folds and ADP-ribose binding modes as shown by structural comparison. All three CoV Mac1 proteins bound to ADP-ribose with low μM affinities. They also demonstrated highly efficient de-MARylating activity, which was greater than that of the human Mdo2 macrodomain. We conclude that the SARS-CoV-2 and other CoV Mac1 proteins are highly efficient ADP-ribosylhydrolases with strikingly similar activity, indicating that compounds targeting CoV Mac1 proteins may have broad antiviral activity against CoVs. [**note: another potential drug target.**]
https://www.biorxiv.org/content/10.1101/2020.05.11.089375v1
- Preliminary data on SARS-CoV-2 infection suggests that some immunocompromised hosts experience worse outcomes. We performed a retrospective matched cohort study to characterize outcomes in HIV-positive patients with SARS-CoV-2 infection. Methods: Leveraging data collected from electronic medical records for all patients hospitalized at NYU Langone Health with COVID-19 between March 2, 2020 and April 23, 2020, we matched 21 HIV-positive

patients to 42 non-HIV patients using a greedy nearest neighbor algorithm. Admission characteristics, laboratory results, and hospital outcomes were recorded and compared between the two groups. Results: While there was a trend toward increased rates of ICU admission, mechanical ventilation, and mortality in HIV-positive patients, these differences were not statistically significant. Rates for these outcomes in our cohort are similar to those previously published for all patients hospitalized with COVID-19. HIV-positive patients had significantly higher admission and peak CRP values. Other inflammatory markers did not differ significantly between groups, though HIV-positive patients tended to have higher peak values during their clinical course. Three HIV-positive patients had superimposed bacterial pneumonia with positive sputum cultures, and all three patients expired during hospitalization. There was no difference in frequency of thrombotic events or myocardial infarction between these groups. Conclusion: This study provides evidence that HIV coinfection does not significantly impact presentation, hospital course, or outcomes of patients infected with SARS-CoV-2, when compared to matched non-HIV patients. A larger study is required to determine if the trends we observed apply to all HIV-positive patients. **[note: this is the first breakdown I have seen about HIV positive patients. According to the paper all of them were on anti-retroviral therapy. Whether this was protective is still not known but the fact that there was no significant difference in outcomes compared to non-HIV positive patients is interesting.**]
https://www.medrxiv.org/content/10.1101/2020.05.07.20094797v1

DIAGNOSTIC DEVELOPMENT

- SARS-CoV-2 has emerged as a previously unknown zoonotic coronavirus that spread worldwide causing a serious pandemic. While reliable nucleic acid-based diagnostic assays were rapidly available, there exists only a limited number of validated serological assays. Here, we evaluated a novel flow cytometric approach based on antigen-expressing HEK 293T cells to assess spike-specific IgG and IgM antibody responses. Analyses of 201 pre-COVID-19 sera proved a high assay specificity in comparison to commercially available CLIA and ELISA systems, while also revealing the highest sensitivity in specimens from PCR-confirmed SARS-CoV-2 infected patients. Additionally, a soluble Angiotensin-Converting-Enzyme 2 (ACE-2) variant was established as external standard to quantify spike-specific antibody responses on different assay platforms. In conclusion, our newly established flow cytometric assay allows sensitive and quantitative detection of SARS-CoV-2-specific antibodies, which can be easily adopted in different laboratories and does not rely on external supply of assay kits. [**note: another approach to SARS-CoV-2 antibody detection.**]
https://www.medrxiv.org/content/10.1101/2020.05.09.20091447v1
- Serologic assays are needed to determine SARS-CoV-2 seroprevalence, but poor specificity can overestimate exposures. Here, we built a pan-human coronavirus proteome-wide programmable phage display assay (VirScan) to profile coronavirus antigens specifically enriched by 20 COVID-19 patient serum IgG. With ReScan, a new diagnostic development workflow which combines the isolation of phage expressing the most immunogenic peptides with paper-based microarrays manufactured via acoustic liquid handling, we identified 9 candidate antigens from a library of 534 SARS-CoV-2 peptides. These arrays could form the basis of a multiplexed COVID-19 serologic assay with enhanced specificity. ReScan has broad applicability for serologic assay

development. [**note: this is a useful approach to identifying antigens to improve serologic assasys.**] https://www.medrxiv.org/content/10.1101/2020.05.11.20092528v1

>>>

2020-05-14

Henri Duparc only wrote 17 songs but each one was masterful in composition and expression. One of my favorites is Phydilé sung here by Sylvia McNair: https://www.youtube.com/watch?v=0Sec4GzrZ9U

Here is a weird story that popped up on my newsfeed yesterday. Some researchers in Texas have vials of a vaccine against the original SARS sitting in a freezer. They are going to test it our to see if it works against SARS-CoV-2, the new variant. I've not seen any information that points to the surface antigens of the two viruses being similar enough that there would be cross reactivity but as I'm fond of saying any port in a storm. Most interesting is that it is a yeast based recombinant DNA vaccine, the same process that is used to make the Hepatitis B vaccine. This is a proven scalable technology and I'm not sure why it hasn't been used for the COVID-19 viral strain. At the time only animal studies were carried out so this will be first in human. I honestly don't understand why they didn't do a small Phase 1 trial back when this was developed just to see whether antibodies were produced.

There are a couple of very good general articles in the Washington Post, here is the story on the Rutgers saliva test and here is one on how the medical community is learning how to better treat hospitalized patients.

Wise words from Atul Gawande on how to set up a reentry regimen.

I have commented about the need for independent validation of clinical diagnostic tests. This is routinely done by most high-level clinical labs before they settle on what test/equipment to use. The Washington Post has a story on the problems the Abbott quick test is having. Some of this may be user error and some may be the inherent limitation of the test system itself. It is always worth remembering that no test is 100% accurate. With the current uncertainty still lingering, proper sanitation, social distancing and the wearing of masks is still recommended.

MODELING

- The SIR (susceptible-infected-removed) model, statistical approach for the parameter identification and the official WHO data about the confirmed cumulative number of cases were used to estimate the characteristics of COVID-19 pandemic in USA, Germany, UK, South Korea and in the world. Epidemic in every country has rather long hidden period before fist cases were confirmed. In particular, the pandemic began in China no later than October, 2019. If current trends continue, the end of the pandemic should be expected no earlier than March 2021, the global number of cases will exceed 5 million. [**note: I need to post this as it is the first contribution from a Ukraine researcher and I like to continue to cite the world-wide efforts that are going on.**] https://www.medrxiv.org/content/10.1101/2020.05.10.20097147v1
- The Chinese horseshoe bat (Rhinolophus sinicus), reservoir host of severe acute respiratory syndrome coronavirus (SARS-CoV), carries many bat SARS-related CoVs (SARSr-CoVs) with high genetic diversity, particularly in the spike gene. Despite these variations, some bat SARSr-CoVs

can utilize the orthologs of human SARS-CoV receptor, angiotensin-converting enzyme 2 (ACE2), for entry. It is speculated that the interaction between bat ACE2 and SARSr-CoV spike proteins drives diversity. Here, we have identified a series of R. sinicus ACE2 variants with some polymorphic sites involved in the interaction with the SARS-CoV spike protein. Pseudoviruses or SARSr-CoVs carrying different spike proteins showed different infection efficiency in cells transiently expressing bat ACE2 variants. Consistent results were observed by binding affinity assays between SARS- and SARSr-CoV spike proteins and receptor molecules from bats and humans. All tested bat SARSr-CoV spike proteins had a higher binding affinity to human ACE2 than to bat ACE2, although they showed a 10-fold lower binding affinity to human ACE2 compared with their SARS-CoV counterpart. Structure modeling revealed that the difference in binding affinity between spike and ACE2 might be caused by the alteration of some key residues in the interface of these two molecules. Molecular evolution analysis indicates that these residues were under strong positive selection. These results suggest that the SARSr-CoV spike protein and R. sinicus ACE2 may have coevolved over time and experienced selection pressure from each other, triggering the evolutionary arms race dynamics. It further proves that R. sinicus is the natural host of SARSr-CoVs. [**note: not a model but an interesting analyitical paper on the origin of SARS-CoV viruses.]** https://www.biorxiv.org/content/10.1101/2020.05.13.093658v1

- Aiming to understand a host genetic component of COVID-19, we focused on variants in genes encoding proteases and genes involved in innate immunity that could be important for susceptibility and resistance to SARS-CoV-2 infection. Analysis of sequence data of coding regions of FURIN, PLG, PRSS1, TMPRSS11a, MBL2 and OAS1 genes in 143 unrelated individuals from Serbian population identified 22 variants with potential functional effect. In silico analyses (PolyPhen-2, SIFT, MutPred2 and Swiss-Pdb Viewer) predicted that 10 variants could impact the structure and/or function of proteins. These protein-altering variants (p.Gly146Ser in FURIN; p.Arg261His and p.Ala494Val in PLG; p.Asn54Lys in PRSS1; p.Arg52Cys, p.Gly54Asp and p.Gly57Glu in MBL2; p.Arg47Gln, p.Ile99Val and p.Arg130His in OAS1) may have predictive value for inter-individual differences in the response to the SARS-CoV-2 infection. Next, we performed comparative population analysis for the same variants using extracted data from the 1000 genomes project. Population genetic variability was assessed using delta MAF and Fst statistics. Our study pointed to 7 variants in PLG, TMPRSS11a, MBL2 and OAS1 genes with noticeable divergence in allelic frequencies between populations worldwide. Three of them, all in MBL2 gene, were predicted to be damaging, making them the most promising population-specific markers related to SARS-CoV-2 infection. Comparing allelic frequencies between Serbian and other populations, we found that the highest level of genetic divergence related to selected loci was observed with African, followed by East Asian, Central and South American and South Asian populations. When compared with European populations, the highest divergence was observed with Italian population. In conclusion, we identified 4 variants in genes encoding proteases (FURIN, PLG and PRSS1) and 6 in genes involved in the innate immunity (MBL2 and OAS1) that might be relevant for the host response to SARS-CoV-2 infection. [**note: we can check the Serbia box now with this paper. This is an interesting finding in that other papers also suggest that there is a genetic predisposition to 'immunity' from SARS-CoV-2 infection.** https://www.biorxiv.org/content/10.1101/2020.05.13.093690v1

