2020-04-06

The music selection for today is a lengthy one, Mahler's 8th symphony. I've seen this performed live on three occasions and always find it incredibly spiritual. This telecast is from the Amsterdam Concertgebouw, recorded last year: https://www.youtube.com/watch?v=nxf3xtH31jA Spectacular and wonderful singing from the soloists.

A quick update on ivermectin. I saw an OHDSI email noting that they will talk with the AMPATH group in Kenya about doing an observational trial as the drug is in use in that country.

Everyone was very busy over the weekend finalizing and posting pre-prints. I'm a little bleary eyed from reading abstracts (not to mention getting up early to go to the grocery/pharmacy to pick up supplies and prescriptions; and yes, they finally had toilet paper!!!). Lots of interesting stuff today. Just when I thought I would not post any more model papers, there a some that are worthy of perusal. Do take a look at the University of Pittsburg press release on their vaccine candidate. It's is the coolest delivery technology I've seen, micro spikes containing the antigen that penetrate the skin and dissolve, all on a small skin patch. Sign me up for the clinical trial, no screwball mRNA vaccines for me!!!

This doesn't fit into the categories I have set out but, here is an interesting paper regarding disinfection of used PPE for healthcare workers. We carried out a rapid review to summarize the existing evidence on SARS-CoV-2 survivorship and methods to disinfect PPE gear, particularly N95 filtering facepiece respirators (FFR). In the absence of data on SARS-CoV-2, we focused on the sister virus SARS-CoV-1. We propose a two-step disinfection process, which is conservative in the absence of robust evidence on SARS-CoV-2. This disinfection protocol is based on an initial storage of PPE for ≥4 days, followed by ultraviolet light (UVC), dry heat treatment, or chemical disinfection. Importantly, each of the two steps is based on independent disinfection mechanisms, so that our proposed protocol is a multiplicative system, maximising the efficacy of our disinfection process. This method could be rapidly implemented in other healthcare settings, while testing of each method is undertaken, increasing the frontline supply of PPE, and avoiding many of the upstream issues of supply chain disruption currently being faced. https://www.medrxiv.org/content/10.1101/2020.04.02.20051409v1

Here is a call from multiple investigators from all over of an International Consortium for Tracking Coronavirus Health Status [**an obvious and needed proposal if it is done correctly**] https://www.medrxiv.org/content/10.1101/2020.04.02.20051284v1

MODELING

- Here is a multi-author paper on the cryptic transmission of SARS-CoV-2 in Washington state. Interesting extrapolation to the first introduction. Here, we analyze 346 SARS-CoV-2 genomes from samples collected between 20 February and 15 March 2020 from infected patients in Washington State, USA. We found that the large majority of SARS-CoV-2 infections sampled during this time frame appeared to have derived from a single introduction event into the state in late January or early February 2020 and subsequent local spread, strongly suggesting cryptic spread of COVID-19 during the months of January and February 2020, before active community

surveillance was implemented. We estimate a common ancestor of this outbreak clade as occurring between 18 January and 9 February 2020. From genomic data, we estimate an exponential doubling between 2.4 and 5.1 days. These results highlight the need for large-scale community surveillance for SARS-CoV-2 introductions and spread and the power of pathogen genomics to inform epidemiological understanding.
https://www.medrxiv.org/content/10.1101/2020.04.02.20051417v1

- Another paper on the impact of higher atmospheric temperatures on SARS-CoV-2. I'm not sure how much to believe in these models given the huge outbreak in Ecuador. Seasonal temperature variation may impact the trajectories of COVID-19 in different global regions. Cumulative data reported by the World Health Organization, for dates up to March 27, 20201, show association between COVID-19 incidence and regions at or above 30° latitude. Historic climate data also show significant reduction of case rates with mean maximum temperature above approximately 22.5 degrees Celsius. Variance at the local level, however, could not be well explained by geography and temperature. These preliminary findings support continued countermeasures and study of SARS-CoV-2/COVID-19 transmission rates by temperature and humidity. https://www.medrxiv.org/content/10.1101/2020.04.02.20051524v1
- Here is a paper for all the epidemiologists, a UK group established a consortium for tracking the outbreak. We established the COronavirus Pandemic Epidemiology (COPE) consortium to bring together scientists with expertise in big data research and epidemiology to develop a COVID-19 Symptom Tracker mobile application that we launched in the UK on March 24, 2020 and the US on March 29, 2020 garnering more than 2.25 million users to date. This mobile application offers data on risk factors, herald symptoms, clinical outcomes, and geographical hot spots. This initiative offers critical proof-of-concept for the repurposing of existing approaches to enable rapidly scalable epidemiologic data collection and analysis which is critical for a data-driven response to this public health challenge.
https://www.medrxiv.org/content/10.1101/2020.04.02.20051334v1
- OK, put your face masks on before you go out in public!!! Here's an ecological study showing the benefit. We hypothesized that population level usage of face masks may be negatively associated SARS CoV2 spread. Methods At a country level, linear regression was used to assess the association between COVID19 diagnoses per inhabitant and the national promotion of face masks in public (coded as a binary variable), controlling for the age of the COVID19 epidemic and testing intensity. Results Eight of the 49 countries with available data advocated wearing face masks in public: China, Czechia, Hong Kong, Japan, Singapore, South Korea, Thailand and Malaysia. In multivariate analysis face mask use was negatively associated with number of COVID19 cases/inhabitant (coef. -326, 95% CI -601- -51, P=0.021). Testing intensity was positively associated with COVID-19 cases (coef. 0.07, 95% CI 0.05-0.08, P<0.001). Conclusion Whilst these results are susceptible to residual confounding, they do provide ecological level support to the individual level studies that found face mask usage to reduce the transmission and acquisition of respiratory viral infections. [**note: if you want to patronize a small business in the DC area, this T-Shirt company has converted to making masks: https://www.customink.com/**]
https://www.medrxiv.org/content/10.1101/2020.03.31.20048652v1
- Yes, do put on your face masks!!!! From NIH, Speaking may be a primary mode of transmission of SARS-CoV-2. Considering that reports of asymptomatic transmission account for 50-80% of