NEWLY REGISTERED CLINICAL TRIALS

- Considering immunomodulatory, anti-inflammatory anti-fibrotic and anti-oxidant actions of vitamin D, it's safety and ease of administration, as well as direct effects of vitamin D on immune cell proliferation and activity, pulmonary ACE2 expression and reducing surface tension, evaluation of vitamin D supplementation as an adjuvant therapeutic intervention could be of substantial clinical and economic significance. High prevalence of vitamin D deficiency in elderly, smokers, patients with chronic diseases and excess uptake by adipose tissue in obesity make investigations of its role as a secondary therapeutic agent in COVID-19 conceivable. It should be necessary to monitor serum 25(OH)D levels in all inpatient and outpatient populations with COVID-19 to identify the importance of maintaining or promptly increasing circulating levels of 25(OH)D into the optimal range of 100-150 nmol/L. The aim of this study is to conduct a double blind, randomized, controlled three weeks clinical trial on the efficacy of vitamin D (daily low dose versus weekly high dose) in COVID-19 patients in order to determine the relationship between baseline vitamin D deficiency and clinical characteristics and to asses patients' response to vitamin D supplementation in week three and determine its association with disease progression and recovery. [**note: somebody had to do a Vitamin D study and the Canadians are ready to go. There are other such studies but this is closest to me.**] NCT04385940
- Genentech have filed for a trial of astegolimab, a monoclonal ST-2. [**note: I didn't find out too much about this mAb other than they have run a trial on COPD.**] NCT04386616
- Here is an Australian study of ifenpodil. [**note: it's an NDMA receptor antagonist and still experimental.**] NCT04382924
- The present study investigates the efficacy of a brief and cost-effective video-intervention that combines bottom-up elements of deep breathing and third-wave cognitive behavioral therapy techniques (i.e., mindfulness and compassion) on coping strategies during the COVID-19 pandemic. [**note: sign me up for this one!! It's worth going to the trial directory and reading the description of the intervention.]** NCT04382560

CLINICAL TRIAL RESULTS

- Acute clinical manifestations of SARS-CoV-2 infection are less frequent and less severe in children than in adults. However, recent observations raised concerns about potential post-viral severe inflammatory reactions in children infected with SARS-CoV-2. Methods: We describe an outbreak of cases of Kawasaki disease (KD) admitted between April 27 and May 7, 2020, in the general paediatrics department of a university hospital in Paris, France. All children prospectively underwent nasopharyngeal swabs for SARS-CoV-2 RT-PCR, SARS-CoV-2 IgG serology testing, and echocardiography. The number of admissions for KD during the study period was compared to that observed since January 1, 2018, based on discharge codes, using Poisson regression. Results: A total of 17 children were admitted for KD over an 11-day period, in contrast with a mean of 1.0 case per 2-week period over 2018-2019 (Poisson incidence rate ratio: 13.2 [95% confidence interval: 7.3-24.1], p <0.001). Their median age was 7.5 (range, 3.7-16.6) years, and 59% of patients originated from sub-Saharan Africa or Caribbean islands. Eleven patients presented with KD shock syndrome (KDSS) requiring intensive care support, and 12 had myocarditis. All children had marked gastrointestinal symptoms at the early stage of illness and high levels of inflammatory markers. Fourteen patients (82%) had evidence of recent SARS-CoV-2 infection (positive RT-PCR 7/17, positive IgG antibody detection 14/16). All patients received

immunoglobulins and some received corticosteroids (5/17). The clinical outcome was favourable in all patients. Moderate coronary artery dilations were detected in 5 cases (29%) during hospitalisation. Conclusions: The ongoing outbreak of KD in the Paris might be related to SARS-CoV2, and shows an unusually high proportion of children with gastrointestinal involvement, KDSS and African ancestry. [**note: this is the first preprint I've seen looking at Kawasaki disease in children with SARS-CoV-2. I'm sure New York City will be reporting on this.**] https://www.medrxiv.org/content/10.1101/2020.05.10.20097394v1

- Background: Convalescent plasma is the only antibody based therapy currently available for COVID-19 patients. It has robust historical precedence and sound biological plausibility. Although promising, convalescent plasma has not yet been shown to be safe as a treatment for COVID-19. Methods: Thus, we analyzed key safety metrics after transfusion of ABO-compatible human COVID-19 convalescent plasma in 5,000 hospitalized adults with severe or life threatening COVID-19, with 66% in the intensive care unit, as part of the US FDA Expanded Access Program for COVID-19 convalescent plasma. Results: The incidence of all serious adverse events (SAEs) in the first four hours after transfusion was <1%, including mortality rate (0.3%). Of the 36 reported SAEs, there were 25 reported incidences of related SAEs, including mortality (n=4), transfusion-associated circulatory overload (TACO; n=7), transfusion-related acute lung injury (TRALI; n=11), and severe allergic transfusion reactions (n=3). However, only 2 (of 36) SAEs were judged as definitely related to the convalescent plasma transfusion by the treating physician. The seven-day mortality rate was 14.9%. Conclusion: Given the deadly nature of COVID-19 and the large population of critically-ill patients included in these analyses, the mortality rate does not appear excessive. These early indicators suggest that transfusion of convalescent plasma is safe in hospitalized patients with COVID-19. [**note: this is a useful paper from the Mayo Clinic showing the putative safety of using convalescent plasma in treating hospitalized patients.**] https://www.medrxiv.org/content/10.1101/2020.05.12.20099879v1
- Background: Systemic corticosteroids are recommended by some treatment guidelines and used in severe and critical COVID-19 patients, though evidence supporting such use is limited. Methods: From December 26, 2019 to March 15, 2020, 1514 severe and 249 critical hospitalized COVID-19 patients were collected from two medical centers in Wuhan, China. We performed multivariable Cox models, Cox model with time-varying exposure and propensity score analysis (both inverse-probability-of-treatment-weighting (IPTW) and propensity score matching (PSM)) to estimate the association of corticosteroid use with the risk of in-hospital mortality among severe and critical cases. Results: Corticosteroids were administered in 531 (35.1%) severe and 159 (63.9%) critical patients. Compared to no corticosteroid use group, systemic corticosteroid use showed no benefit in reducing in-hospital mortality in both severe cases (HR=1.77, 95% CI: 1.08-2.89, p=0.023), and critical cases (HR=2.07, 95% CI: 1.08-3.98, p=0.028). In the time-varying Cox analysis that with time varying exposure, systemic corticosteroid use still showed no benefit in either population (for severe patients, HR=2.83, 95% CI: 1.72-4.64, p< 0.001; for critical patients, HR=3.02, 95% CI: 1.59-5.73, p=0.001). Baseline characteristics were matched after IPTW and PSM analysis. For severe COVID-19 patients at admission, corticosteroid use was not associated with improved outcome in either the IPTW analysis. For critical COVID-19 patients at admission, results were consistent with former analysis that corticosteroid use did not reduce in-hospital mortality. Conclusions: Corticosteroid use showed no benefit in reducing in-hospital mortality for severe or critical cases. The routine use of systemic corticosteroids among severe

and critical COVID-19 patients was not recommended. [**note: this runs counter to at least two other preprints I've read about corticosteroid therapy. I guess we need to wait for more trials to be completed.**] https://www.medrxiv.org/content/10.1101/2020.05.11.20097709v1

DRUG DEVELOPMENT

- Vaccines are an essential countermeasure urgently needed to control the pandemic4. Here, we show that the adenovirus-vectored vaccine ChAdOx1 nCoV-19, encoding the spike protein of SARS-CoV-2, is immunogenic in mice, eliciting a robust humoral and cell-mediated response. This response was not Th2 dominated, as demonstrated by IgG subclass and cytokine expression profiling. A single vaccination with ChAdOx1 nCoV-19 induced a humoral and cellular immune response in rhesus macaques. We observed a significantly reduced viral load in bronchoalveolar lavage fluid and respiratory tract tissue of vaccinated animals challenged with SARS-CoV-2 compared with control animals, and no pneumonia was observed in vaccinated rhesus macaques. Importantly, no evidence of immune-enhanced disease following viral challenge in vaccinated animals was observed. ChAdOx1 nCoV-19 is currently under investigation in a phase I clinical trial. Safety, immunogenicity and efficacy against symptomatic PCR-positive COVID-19 disease will now be assessed in randomised controlled human clinical trials. [**note: good news here. Animal data show the validity of the UK adenovirus vaccine!**] https://www.biorxiv.org/content/10.1101/2020.05.13.093195v1
- Multiple vaccine candidates against SARS-CoV-2 based on viral spike protein are under development. However, there is limited information on the quality of antibody response generated following vaccination by these vaccine modalities. To better understand antibody response induced by spike protein-based vaccines, we immunized rabbits with various SARS-CoV-2 spike protein antigens: S-ectodomain (S1+S2) (aa 16-1213), which lacks the cytoplasmic and transmembrane domains (CT-TM), the S1 domain (aa 16-685), the receptor-binding domain (RBD) (aa 319-541), and the S2 domain (aa 686-1213 as control). Antibody response was analyzed by ELISA, Surface Plasmon Resonance (SPR) against different Spike proteins in native conformation, and a pseudovirion neutralization assay to measure the quality and function of the antibodies elicited by the different Spike antigens. All three antigens (S1+S2 ectodomain, S1 domain, and RBD) generated strong neutralizing antibodies against SARS-CoV-2. Vaccination induced antibody repertoire was analyzed by SARS-CoV-2 spike Genome Fragment Phage Display Libraries (SARS-CoV-2 GFPDL), which identified immunodominant epitopes in the S1, S1-RBD and S2 domains. Furthermore, these analyses demonstrated that surprisingly the RBD immunogen elicited a higher antibody titer with 5-fold higher affinity antibodies to native spike antigens compared with other spike antigens. These findings may help guide rational vaccine design and facilitate development and evaluation of effective therapeutics and vaccines against COVID-19 disease. [**note: this is some fine work from the CBER folks at FDA. They looked at several spike protein antigens and measured immunogenicity. This can be used for rational vaccine design and perhaps quicker production ramp up.**] https://www.biorxiv.org/content/10.1101/2020.05.12.091918v1
- Antibodies are a principal determinant of immunity for most RNA viruses and have promise to reduce infection or disease during major epidemics. The novel coronavirus SARS-CoV-2 has caused a global pandemic with millions of infections and hundreds of thousands of deaths to date1,2. In response, we used a rapid antibody discovery platform to isolate hundreds of human

monoclonal antibodies (mAbs) against the SARS-CoV-2 spike (S) protein. We stratify these mAbs into five major classes based on their reactivity to subdomains of S protein as well as their cross-reactivity to SARS-CoV. Many of these mAbs inhibit infection of authentic SARS-CoV-2 virus, with most neutralizing mAbs recognizing the receptor-binding domain (RBD) of S. This work defines sites of vulnerability on SARS-CoV-2 S and demonstrates the speed and robustness of existing antibody discovery methodologies [**note: some good work on isolation of mAbs. It also partially confirms the FDA finding above about the RBD antigen.**]
https://www.biorxiv.org/content/10.1101/2020.05.12.091462v1

- Neutralizing antibodies could be antivirals against COVID-19 pandemics. Here, we report isolation of four human-origin monoclonal antibodies from a convalescent patient, all of which display neutralization abilities. B38 and H4 block the binding between virus S-protein RBD and cellular receptor ACE2. A competition assay indicates their different epitopes on the RBD, making them a potential virus-targeting MAb-pair to avoid immune escape in future clinical applications. Moreover, a therapeutic study in a mouse model validated that these antibodies can reduce virus titers in infected lungs. The RBD-B38 complex structure revealed that most residues on the epitope overlap with the RBD-ACE2 binding interface, explaining the blocking effect and neutralizing capacity. Our results highlight the promise of antibody-based therapeutics and provide a structural basis for rational vaccine design. [**note: and more from China on the isolation of mAbs with therapeutic potential.**]
https://science.sciencemag.org/content/early/2020/05/12/science.abc2241

DIAGNOSTIC DEVELOPMENT

- Accurate serological assays can improve the early diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, but few studies have compared performance characteristics between assays in symptomatic and recovered patients. Methods: We recruited 32 patients who had 2019 coronavirus disease (COVID-19; 18 hospitalized and actively symptomatic, 14 recovered mild cases), and measured levels of IgM (against the full-length S1 or the highly homologous SARS-CoV E protein) and IgG (against S1 receptor binding domain [RBD]). We performed the same analysis in 103 pre-2020 healthy adult control (HC) participants and 13 participants who had negative molecular testing for SARS-CoV-2. Results: Anti-S1-RBD IgG levels were very elevated within days of symptom onset for hospitalized patients (median 2.04 optical density [OD], vs. 0.12 in HC). People who recovered from milder COVID-19 only reached similar IgG levels 28 days after symptom onset. IgM levels were elevated early in both groups (median 1.91 and 2.12 vs. 1.14 OD in HC for anti-S1 IgM, 2.23 and 2.26 vs 1.52 in HC for anti-E IgM), with downward trends in hospitalized cases having longer disease duration. The combination of the two IgM levels showed similar sensitivity for COVID-19 as IgG but greater specificity, and identified 4/10 people (vs. 3/10 by IgG) with prior symptoms and negative molecular testing to have had COVID-19. Conclusions: Disease severity and timing both influence levels of IgM and IgG against SARS-CoV-2, with IgG better for early detection of severe cases but IgM more suited for early detection of milder cases. [**note: this is a combination clinical and diagnostic paper. It shows the utility of measuring both IgG and IgM.**]
https://www.medrxiv.org/content/10.1101/2020.05.10.20097535v1
- The COVID-19 pandemic has brought the world to a halt, with cases observed around the globe causing significant mortality. There is an urgent need for serological tests to detect antibodies

against SARS-CoV-2, which could be used to assess the prevalence of infection, as well as ascertain individuals who may be protected from future infection. Current serological tests developed for SARS-CoV-2 rely on traditional technologies such as enzyme-linked immunosorbent assays (ELISA) and lateral flow assays, which may lack scalability to meet the demand of hundreds of millions of antibody tests in the coming year. Herein, we present an alternative method of antibody testing that just depends on one protein reagent being added to patient serum/plasma or whole blood and a short five-minute assay time. A novel fusion protein was designed that binds red blood cells (RBC) via a single-chain variable fragment (scFv) against the H antigen and displays the receptor-binding domain (RBD) of SARS-CoV-2 spike protein on the surface of RBCs. Upon mixing of the fusion protein, RBD-scFv with recovered COVID-19 patient serum and RBCs, we observed agglutination of RBCs, indicating the patient developed antibodies against SARS-CoV-2 RBD. Given that the test uses methods routinely used in hospital clinical labs across the world, we anticipate the test can be rapidly deployed with only the protein reagent required at projected manufacturing cost at U.S. cents per test. We anticipate our agglutination assay may find extensive use in low-resource settings for detecting SARS-CoV-2 antibodies. [**note: here is a new approach to serology testing based on agglutination of red blood cells. Good out of the box thinking.**]
https://www.biorxiv.org/content/10.1101/2020.05.13.094490v1

- The test performance characteristics of the Accula (Mesa Biotech) SARS-CoV-2 POC test need to be evaluated to inform its optimal use. Objectives: The aim of this study was to assess test performance of the Accula SARS-CoV-2 test. Study design: The performance of the Accula test was assessed by comparing results of 100 nasopharyngeal swab samples previously characterized by the Stanford Health Care EUA laboratory-developed test (SHC-LDT) targeting the envelope (E) gene. Assay concordance was assessed by overall percent agreement, positive percent agreement (PPA), negative percent agreement (NPA), and Cohen's kappa coefficient. Results: Overall percent agreement between the assays was 84.0% (95% confidence interval [CI] 75.3 to 90.6%), PPA was 68.0% (95% CI 53.3 to 80.5%) and the kappa coefficient was 0.68 (95% CI 0.54 to 0.82). Sixteen specimens detected by the SHC-LDT were not detected by the Accula test, and showed low viral load burden with a median cycle threshold value of 37.7. NPA was 100% (95% CI 94.2 to 100%). Conclusion: Compared to the SHC-LDT, the Accula SARS-CoV-2 test showed excellent negative agreement. However, positive agreement was low for samples with low viral load. The false negative rate of the Accula POC test calls for a more thorough evaluation of POC test performance characteristics in clinical settings, and for confirmatory testing in individuals with moderate to high pre-test probability of SARS-CoV-2 who test negative on Accula. [**note: in house validation of diagnostic tests is critical.**]
https://www.biorxiv.org/content/10.1101/2020.05.12.092379v1
- As the Coronavirus 2019 (COVID-19) pandemic evolves, the development of immunoassays to help determine exposure and potentially predict immunity has become a pressing priority. In this report we present the performance of the EUROIMMUN enzyme-linked immunosorbent assay (ELISA) for semi-quantitative detection of IgA and IgG antibodies in serum and plasma samples using recombinant S1 domain of the SARS-CoV-2 spike protein as antigen. Specimens from patients, with and without COVID-19 infection, were tested at the University of Chicago Clinical Microbiology and Immunology Laboratory. Of 57 samples from COVID-19 PCR-negative patients, including 28 samples positive for common human coronavirus strains, 53 tested

negative and 4 tested positive for IgA (93.0% agreement) while 56 tested negative and 1 tested positive for IgG (98.2% agreement). For COVID-19 PCR-positive patients, 29 of 30 (96.7%) samples collected ≥3 days after positive PCR were positive for IgA, and 28 of 28 samples collected ≥4 days after positive PCR were positive for IgG. The EUROIMMUN Anti-SARS-CoV-2 ELISA Assay demonstrates excellent sensitivity for detection of IgA and IgG antibodies from samples collected ≥3 days and ≥4 days, respectively, after COVID-19 diagnosis by PCR. This assay did not demonstrate cross reaction in any of the 28 samples from patients with common human coronaviruses, including types HKU1, NL63, CV229E, and OC43. [**note: in house validation of the main European serology test.**] https://www.biorxiv.org/content/10.1101/2020.05.11.089862v1

>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>

2020-05-15

I love opera duets!!! One of my faves is the Flower Duet from Leo Delibes's opera Lakme. Here is a nice version from the recording studio with Sabine Devieilhe and Marianne Cregassa: https://www.youtube.com/watch?v=C1ZL5AxmK_A

Two important editorials from British medical journals. The BMJ astutely weighs in on the waste in COVID-19 research, something your humble correspondent has noted from time to time. More importantly, The Lancet has a very direct commentary on reviving the US Centers for Disease Control. The money quote is the last line of the editorial and I'll let you read that for yourself!!!

This story on the Walter Reed lab vaccine efforts came across my news feed yesterday. It is interesting that they chose ferritin as the vaccine platform

Derek Lowe with a Q&Q on monoclonal antibodies and vaccines against SARS-CoV-2.

There has been a lot of press about aerosol transmission of SARS-CoV-2. The Scientific American has one of the better stories on this.

MODELING

- Summary Background With confirmed cases of COVID-19 declining in many countries, lockdown measures are gradually being lifted. However, even if most social distancing measures are continued, other public health measures will be needed to control the epidemic. Contact tracing either via conventional methods or via mobile app technology is central to control strategies during de-escalation of social distancing. It is therefore essential to identify key factors for a contact tracing strategy (CTS) to be successful. Methods We evaluated the impact of timeliness and completeness in various steps of a CTS using a stochastic mathematical model with explicit time delays between time of infection, symptom onset, diagnosis by testing, and isolation. The model also includes tracing of close contacts (e.g. household members) and casual contacts with different delays and coverages. We computed effective reproduction numbers of a CTS (Rcts) for a population with social distancing measures and various scenarios for isolation of index cases and tracing and quarantine of its contacts. Findings In the best-case scenario (testing and tracing delays of 0 days and tracing coverage of 100%) the effective reproduction number will be reduced with 50% from 1.2 (with social distancing only) to 0.6 (Rcts) by contact tracing. A testing

delay of 3 days requires tracing delay or coverage to be at most 1 day or at least 80% to keep Rcts below 1, with the Rcts reduction being 15% and 17%, respectively. With a testing delay of 4 days, even the most efficient CTS cannot reach Rcts values below 1. The effect of minimizing tracing delay (e.g., with app-based technology) declines with declining coverage of app use, but app-based tracing remains more effective than conventional contact tracing even with 20% coverage. The proportion of transmissions per index case that can be prevented depending on testing and tracing delay and isolation of index cases ranges from above 80% in the best-case scenario (testing and tracing delays of 0 days) to 40% and 17% with testing delays of 3 and 5 days, respectively. Interpretation Minimizing testing delay is of key importance for the effectiveness of CTS. Optimizing testing and tracing coverage and minimizing tracing delays, for instance with app-based technology further enhances effectiveness of CTS, with a potential to prevent up to 80% of all transmissions. The process of conventional contact tracing should be reviewed and streamlined, while mobile app technology may offer a tool for gaining speed in the process. [**note: this is a nice paper from a Dutch group on contact tracing and the impact of delays. Now you might argue that is something that is easy to do in a small country such as The Netherlands but won't be applicable to the US. My view is that we need to try to do this as it's the only reasonable way forward. Train more public health workers and get this job done.**] https://www.medrxiv.org/content/10.1101/2020.05.09.20096289v1

- The COVID-19 pandemic has created an urgent need for models that can project epidemic trends, explore intervention scenarios, and estimate resource needs. Here we describe the methodology of Covasim (COVID-19 Agent-based Simulator), an open-source model developed to help address these questions. Covasim includes demographic information on age structure and population size; realistic transmission networks in different social layers, including households, schools, workplaces, and communities; age-specific disease outcomes; and intrahost viral dynamics, including viral-load-based transmissibility. Covasim also supports an extensive set of interventions, including non-pharmaceutical interventions, such as physical distancing, hygiene measures, and protective equipment; and testing interventions, such as symptomatic and asymptomatic testing, isolation, contact tracing, and quarantine. These interventions can incorporate the effects of delays, loss-to-follow-up, micro-targeting, and other factors. In collaboration with local health agencies and policymakers, Covasim has already been applied to examine disease dynamics and policy options in Africa, Europe, Oceania, and North America. [**note: here is a good open source modeling project that looks at disease dynamics and options. Open source models are always the way to go.**] https://www.medrxiv.org/content/10.1101/2020.05.10.20097469v1
- In the absence of medical treatment and vaccination, the mitigation and containment of the ongoing COVID-19 pandemic relies on behavioral changes. Timely data on attitudes and behaviors are thus necessary to develop optimal intervention strategies and to assess the consequences of the pandemic for different demographic groups. We developed a rapid response monitoring system via a continuously run online survey (the "COVID-19 Health Behavior Survey") across eight countries (Belgium, France, Germany, Italy, the Netherlands, Spain, the United Kingdom, the United States). The survey was specifically designed to collect key information on people's health status, behaviors, close social contacts, and attitudes in response to the COVID-19 pandemic. We developed an innovative approach to recruit participants via targeted Facebook advertisement campaigns in order to generate balanced

samples for post-stratification. In this paper, we present results for the period from March 13-April 19, 2020. We estimate important differences by sex: women show a substantially higher perception of threat along with a lower level of confidence in the health system. This is paralleled by sex-specific behaviors, with women more likely to adopt a wide range of preventive behaviors. We thus expect behavior to serve as a protective factor for women. Our findings also show a higher level of awareness and concern among older respondents, in line with the evidence that the elderly are at highest risk of severe complications following infection from COVID-19. While across all the samples respondents were less concerned for themselves than for their country or for the world, we also observed substantial temporal and spatial heterogeneity in terms of confidence in institutions and responses to non-pharmaceutical interventions. [**note: this will be the first and last Facebook survey post!!** 😊]
https://www.medrxiv.org/content/10.1101/2020.05.09.20096388v1

- The pandemic inflicted by coronavirus disease 2019 (COVID-19) resulted in physical isolation measures in many parts of the world. In Australia, nationwide restrictions included staying at home, unless seeking medical care, providing care, purchasing food, undertaking exercise, or attending work in an essential service. All undergraduate university classes transitioned to online, mostly home-based learning. This disruption to daily life may have consequences for eating and physical activity patterns. Methods: In this observational study, we examined the effect of isolation measures, during the early phase of the COVID-19 pandemic in Australia (March/April), on diet (24-hour diet recall, ASA-24) and physical activity (Active Australia Survey) patterns among third-year biomedical students in Brisbane, Australia. Findings were compared to students enrolled in the same course in the previous two years. Results: In females, energy intake was ~20% greater in 2020 compared with 2018 and 2019, and the frequency of snacking and energy density of consumed snacks were also increased. In males, there was no difference in energy intake or snacking behaviour. Physical activity was impacted for both sexes, whereby fewer students undertook any walking activity and, of those that did, time spent doing so was less compared with 2018 and 2019. The proportion of students reporting any vigorous activity was not different for males or females but, among males who participated in this level of activity, the duration was less in 2020 compared with previous years. The proportion of male and female students achieving sufficient levels of activity, defined by at least 150 mins over at least 5 sessions, was ~30% less in 2020. Indeed, the majority of students reported as having undertaken less physical activity than usual. Conclusions: Increased energy intake for females and reduced physical activity for males and females demonstrate impacts of isolation measures that may have deleterious consequences for physical and mental wellbeing, with the potential to affect long-term nutrition and activity patterns. [**note: this pretty much confirms the survey that my wife and I have been conducting. I just avoid the bathroom scale and as long as my pants are not too tight, I figure I'm doing the right amount of exercise!**]
https://www.medrxiv.org/content/10.1101/2020.05.10.20076414v1
- The world is going through a global viral pandemic with devastating effects on human life and socioeconomic activities. This pandemic is the result of a zoonotic coronavirus, Severe Acute Respirsatory Syndrom Coronavirus 2 (SARS-CoV-2) which is believed to have originated in bats and transferred to humans possibly through an intermediate host species (Zhou et al. 2020; Coronaviridae Study Group of the International Committee on Taxonomy of Viruses 2020). The virus attacks host cells by attaching to a cell membrane surface protein receptor called ACE2 (Ge

et al. 2013; Zhou et al. 2020). Given the critical role of ACE2 as a binding receptor for a number of coronaviruses, we studied the molecular evolution of ACE2 in a diverse range of mammalian species. Using ACE2 as the target protein, we wanted to specifically test the Red Queen hypothesis (Dawkins and Krebs 1979) where the parasite and host engage in an evolutionary arms race which can result in positive selection of their traits associated to their fitness and survival. Our results clearly show a phylogenetically broad evolutionary response, in the form of positive selection detected at the codon-level in ACE2. We see positive selection occurring at deep branches as well as 13 incidents at the species level. We found the strongest level of positive selection in Tasmanian devil (Sarcophilus harrisii), donkey (Equus asinus), large flying fox (Pteropus vampyrus), Weddell seal (Leptonychotes weddellii), and dog (Canis lupus familiaris). At the codon-level, we found up to 10% of ACE2 codons are impacted by positive selection in the mammalian lineages studied. This phylogenetically broad evolutionary arms race can contribute to the emergence of new strains of coronaviruses in different mammalian lineages with a potential to transfer between species given the common binding receptor ACE2. Our study provides a molecular evolutionary perspective to the current pandemic and sheds light on its evolutionary mechanisms. [**note: any paper with a reference to "Alice in Wonderland" gets an automatic citation from me!!!**]
https://www.biorxiv.org/content/10.1101/2020.05.14.096131v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow. Lots of sera from recovered patients are being registered.

CLINICAL TRIAL RESULTS

- Cardiometabolic morbidity and medications, specifically Angiotensin Converting Enzyme inhibitors (ACEi) and Angiotensin Receptor Blockers (ARBs), have been linked with adverse outcomes from coronavirus disease 2019 (COVID-19). This study aims to investigate factors associated with COVID-19 positivity for the first 669 UK Biobank participants; compared with individuals who tested negative, and with the untested, presumed negative, rest of the population. Methods: We studied 1,474 participants from the UK Biobank who had been tested for COVID-19. Given UK testing policy, this implies a hospital setting, suggesting at least moderate to severe symptoms. We considered the following exposures: age, sex, ethnicity, body mass index (BMI), diabetes, hypertension, hypercholesterolaemia, ACEi/ARB use, prior myocardial infarction (MI), and smoking. We undertook comparisons between: 1) COVID-19 positive and COVID-19 tested negative participants; and 2) COVID-19 tested positive and the remaining participants (tested negative plus untested, n=501,837). Logistic regression models were used to investigate univariate and mutually adjusted associations. Results: Among participants tested for COVID-19, non-white ethnicity, male sex, and greater BMI were independently associated with COVID-19 positive result. Non-white ethnicity, male sex, greater BMI, diabetes, hypertension, prior MI, and smoking were independently associated with COVID-19 positivity compared to the remining cohort (test negatives plus untested). However, similar associations were observed when comparing those who tested negative for COVID-19 with the untested cohort; suggesting that these factors associate with general hospitalisation rather than specifically with COVID-19. Conclusions: Among participants tested for COVID-19 with presumed moderate to severe symptoms in a hospital setting, non-white ethnicity, male sex, and higher

BMI are associated with a positive result. Other cardiometabolic morbidities confer increased risk of hospitalisation, without specificity for COVID-19. Notably, ACE/ARB use did not associate with COVID-19 status. [**note: more evidence that pharmaceuticals used to control blood pressure via renin/angiotensin pathway don't impact medical outcomes but non-white ethnicity, male sex and increase BMI do. Good stuff from the UK where they can do these observational studies pretty quickly.**]
https://www.medrxiv.org/content/10.1101/2020.05.10.20096925v1 **but wait, there's more:** Our analysis suggests there are several demographic, clinical and laboratory findings associated with a symptomatic presentation of COVID-19. Moreover, *significant associations between patient deterioration were found with age, sex and specific blood markers, chiefly C-reactive protein, and could help early identification of patients at risk of poorer prognosis*. Further work is required to clarify the extent to which our observations are relevant beyond current settings.
https://www.medrxiv.org/content/10.1101/2020.05.11.20093096v1

- Elucidating the role of T cell responses in COVID-19 is of utmost importance to understand the clearance of SARS-CoV-2 infection. Methods: 90 individuals were enrolled in this study, 30 hospitalized COVID-19 patients and 60 age- and gender-matched healthy controls (HC). Using two comprehensive 11-color flow cytometric panels conforming to Good Laboratory Practice (GLP) and approved for clinical diagnostics, we longitudinally examined cell count differences in lymphocyte populations and T cell activation in COVID-19 patients. Findings: Absolute numbers of lymphocyte subsets were differentially decreased in COVID-19 patients according to clinical severity. In severe disease (SD) patients, all lymphocyte subsets were reduced, whilst in mild disease (MD) NK, NKT and γδ T cells were at the level of HC. Additionally, we provide evidence of T cell activation in MD but not SD, when compared to HC. Interestingly, follow up samples revealed a marked increase in effector T cells and memory subsets in convalescing but not in non-convalescing patients. Interpretation: *Our data suggest that activation and expansion of innate and adaptive lymphocytes play a major role in COVID-19. Additionally, recovery is associated with formation of T cell memory as suggested by the missing formation of effector and central memory T cells in SD but not in MD.* Our data imply that the presence of SARS-CoV-2 responsive T cells contributes to convalescence in MD. Thus, understanding the T cell-response in the context of clinical severity might serve as foundation to overcome the lack of effective anti-viral immune response in severely affected COVID-19 patients and can offer prognostic value as biomarker for disease outcome and control. [**note: T cell activation and response if important in terms of positive clinial outcomes.**]
https://www.medrxiv.org/content/10.1101/2020.05.11.20096263v1

DRUG DEVELOPMENT

- Background& Aims: The Coronavirus Disease 2019 (COVID-19) has become a global epidemic and has caused a lasting and huge loss of life security, economic development and social stability in more than 180 countries around the world. Unfortunately, there is still no specific treatment for COVID-19 till now, therefore, at this point, all potential therapies need to be critically considered. LL-37 is one of the best-studied human antimicrobial peptide (AMPs) that has a broad-spectrum activity against bacteria and viruses. The use of living, genetically modified organisms (GMOs) is an effective approach for delivery of therapeutic proteins. The aim of this study was to determine the safety and efficacy of the Lactococcus lactis which has

been genetically modified to produce the therapeutic human antimicrobial peptide LL-37 (herein after referred to cas001) in the patients of COVID-19. Methods: Firstly we constructed genetically modified food-grade probiotic, Lactococcus lactis, with sequence of seven tandem repeats of mature human LL-37 under control of the nisin-inducible nisA promoter to produce the cas001. A total of 20 healthy SD rats, half male and half female (There were five male and five female in the control group, the same in treatment group) were used to observe the acute toxic reaction and death after daily administration of cas001 for three weeks, which helps to provide necessary reference basis for clinical dose selection, verificaition of toxic reaction and possible target organs. According to the estimated clinical dosage of 1 x 108CFU /kg/day, considering the conversion of body surface area, the dose for rats should be multiplied by 6.17 to 6 x 108 CFU/kg/day. We administrated 100 times higher dose at 6 x 1010 CFU/kg/day to rats. In order to investigate the pharmacokinetics of cas001, male SD rats (body weight 250-300g, 1 x 1010 /animal, n=3) were given oral administration of LL-37 bacteria powder. The concentration of LL-37 in the blood before and after gavage was detected by ELISA kit (Hycult biotechnology Cat# HK321). Human clinical study was approved by Ethics committee of Chinese PLA General Hospital (S2020-074-04) and a total of 11 patients with mild symptoms were enrolled in Wuhan hankou hospital and Huoshenshan hospital. They were enrolled voluntarily and all patients signed informed consent. Among them, there were 5 males and 6 females, aged 55 ± 12 (36-70) years old, and the duration from onset to medication enrollment was 35 ± 19 (5-68) days. 6 patients were nucleic acid positive and 5 patients were nucleic acid negative when they were enrolled. All patients received the oral drug cas001 treatment according to requirement(1 x 109 CFU/capsule, 3 capsules/time, three times a day for 3weeks), with an average follow-up time of 33 ± 15 days (see table 1 for the results). Findings: Western blot analysis shows that reasonable amount of LL-37 were induced by different concentrations of nisin, which means we have successfully constructed cas001. In the pre-clinical safety evaluation test, after three weeks administration of cas001, no adverse effects were observed on the rat's body weight, food and water intake, hematological or serum biochemical parameters. The results showed that the LD50 of cas001 was higher than that of the 100 times of the expected clinical dose of 6 x 1010 CFU/day. These results showed that cas001 could be safe in animal experiments. In addition, rat pharmacokinetics results showed that the serum concentration of LL-37 reached peak 2 hours after gavage of cas001 and returned to basal level 6 hours after gavage. During study period, the volunteers did not feel any discomfort while taking the cas001 capsules, and two hours after oral administration, the concentration of LL-37 were increased in healthy volunteers. cas001 shows definite effect in the improvement of gastrointestinal symptoms and is possible to have effects in improving the systemic symptoms and respiratory symptoms and may play a role in the improvement of results of nucleic acid test and lung CT test. 11 patients enrolled showed good compliance, tolerance, subjective feeling and actively interacted with the doctors. None of the patients had any adverse reactions. Conclusions Based on above observations, we conclude here that as an oral anti-viral agent, cas001 displayed good safety profiles. It is very hard to reach conclusion of clinical outcomes related to the cas001, although changes of several symptoms indicate encouraging findings. [**note: what a creative approach to drug delivery! I don't know too much about the LL-37 peptide and this is the best review I could find: https://www.frontiersin.org/articles/10.3389/fimmu.2013.00143/full I would note that at**

least one vaccine approach is using similar technology delivery platform.]
https://www.medrxiv.org/content/10.1101/2020.05.11.20064584v1

- The immediate call for translational research in the field of coronavirus disease (COVID-19) pandemic, needs new and unexplored angles to support and contribute to this important worldwide health problem. The aim of this study is to better understand the pathogenic mechanisms underlying COVID-19, deciphering the carbohydrate-mediated interactions of the SARS-CoV-2 spike protein. We studied the carbohydrate-binding receptors that could be important for viral entry and for immune-modulatory responses, and we studied the interactions of the spike protein with the host lung microbiota. Exploring solid-phase immunoassays, we evaluated the interactions between the SARS-CoV-2 spike protein and a library of 12 different human carbohydrate-binding proteins (C-type lectins and Siglecs) involved in binding, triggering and modulation of innate and adaptive immune-responses. We revealed a specific binding of the SARS-CoV-2 spike protein to the receptors DC-SIGN, MGL, Siglec-9 and Siglec-10 that are all expressed on myeloid immune cells. In addition, because the lung microbiota can promote or modulate viral infection, we studied the interactions between the SARS-CoV-2 spike protein and a library of Streptococcus pneumoniae capsular polysaccharides, as well as other bacterial glyco-conjugates. We show specific binding of the spike protein to different S. pneumoniae capsular polysaccharides (serotypes 19F and 23F but not to serotype 14). Moreover we demonstrated a specific binding of SARS-CoV-2 spike protein to the lipopolysaccharide from the opportunistic human pathogen Pseudomonas aeruginosa, one of the leading cause of acute nosocomial infections and pneumonia. Interestingly, we identified rhamnosylated epitopes as one of the discriminating structures in lung microbiota to bind SARS-CoV-2 spike protein. In conclusion, we revealed novel ACE2-independent carbohydrate-mediated interactions with immune modulating lectins expressed on myeloid cells, as well as host lung microbiota glyco-conjugates. Our results identified new molecular pathways using host lectins and signalling, that may contribute to viral infection and subsequent immune exacerbation. Moreover we identified specific rhamnosylated epitopes in lung microbiota to bind SARS-CoV-2, providing a hypothetical link between the presence of specific lung microbiota and SARS-CoV-2 infection and severity. [**note: this is a somewhat complicated paper. It's clear that binding affinities are critical for infection. I still wonder if there is any hose defense from mucopolysaccharide binding. I continue to stand ready to donate some of my precious nasal secretions for this study.**] https://www.biorxiv.org/content/10.1101/2020.05.13.092478v1

DIAGNOSTIC DEVELOPMENT

- The use of saliva collection for SARS-CoV-2 diagnostics in the ambulatory setting provides several advantages when compared to nasopharyngeal swabs (NPS), including ease of self-collection and reduced use of personal protective equipment (PPE). In addition saliva collection could be advantageous in advising if a convalescent patient is able to return to work after a period of self-quarantine. We investigated the utility of saliva collection in the community setting at Renown Health in a prospective Diagnostic Cohort of 88 patients and in a Convalescent Cohort of 24 patients. In the Diagnostic Cohort, we find that saliva collection has reduced sensitivity (~15% less) relative than NPS. And in our convalescent cohort of patients greater than 8 days and less than 21 days from first symptom, we find that saliva has ~ 50% sensitivity relative to NPS. Our results suggest that rigorous studies in the intended populations

should be performed before large-scale screening using saliva as the test matrix is initiated. [**note: this is a bit of a bummer but as we know, validation of any diagnostic test is imperative!!**] https://www.medrxiv.org/content/10.1101/2020.05.11.20092338v1

>>

2020-05-16

No introduction about today's music selection, just the name – Ella Fitzgerald: https://www.youtube.com/watch?v=lMFIejZgJSM

On top of yesterday's medical journal editorials, here is one that arrived in my mailbox after the newsletter was sent. This time it is the venerable New England Journal of Medicine discussing the tragic data gap underminng the US pandemic response.

Here is a commentary from two retired CDC scientists on the current state of affairs.

Derek Lowe has a very good post on the human immune response citing a number of the papers I have already highlighted. In all, this is good news, indicating a vaccine is likely to work quite well.

Here is more on the race for a SARS-CoV-2 vaccine from the Washington Post.

MODELING

- Objectives: Mortality from Covid-19 is monitored in detail both within as well as between countries with different strategies against the virus. However, death counts and relative risks based on crude numbers can be misleading. Instead, age specific death rates should be used for comparability. Given the difficulty of ascertainment of Covid-19 specific deaths, excess all-cause mortality is currently more appropriate for comparisons. By estimating age- and sex-specific death rates we aim to get more accurate estimates of the excess mortality attributed to Covid-19, as well as the difference between men and women in Sweden. Design: We make use of Swedish register data about total weekly deaths, total population at risk, and estimate age- and sex-specific weekly death rates for 2020 and the 5 previous years. The data is provided by Statistics Sweden. Results: From the first week of April and onwards, the death rates at all ages above 60 are higher than those in previous years in Sweden. Persons above age 80 are dis-proportionally more affected, and men suffer higher levels of excess mortality than women at all ages with 75% higher death rates for males and 50% higher for females. Current excess mortality corresponds to a decline in remaining life expectancy of 3 years for men and 2 years for women. Conclusion: The Covid-19 pandemic has so far had a clear and consistent effect on total mortality in Sweden, with male death rates being comparably more affected. What consequences the pandemic will eventually have on mortality and life expectancy will depend on the progression of the pandemic, the extent that some of the deaths would have occurred in

the absence of the pandemic, only somewhat later, the consequences for other health conditions, as well as the health care sector at large. [**note: Sweden has taken a different approach and this is the first paper I've seen that looks at the mortality statistics.**] https://www.medrxiv.org/content/10.1101/2020.05.10.20096909v1

NEWLY REGISTERED CLINICAL TRIALS

- We propose to evaluate the efficacy of Montelukast in attenuating cytokine storm syndrome and ARDS via a randomized, blinded, placebo-controlled clinical trial. Specifically, our primary objective is comparing the efficacy of low-dose Montelukast versus placebo in reducing the risk of acute care visits and hospital admissions among COVID-19 positive patients in the general population. Two of the main complications observed in severe patients are the development of acute respiratory distress syndrome (ARDS) and a hyperinflammatory cytokine profile that is often termed a cytokine storm. NF-B (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex involved in cytokine production, among other biological processes, including control of many genes involved in innate and adaptive immune responses, and inflammation. NF-B is known to be a key player in inflammatory responses and is a rapid-acting 'first responder' to harmful stimuli, such as viral infections. Inhibition of the NF-B signaling pathway has been explored for its therapeutic potential in inflammatory diseases, with attenuation of NF-B activation often associated with a reduction in cytokine production. Montelukast, an FDA and Health Canada approved asthma drug, has been shown to inhibit the signalling of NF-B, such as interleukin-6,8,10, TNF-alpha, MCP-1, and other proinflammatory mediators. Montelukast is available as a low-cost generic and has an excellent safety profile. We hypothesize that repurposing Montelukast to target suppression of NF-B activation in COVID-19 positive patients will result in a corresponding reduction of proinflammatory mediators, thereby attenuating cytokine production, and taming the cytokine storm. Additionally, we are proposing that a reduction in proinflammatory mediators by Montelukast will result in a mitigation of severe COVID-19 symptoms, and serve as a therapeutic for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, thereby alleviating severe complications/symptoms of the viral infection, such as ARDS and cytokine storm syndrome. [**note: ionically montelukast came up in some of the early AI studies of viral inhibition. Now we can see if it works.** NCT04389411
- Novartis have launched a trial of efficacy and safety of MAS825 for the treatment of SARS-CoV-2 infected patients with COVID-19 pneumonia and impaired respiratory function [**note: I have not found anything else about this compound.]** NCT04382651

- Over the past years, evidence has shown the effectivity of photobiomodulation therapy (PBMT) combined with static magnetic field (sMF) (PBMT/sMF) in delaying muscle fatigue, decrease in markers of inflammatory damage and oxidative stress of skeletal muscle. These effects result in an improvement in the functional capacity of the irradiated muscles by PBMT/sMF. However, do date, there is a lack of evidence regarding the effects of PBMT/sMF on the respiratory muscles. Therefore, the irradiation of PBMT/sMF may result in improvement in the functional capacity of respiratory muscles in patients with COVID-19, accelerating the ventilatory weaning process of the patients intubated due to respiratory failure. In addition, the irradiation of PBMT/sMF may induce the increase of anti-inflammatory mediators' activity in patients with COVID-19. Thus, the aim of this project is to investigate the effects of PBMT/sMF on respiratory muscles of

patients admitted to the Intensive Care Unit (ICU) with COVID-19 using invasive mechanical ventilation. [**note: I have no idea about the viability of this trial but the description is cool.**] NCT04386694

- I missed this one and was reminded by a Washington Post story today. NIAID is running a moderate 2000 patient clinical trial with hydroxychloroquine and azithromycin (A Randomized, Double-blind, Placebo-controlled Trial to Evaluate the Efficacy of Hydroxychloroquine and Azithromycin to Prevent Hospitalization or Death in Persons With COVID-19). [**note: WHY??? There are already a boatload of these trials going on. Is this one going to add anything more to the discussion?**] NCT04358068

CLINICAL TRIAL RESULTS

- Several related human coronaviruses (HCoVs) are endemic in the human population, causing mild respiratory infections. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the etiologic agent of Coronavirus disease 2019 (COVID-19), is a recent zoonotic infection that has quickly reached pandemic spread. Zoonotic introduction of novel coronaviruses is thought to occur in the absence of pre-existing immunity in the target human population. Using diverse assays for detection of antibodies reactive with the SARS-CoV-2 Spike (S) glycoprotein, we demonstrate the presence of pre-existing immunity in uninfected and unexposed humans to the new coronavirus. SARS-CoV-2 S-reactive antibodies, exclusively of the IgG class, were readily detectable by a sensitive flow cytometry-based method in SARS-CoV-2-uninfected individuals with recent HCoV infection and targeted the S2 subunit. In contrast, SARS-CoV-2 infection induced higher titres of SARS-CoV-2 S-reactive IgG antibodies, as well as concomitant IgM and IgA antibodies throughout the observation period of 6 weeks since symptoms onset. HCoV patient sera also variably reacted with SARS-CoV-2 S and nucleocapsid (N), but not with the S1 subunit or the receptor binding domain (RBD) of S on standard enzyme immunoassays. Notably, HCoV patient sera exhibited specific neutralising activity against SARS-CoV-2 S pseudotypes, according to levels of SARS-CoV-2 S-binding IgG and with efficiencies comparable to those of COVID-19 patient sera. Distinguishing pre-existing and de novo antibody responses to SARS-CoV-2 will be critical for serology, seroprevalence and vaccine studies, as well as for our understanding of susceptibility to and natural course of SARS-CoV-2 infection. [**note: don't know what to make of this one. It's interesting in that there may be some pre-existing humoral immunity and how did this arise? It may explain the differential clinical progressions in humans.**] https://www.biorxiv.org/content/10.1101/2020.05.14.095414v1
- Background. COVID-19 infection has limited preventive or therapeutic drug options at this stage. Some of common existing drugs like angiotensin-converting enzyme inhibitors (ACEi), angiotensin II receptor blockers (ARB) and the HMG-CoA reductase inhibitors (statins) have been hypothesised to impact on disease severity. However, up till now, no studies investigating this association were conducted in the most vulnerable and affected population groups, i.e. older people residing in nursing homes. The purpose of this study has been to explore the association of ACEi/ARB and/or statins with clinical manifestations in COVID-19 infected older people residing in nursing homes. Methods and Findings. We undertook a retrospective multi-centre cohort study in two Belgian nursing homes that experienced similar COVID-19 outbreaks. COVID-19 diagnoses were based on clinical suspicion and/or viral presence using PCR of nasopharyngeal samples. A total of 154 COVID-19 positive subjects was identified. The

outcomes were defined as 1) serious COVID-19 defined as a long-stay hospital admission (length of stay ≥ 7 days) or death (at hospital or nursing home) within 14 days of disease onset, and 2) asymptomatic, i.e. no disease symptoms in the whole study-period while still being PCR diagnosed. Disease symptoms were defined as any COVID-19-related clinical symptom (e.g. coughing, dyspnoea, sore throat) or sign (low oxygen saturation and fever) for ≥ 2 days out of 3 consecutive days. Logistic regression models with Firth corrections were applied on these 154 subjects to analyse the association between ACEi/ARB and/or statin use with the outcomes. Age, sex, functional status, diabetes and hypertension were used as covariates. Sensitivity analyses were conducted to evaluate the robustness of our statistical significant findings. We found a statistically significant association between statin intake and the absence of symptoms during COVID-19 infection (unadjusted OR 2.91; CI 1.27-6.71; p=0.011), which remained statistically significant after adjusting for age, sex, functional status, diabetes mellitus and hypertension. The strength of this association was considerable and clinically important. Although the effects of statin intake on serious clinical outcome (long-stay hospitalisation or death) were in the same beneficial direction, these were not statistically significant (OR 0.75; CI 0.25-1.85; p=0.556). There was also no statistically significant association between ACEi/ARB and asymptomatic status (OR 1.52; CI 0.62-3.50; p=0.339) or serious clinical outcome (OR 0.79; CI 0.26-1.95; p=0.629). Conclusions. Our data indicate that statin intake in old, frail people could be associated with a considerable beneficial effect on COVID-19 related clinical symptoms. The role of statins and any interaction with renin-angiotensin system drugs need to be further explored in larger observational studies as well as randomised clinical trials. **[note: from Belgium, statin medication may be beneficial in an elderly population though much more data is needed.**] https://www.medrxiv.org/content/10.1101/2020.05.11.20096347v1

DRUG DEVELOPMENT

- We developed a severe acute respiratory syndrome (SARS) subunit recombinant protein vaccine candidate based on a high-yielding, yeast-engineered, receptor-binding domain (RBD219-N1) of the SARS beta-coronavirus (SARS-CoV) spike (S) protein. When formulated with Alhydrogel®, RBD219-N1 induced high-level neutralizing antibodies against both pseudotyped virus and a clinical (mouse-adapted) isolate of SARS-CoV. Here, we report that mice immunized with RBD219-N1/Alhydrogel® were fully protected from lethal SARS-CoV challenge (0% mortality), compared to ~ 30% mortality in mice when immunized with the SARS S protein formulated with Alhydrogel®, and 100% mortality in negative controls. An RBD219-N1 formulation Alhydrogel® was also superior to the S protein, unadjuvanted RBD, and AddaVax (MF59-like adjuvant)-formulated RBD in inducing specific antibodies and preventing cellular infiltrates in the lungs upon SARS-CoV challenge. Specifically, a formulation with a 1:25 ratio of RBD219-N1 to Alhydrogel® provided high neutralizing antibody titers, 100% protection with non-detectable viral loads with minimal or no eosinophilic pulmonary infiltrates. As a result, this vaccine formulation is under consideration for further development against SARS-CoV and other emerging and re-emerging beta-CoVs such as SARS-CoV-2. [**note: this is the vaccine that was mentioned the other day against the first SARS virus. The yeast production system is well documented having previously been used for Hepatitis B.**]
https://www.biorxiv.org/content/10.1101/2020.05.15.098079v1

- Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) for which a vaccine is urgently needed to control its spreading. To facilitate the representation of a native-like immunogen without being infectious, here, we reported a SARS-CoV-2 vaccine candidate (designated ShaCoVacc) by incorporating spike-encoding mRNA inside and decorating spike protein on the surface of the virus simulating particles (VSPs) derived from lentiviral particles. We characterized the mRNA copy number, glycosylation status, transduction efficiency, and innate immune property of the new vaccine platform. Importantly, we showed the ShaCoVacc induced strong spike-specific humoral immune responses and potent neutralizing activities by a single injection. Additionally, we disclosed the epitopes of spike-specific antibodies using peptide microarray and revealed epitopes susceptible to specific neutralizing antibodies. These results support further development of ShaCoVacc as a candidate vaccine for COVID-19 and VSP may serve as a new vaccine platform for emerging infectious diseases. [**note: this is a new approach to creating a SARS-CoV-2 vaccine. Good luck with the project!**]
  https://www.biorxiv.org/content/10.1101/2020.05.14.093054v1
- The ongoing Corona Virus Disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has emphasized the urgent need for antiviral therapeutics. The viral RNA-dependent-RNA-polymerase (RdRp) is a promising target with polymerase inhibitors successfully used for the treatment of several viral diseases. Here we show that Favipiravir exerts an antiviral effect as a nucleotide analogue through a combination of chain termination, slowed RNA synthesis and lethal mutagenesis. The SARS-CoV RdRp complex is at least 10-fold more active than any other viral RdRp known. It possesses both unusually high nucleotide incorporation rates and high-error rates allowing facile insertion of Favipiravir into viral RNA, provoking C-to-U and G-to-A transitions in the already low cytosine content SARS-CoV-2 genome. The coronavirus RdRp complex represents an Achilles heel for SARS-CoV, supporting nucleoside analogues as promising candidates for the treatment of COVID-19. [**note: good information of favpiravir, an oral anti-viral.**]
  https://www.biorxiv.org/content/10.1101/2020.05.15.098731v1
- Broadly protective vaccines against known and pre-emergent coronaviruses are urgently needed. Critical to their development is a deeper understanding of cross-neutralizing antibody responses induced by natural human coronavirus (HCoV) infections. Here, we mined the memory B cell repertoire of a convalescent SARS donor and identified 200 SARS-CoV-2 binding antibodies that target multiple conserved sites on the spike (S) protein. A large proportion of the antibodies display high levels of somatic hypermutation and cross-react with circulating HCoVs, suggesting recall of pre-existing memory B cells (MBCs) elicited by prior HCoV infections. Several antibodies potently cross-neutralize SARS-CoV, SARS-CoV-2, and the bat SARS-like virus WIV1 by blocking receptor attachment and inducing S1 shedding. These antibodies represent promising candidates for therapeutic intervention and reveal a new target for the rational design of pan-sarbecovirus vaccines. [**note: more good information on the isolation of potential mAbs.**]
  https://www.biorxiv.org/content/10.1101/2020.05.15.096511v1 and even more from these UMass researchers: https://www.biorxiv.org/content/10.1101/2020.05.15.096719v1
- However, little is known about the human antibody response to SARS-CoV-2. Here we report on 68 COVID-19 convalescent individuals. Plasmas collected an average of 30 days after the onset of symptoms had variable half-maximal neutralizing titers ranging from undetectable in 18% to

below 1:1000 in 78%, while only 3% showed titers >1:5000. Antibody cloning revealed expanded clones of RBD-specific memory B cells expressing closely related antibodies in different individuals. Despite low plasma titers, antibodies to distinct epitopes on RBD neutralized at half-maximal inhibitory concentrations (IC50s) as low as few ng/mL. Thus, most convalescent plasmas obtained from individuals who recover from COVID-19 without hospitalization do not contain high levels of neutralizing activity. Nevertheless, rare but recurring RBD-specific antibodies with potent antiviral activity were found in all individuals tested, suggesting that a vaccine designed to elicit such antibodies could be broadly effective. [**note: more on antibodies and this one is interesting in that a lot of convalescent plasma does not contain high levels of neutralizing antibodies.**]
https://www.biorxiv.org/content/10.1101/2020.05.13.092619v1

DIAGNOSTIC DEVELOPMENT

- No news today.

>>

2020-05-17

For reflection Sunday, one of the masterpieces of Western Music. To me Bach is the embodiment of spirituality. Looking at his vast output, there had to be something more than musical genius and his B minor Mass is a piece I can listen to over and over. Here is a French group, Ensemble Pygmalion, playing on period instruments with a great choir and soloists:
https://www.youtube.com/watch?v=D5ttwUX4zWg

Even the Libertarian economists are getting on the testing bandwagon!! Here is a very nice Washington Post Op-Ed by Alex Tabarrok and Puja Ahluwalia Ohlhaver on the need to ramp up testing. The call for more money to be directed towards more public health people is resonating. Let's get on with it!!!!

Boston is not waiting around and has done their own city wide testing. One in ten have antibodies and 1 in 40 asymptomatic individuals tested positive for the virus. I suspect the 1 in 10 ratio for antibodies is the base line scenario and it may be higher as more testing is completed.

This is an accurate and sad op-ed from STAT about the loss of scientific decision-making in the present Administration. It's well worth reading and pondering on how we arrived at this point.

There are some very good abstracts in the Clinical Trials section today.

MODELING

- Interesting conjecture from Israel. Discovering the mechanism that enables pre-symptomatic individuals to transmit the SARS-CoV-2 virus has a significant impact on the possibility of controlling COVID-19 pandemic. To this end, we have developed an evidence based quantitative

mechanistic mathematical model. The model explicitly tracks the dynamics of contact and airborne transmission between individuals indoors, and was validated against the observed fundamental attributes of the epidemic, the secondary attack rate (SAR) and serial interval distribution. Using the model we identified the dominant driver of pre-symptomatic transmission, which was found to be contact route, while the contribution of the airborne route is negligible. We provide evidence that a combination of rather easy to implement measures of frequent hand washing, cleaning fomites and avoiding physical contact decreases the risk of infection by an order of magnitude, similarly to wearing masks and gloves. [**note: I assume that they are not figuring in coughing or sneezing from carriers which ought to transmit viral particles to a naïve individual. I on my second refill of the fine Clorox product Formula 409 right now. It cleans everything and I really should have bought more Clorox stock back in early March.**] https://www.medrxiv.org/content/10.1101/2020.05.12.20099085v1

- To optimize epidemiologic interventions, predictors of mortality should be identified. The US COVID-19 epidemic data, reported up to 31 March 2020, were analyzed using kernel regularized least squares regression. Six potential predictors of mortality were investigated: (i) the number of diagnostic tests performed in testing week I; (ii) the proportion of all tests conducted during week I of testing; (iii) the cumulative number of (test-positive) cases through 3-31-2020, (iv) the number of tests performed/million citizens; (v) the cumulative number of citizens tested; and (vi) the apparent prevalence rate, defined as the number of cases/million citizens. Two metrics estimated mortality: the number of deaths and the number of deaths/million citizens. While both expressions of mortality were predicted by the case count and the apparent prevalence rate, the number of deaths/million citizens was ≈3.5 times better predicted by the apparent prevalence rate than the number of cases. In eighteen states, early testing/million citizens/population density was inversely associated with the cumulative mortality reported by 31 March, 2020. Findings support the hypothesis that early and massive testing saves lives. Other factors —e.g., population density— may also influence outcomes. To optimize national and local policies, the creation and dissemination of high resolution geo-referenced, epidemic data is recommended. [**note: to restate the obvious, TEST, TEST, TEST**] https://www.medrxiv.org/content/10.1101/2020.05.14.20102483v1
- Knowing the true infected and symptomatic case fatality ratios (IFR and CFR) for COVID-19 is of high importance for epidemiological modeling and public health planning, but is difficult to calculate for countries and regions where there is limited testing for the disease. The large majority of reports have used modeling to correct the reported values for missed infections. However even for the same region, a wide range in the calculated correction factors have been proposed, for example from 6.6 to 50 for the United States which presently has a non-corrected CFR of 5.96%. The large correction factors have been justified based on findings in countries with extensive testing and case tracking, and therefore likely to capture most symptomatic cases, of CFR values below 1.0% early in their outbreaks. However as of May 7, 2020, the reported CFR values for 7 of these countries had risen several fold and had a wide CFR range of 0.6 - 4.3%. We tested whether this variation could be explained based upon a common age dependence of the CFR and the age distribution of cases in each country. Methods. We calculated the corrected CFR for these 7 countries using standard time between diagnosis to death methods and a new method that uses the closed case CFR time course. Corrected CFR values for cases between ages 0 - 69, 70 - 79, and 80 and above were then separately calculated

for each country. A linear model was generated that predicts the total CFR based on the mean and variation of these coefficients and the age distribution of cases. The model was tested by linear regression of each countries CFR against case percentage 70 and over. It was further tested by calculating the CFR and IFR for China and New York City and calculating the percent of the population that has been infected by COVID-19 based on number of deaths up to April 22, 2020. Results. Corrected CFR values by both methods were consistent and ranged from 0.58% to 5.0%. The large majority of deaths in each country were in the 70 and above groups (81% +/- 8%). Despite the range in corrected CFR values, 89% of the variation was explained by age distribution. Using the linear model, we calculated an IFR for NYC of 1.80% (95% CI of mean: 1.36% - 2.23%) and predicted that as of April 22, 2020 between 14.69% and 22.01% of the adult population in NYC had been infected by COVID-19. This prediction was in good agreement with recent studies performing random testing (15.3% - 21%). In contrast, previous IFR estimates predicted a minimum infected population percentage between 29% - 222%. Conclusion. We conclude that a model using age specific corrected CFR values from countries with extensive testing, based on the total to positive test ratio, provides a conservative but robust estimate of the true CFR and IFR values that is between 2 to 14 fold higher than previous estimates for the US. Furthermore, there is an extraordinarily high dependence of CFR with age that should be taken into account in measures targeted at mitigating the health and societal impact of the pandemic. [**note: we already knew about the age dependent mortality and I'm not sure that there is any good data from any country to really identify the correct CRF.**]
https://www.medrxiv.org/content/10.1101/2020.05.13.20101022v1

- The disease is thought to spread mainly from person-to-person through respiratory droplets produced when an infected person coughs, sneezes, or talks. The pathogen of COVID-19 is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It infects the cells binding to the angiotensin-converting enzyme 2 receptor (ACE2) which is expressed by cells throughout the airways as targets for cellular entry. Although the majority of persons infected with SARS-CoV-2 experience symptoms of mild upper respiratory tract infection, in some people infections of the peripheral airways result in severe, potentially fatal pneumonia. However, the induction of COVID-19 pneumonia requires that SARS-CoV-2 reaches the peripheral airways. While huge efforts have been made to understand the spread of the disease as well as the pathogenesis following cellular entry, much less attention is paid how SARS-CoV-2 from the environment reach the receptors of the target cells. The aim of the present study is to characterize the deposition distribution of SARS-CoV-2 in the airways upon exposure to cough-generated aerosol. For this purpose, the Stochastic Lung Deposition Model has been applied. Aerosol size distribution and breathing parameters were taken from the literature supposing normal breathing through the nose. We found that the probability of direct infection of the peripheral airways due to inhalation of aerosol generated by a bystander cough is very low. As the number of pathogens deposited in the extrathoracic airways is ~10 times higher than in the peripheral airways, we concluded that in most cases COVID-19 pneumonia must be preceded by SARS-CoV-2 infection of the upper airways. Our results suggest that without the enhancement of viral load in the upper airways, COVID-19 would be much less dangerous. *The period between the onset of initial symptoms and the potential clinical deterioration could provide an opportunity for prevention of pneumonia by blocking or significantly reducing the transport of viruses towards the peripheral airways. Coughing into a tissue or cloth even at home in order to absorb the*

emitted aerosol is highly recommended to avoid the continuous re-inhalation of own cough. [**note: wise words from these Hungarian scientists, don't re-inhale your own cough!!!]** https://www.medrxiv.org/content/10.1101/2020.05.13.20100057v1

- Research suggests that epidemics and corresponding containment measures have negative consequences to the individual and cause stress. The psychological mechanisms that determine stress, caused by the COVID-19 pandemic and containment measures, are not yet clear. In a survey during the lockdown in Switzerland (n=1565), we found substantially increased levels of stress in the population. In particular, individuals who did not agree with the containment measures, as well as those who saw nothing positive in the crisis, experienced even higher levels of stress. In contrast, individuals who are part of a risk group or who are working in healthcare or in essential shops experienced similar stress levels as the general public. We conducted a path analysis to gain a deeper understanding of the psychological mechanisms during lockdown. Experiencing fear of the disease is a key driver for being worried. Our model further shows that worries about the individual, social, and economic consequences of the crisis, strongly boost stress. *The infection rate in the canton (i.e. state) of residence also contributes to stress. Positive thinking and perceived social, organizational, and governmental support mitigate worries and stress. To prevent stress, authorities should explain containment measures well, highlight positive aspects of the crisis, address worries, and facilitate support.* [**note: WISE WORDS FOR OUR STATE AND NATIONAL LEADERS!!**] https://www.medrxiv.org/content/10.1101/2020.05.13.20100313v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- The abnormal liver function and even liver failure related death were reported in the COVID-19 patients, but less of studies focus on the dynamic liver function changes. We analysed the liver function indexes of COVID-19 patients to explore the characteristics of liver function changes in patients with different severity. Methods: This study included 54 moderate, 50 severe, and 31 death nucleic acid-confirmed COVID-19 patients hospitalized at the central hospital of Wuhan, China. Epidemiological histories, clinical features, imaging materials, medications and especially major liver function laboratory tests were collected for analysis. Results: The clinical symptoms did not present any significant difference in the patients at admission, but the older male patients had pronounced mortality risk. The normal ratio of ALT, TB, and DBIL of moderate patients was 96.3%, 94.44%, and 98.15% separately at the first test, but 59.26% of patients showed declined ALB levels. The normal ratio of all liver function indexes declined after admission, but most abnormalities were mild (1-2 times of upper limit unit) and went back normal before discharge. In severe patients, the normal ratio of ALB dropped down to 30.61% at admission along with the dramatic impaired normal ratio of bilirubin at the second test. The severe patients liver function dysfunction was worse than the moderate patients but without a significant difference. The dead patients showed a significantly higher level of DBIL, AST, GGT and CRP than other groups patients in the final test, along with the hypoalbuminemia. What is worse, 16.13% of non-survivors were diagnosed with liver failure. No medication was found to be related to ALT, AST, and GGT abnormality in our study. Conclusion: In moderate and severe

patients, liver dysfunction was mild. Patients widely presented lower level of ALB. The higher level of bilirubin, AST, and GGT was likely to indicate the worse outcome. Dynamic monitoring of liver function indexes could be considered and liver failure related death should be noticed and prevented in the early stage. [**note: more important clinical information, this time liver function decay in seriously ill patients.**]
https://www.medrxiv.org/content/10.1101/2020.05.13.20099614v1

- Of those patients afflicted in the United States, many have required treatment with ventilator secondary to acute respiratory distress syndrome (ARDS). Data are needed regarding the benefit of treatment and prevention of the cytokine storms in COVID-19 patients with Tocilizumab. Methods: Clinical outcomes data for patients admitted to Orange Regional Medical Center with confirmed COVID-19 from Mar 15, 2020 to Apr 20, 2020 were identified through electronic health record chart review. We conducted a retrospective case-control study in confirmed COVID 19 positive patients with ARDS requiring mechanical ventilation and compared outcome in terms of mortality and length of stay amongst those who received Tocilizumab as treatment modality opposed to those that did not. Results: A total of 94 patients with COVID-19 ARDS were analyzed. 44 were in the study group and 50 in the control group. We tried to match both group as close as possible in terms of age, sex, BMI and HS score- calculated using inflammatory markers- ferritin, triglycerides, AST and fibrinogen. The median age was 55.5 years in the study group and 66 in the control group, difference was not statistically significant. Average HS score was 114 in the Tocilizumab group and 92 in the control group, difference was statistically significant with P<0.0001. Also, the patients in the study group had elevated levels of IL-6, triglycerides, AST, ferritin which were statistically significant with $p < 0.0001$ when compared to the control group. *Length of stay was longer, average 17.9 days in the Tocilizumab. Survival rate was much lower at 48 % in the control group and 61.36 % in patients who received Tocilizumab with significant P value of < 0.00001. The number needed to treat (NNT) was 7.48, if we treat 8 patients with Tocilizumab, 1 will not die.* Conclusions: Cytokine Release Syndrome (CRS) occurs in a large number of patients with severe COVID-19, which is also an important cause of death. IL- 6 is the key molecule of CRS, so IL-6R antagonist Tocilizumab may be of value in improving outcomes. In our study Tocilizumab group seemed to have improved survival outcome. Results have to be interpreted with caution since this is a retrospective study and mortality is affected by multiple, confounding factors. We await the results of ongoing randomized controlled trials to definitely answer the question of whether Tocilizumab improves survival in COVID-19 ARDS patients. [**note: this data comes from a regional Hudson Valley, NY center. The clinical trials should confirm whether these results hold up.**]
https://www.medrxiv.org/content/10.1101/2020.05.13.20100081v1
- Spanish case-fatality rate is 11.86%, far higher to those reported in Asia or by other European countries. A multicenter retrospective study of demographic, clinical, laboratory and immunological features of 574 Spanish COVID-19 inpatients (59.4% males) and their outcomes was performed. 27.7% cases presented a mild curse, 42% a moderate one and 30.3%, severe. Ages ranged from 18 to 98 (average 63.2). Interleukin 6 was higher as increasing severity. On the other hand, CD8 lymphocyte count was significantly lower as severity grew and subpopulations CD4, CD8, CD19 and NK showed concordant lowering trends. Severity-related natural killer percent descents were evidenced just within aged cases. A significant severity-related decrease of CD4 lymphocytes was found in males. The use of renin-angiotensin system blockers was

associated with moderate or mild disease courses. Clinical course of the disease is more severe in this study than in previous literature cohorts. Age and age-related comorbidities, such as dyslipidemia, hypertension or diabetes, were also higher. Immunosenescence might be therefore a suitable explanation for immune system effectors severity-related hampering. Adaptive immunity would go exhausted and a huge ineffective and almost deleterious innate response would account for COVID-19 severity. Hypertensive patients treated with renin-angiotensin system blockers developed milder forms of the disease. [**note: case level aggregate data from Spain is beginning to come in. Interesting finding that renin-angiotensin system blockers seemed to be mildly protective.**]
https://www.medrxiv.org/content/10.1101/2020.05.13.20101345v1

- Previous studies suggest a role for systemic reprogramming of host metabolism during viral pathogenesis to fuel rapidly expanding viral proliferation, for example by providing free amino acids and fatty acids as building blocks. In addition, general alterations in metabolism can provide key understanding of pathogenesis. However, little is known about the specific metabolic effects of SARS-COV-2 infection. The present study evaluated the serum metabolism of COVID-19 patients (n=33), identified by a positive nucleic acid test of a nasopharyngeal swab, as compared to COVID-19-negative control patients (n=16). Targeted and untargeted metabolomics analyses specifically identified alterations in the metabolism of tryptophan into the kynurenine pathway, which is well-known to be involved in regulating inflammation and immunity. Indeed, the observed changes in tryptophan metabolism correlated with serum interleukin-6 (IL-6) levels. Metabolomics analysis also confirmed widespread dysregulation of nitrogen metabolism in infected patients, with decreased circulating levels of most amino acids, except for tryptophan metabolites in the kynurenine pathway, and increased markers of oxidant stress (e.g., methionine sulfoxide, cystine), proteolysis, and kidney dysfunction (e.g., creatine, creatinine, polyamines). Increased circulating levels of glucose and free fatty acids were also observed, consistent with altered carbon homeostasis in COVID-19 patients. Metabolite levels in these pathways correlated with clinical laboratory markers of inflammation and disease severity (i.e., IL-6 and C-reactive protein) and renal function (i.e., blood urea nitrogen). In conclusion, this initial observational study of the metabolic consequences of COVID-19 infection in a clinical cohort identified amino acid metabolism (especially kynurenine and cysteine/taurine) and fatty acid metabolism as correlates of COVID-19, providing mechanistic insights, potential markers of clinical severity, and potential therapeutic targets. [**note: yet more markers, this time amino acid metabolism.**] https://www.medrxiv.org/content/10.1101/2020.05.14.20102491v1
- It is known that exacerbated inflammation and dysregulated immune responses involving T and myeloid cells occur in COVID-19 patients with severe clinical progression. However, the differential contribution of specific subsets of dendritic cells and monocytes to ARDS is still poorly understood. In addition, the role of CD8+ T cells present in the lung of COVID-19 patients and relevant for viral control has not been characterized. With the aim to improve the knowledge in this area, we developed a cross-sectional study, in which we have studied the frequencies and activation profiles of dendritic cells and monocytes present in the blood of COVID-19 patients with different clinical severity in comparison with healthy control individuals. Furthermore, these subpopulations and their association with antiviral effector CD8+ T cell subsets were also characterized in lung infiltrates from critical COVID-19 patients. Collectively, our results suggest that inflammatory transitional and non-classical monocytes preferentially

migrate from blood to lungs in patients with severe COVID-19. CD1c+ conventional dendritic cells also followed this pattern, whereas CD141+ conventional and CD123hi plasmacytoid dendritic cells were depleted from blood but were absent in the lungs. Thus, this study increases the knowledge on the pathogenesis of COVID-19 disease and could be useful for the design of therapeutic strategies to fight SARS-CoV-2 infection. [**note: more from Spain, this time activation of dendritic cells and monocytes in the blood of COVID-19 patients.**] https://www.medrxiv.org/content/10.1101/2020.05.13.20100925v1

- Olfactory and taste dysfunctions have emerged as symptoms of COVID-19. Among individuals with COVID-19 enrolled in a household study, loss of taste and/or smell was the fourth most commonly reported symptom (26/42; 62%), and among household contacts, it had the highest positive predictive value (83%; 95% CI: 55-95%) for COVID-19. These findings support consideration of loss of taste and/or smell in possible case identification and testing prioritization for COVID-19. [**note to self: time to work on the COVID-19 Scent Strip™ and advance the public health**.] https://www.medrxiv.org/content/10.1101/2020.05.13.20101006v1 and also this one from China, France and Germany!!! https://www.medrxiv.org/content/10.1101/2020.05.13.20100198v1
- Elevated levels of inflammatory cytokines have been associated with poor outcomes among COVID-19 patients. It is unknown, however, how these levels compare to those observed in critically ill patients with ARDS or sepsis due to other causes. Objectives: To directly compare plasma levels of inflammatory cytokines, with a focus on 6 cytokines associated with cytokine storm (IL-1b, IL-1RA, IL-6, IL-8, IL-18, and TNFα), between hospitalized COVID-19 patients and banked plasma samples from ARDS and sepsis patients from prior to the COVID-19 pandemic. Findings: 15 hospitalized COVID-19 patients, 9 of whom were critically ill, were compared to 28 critically ill patients with ARDS or sepsis. There were no statistically significant differences in baseline levels of IL-1b, IL-1RA, IL-6, IL-8, IL-18, and TNFα between patients with severe COVID-19 and critically ill controls with ARDS or sepsis. Conclusions: Levels of inflammatory cytokines IL-1b, IL-1RA, IL-6, IL-8, IL-18, and TNFα were not higher in critically ill COVID-19 patients than in critically ill patients admitted with ARDS or sepsis due to other causes in this small cohort. Broad use of immunosuppressive therapies in ARDS has failed in numerous Phase 3 studies; use of these therapies in unselected patients with COVID-19 is likely unwarranted. [**note: cautionary information from Stanford as they compare cytokine levels in COVID-19 patients to those with sepsis or ARDS. It's a small sample and as with everything, we need to wait for better clinical data.**] https://www.medrxiv.org/content/10.1101/2020.05.15.20103549v1
- The severity of the new COVID-19 pandemic caused by the SARS-CoV-2 virus is strikingly variable in different global populations. SARS-CoV-2 uses ACE2 as a cell receptor, TMPRSS2 protease, and FURIN peptidase to invade human cells. Here, we investigated 1,378 whole-exome sequences of individuals from the Middle Eastern populations (Kuwait, Qatar, and Iran) to explore natural variations in the ACE2, TMPRSS2, and FURIN genes. We identified two activating variants (K26R and N720D) in the ACE2 gene that are more common in Europeans than in the Middle Eastern, East Asian, and African populations. We postulate that K26R can activate ACE2 and facilitate binding to S-protein RBD while N720D enhances TMPRSS2 cutting and, ultimately, viral entry. We also detected deleterious variants in FURIN that are frequent in the Middle Eastern but not in the European populations. This study highlights specific genetic variations in the ACE2 and

FURIN genes that may explain SARS-CoV-2 clinical disparity. We showed structural evidence of the functionality of these activating variants that increase the SARS-CoV-2 aggressiveness. Finally, our data illustrate a significant correlation between ACE2 variants identified in people from Middle Eastern origins that can be further explored to explain the variation in COVID-19 infection and mortality rates globally. [**note: Kuwait joins the list of cited abstracts. Interesting stuff about the genetic linkage and infection aggressiveness.**]
https://www.biorxiv.org/content/10.1101/2020.05.16.099176v1

DRUG DEVELOPMENT

- No news today.

DIAGNOSTIC DEVELOPMENT

- Scalable, inexpensive, accurate, and secure testing for SARS-CoV-2 infection is crucial for control of the novel coronavirus pandemic. Recently developed highly multiplexed sequencing assays that rely on high-throughput sequencing (HMSAs) can, in principle, meet these demands, and present promising alternatives to currently used RT-qPCR-based tests. However, the analysis and interpretation of HMSAs requires overcoming several computational and statistical challenges. Using recently acquired experimental data, we present and validate an accurate and fast computational testing workflow based on kallisto and bustools, that utilize robust statistical methods and fast, memory efficient algorithms for processing high-throughput sequencing data. We show that our workflow is effective at processing data from all recently proposed SARS-CoV-2 sequencing based diagnostic tests, and is generally applicable to any diagnostic HMSAs. [**note: another approach to large scale testing. Get it Done!**]
https://www.medrxiv.org/content/10.1101/2020.05.13.20100131v1