COVID-19 cases and that saliva has peak viral loads at time of patient presentation, droplet emission while speaking could be a significant factor driving transmission and warrants further study. We used a planar beam of laser light passing through a dust-free enclosure to detect saliva droplets emitted while speaking. We found that saying the words 'Stay Healthy' generates thousands of droplets that are otherwise invisible to the naked eye. A damp homemade cloth face mask dramatically reduced droplet excretion, with none of the spoken words causing a droplet rise above the background. Our preliminary findings have important implications for pandemic mitigation efforts.
https://www.medrxiv.org/content/10.1101/2020.04.02.20051177v1

- The Chinese researchers continue to amaze! Here is a new method for rapid identification of infection. Due to the lack of constructive information on the pathogenesis of COVID-19 and specific treatment, it highlights the importance of early diagnosis and timely treatment. In this study, 11 key blood indices were extracted through random forest algorithm to build the final assistant discrimination tool from 49 clinical available blood test data which were derived by commercial blood test equipments. The method presented robust outcome to accurately identify COVID-19 from a variety of suspected patients with similar CT information or similar symptoms, with accuracy of 0.9795 and 0.9697 for the cross-validation set and test set, respectively. The tool also demonstrated its outstanding performance on an external validation set that was completely independent of the modeling process, with sensitivity, specificity, and overall accuracy of 0.9512, 0.9697, and 0.9595, respectively. Besides, 24 samples from overseas infected patients with COVID-19 were used to make an in-depth clinical assessment with accuracy of 0.9167. After multiple verification, the reliability and repeatability of the tool has been fully evaluated, and it has the potential to develop into an emerging technology to identify COVID-19 and lower the burden of global public health. The proposed tool is well-suited to carry out preliminary assessment of suspected patients and help them to get timely treatment and quarantine suggestion. The assistant tool is now available online at http://lishuyan.lzu.edu.cn/COVID2019_2/ [**note: I have not investigated the tool**]
https://www.medrxiv.org/content/10.1101/2020.04.02.20051136v1

NEWLY REGISTERED CLINICAL TRIALS

- Nothing new today.

CLINICAL TRIAL RESULTS

- We don't have to wait for a SARS-CoV-2 vaccine; let's just do a mass BCG vaccine program. Here's another conjectural article. The amount of variance in cases and deaths explained by BCG vaccination policy ranged between 12.5% and 38%. Importantly, this effect remained significant after controlling for the country's life expectancy and the average temperature in February and March 2020, which themselves are significantly correlated with the cases and deaths indices, respectively. By contrast, the ratio between deaths and cases was weakly affected. This latter outcome suggested that BCG vaccination may have hindered the overall spread of the virus or progression of the disease rather than reducing mortality rates (i.e.,

deaths/cases ratio). Finally, by roughly dividing countries into three categories showing high, middle, or low growth rate of the cases, we found a highly significant difference between the slope categories among the BCG groups, suggesting that the time since the onset of the spread of the virus was not a major confounding factor. While this study potentially suffers from a number of unknown confounding factors, these associations support the idea that BCG vaccination may provide protection against SARS-CoV-2, which, together with its proven safety, encourages consideration of further detailed epidemiological studies, large-scale clinical trials on the efficacy of this vaccine on COVID-19, and/or re-introduction of BCG vaccination practice in the countries which are currently devoid of the practice. [**note: Personally, I want to get not the trial for the nifty Pittsburg patch vaccine!**]
https://www.medrxiv.org/content/10.1101/2020.03.30.20048165v1 &
https://www.medrxiv.org/content/10.1101/2020.04.01.20049478v1

- Another paper from China, this time looking at neutralizing antibody in recovered patients and the implications. The variations of SARS-CoV-2 specific NAbs in recovered COVID-19 patients may raise the concern about the role of NAbs on disease progression. The correlation of NAb titers with age, lymphocyte counts, and blood CRP levels suggested that the interplay between virus and host immune response in coronavirus infections should be further explored for the development of effective vaccine against SARS-CoV-2 virus. Furthermore, titration of NAb is helpful prior to the use of convalescent plasma for prevention or treatment.
https://www.medrxiv.org/content/10.1101/2020.03.30.20047365v1
- This is for the clinicians in the audience! More work from China with the development and validation of a diagnostic nomogram to predict SARS-COV-2 pneumonia. We used the LASSO aggression and multivariable logistic regression methods to explore the predictive factors associated with COVID-19 pneumonia, and established the diagnostic nomogram for COVID-19 pneumonia using multivariable regression. This diagnostic nomogram was assessed by the internal and external validation data set. Further, we plotted decision curves and clinical impact curve to evaluate the clinical usefulness of this diagnostic nomogram. RESULTS: The predictive factors including the epidemiological history, wedge-shaped or fan-shaped lesion parallel to or near the pleura, bilateral lower lobes, ground glass opacities, crazy paving pattern and white blood cell (WBC) count were contained in the nomogram. In the primary cohort, the C-statistic for predicting the probability of the COVID-19 pneumonia was 0.967, even higher than the C-statistic (0.961) in initial viral nucleic acid nomogram which was established using the univariable regression. The C-statistic was 0.848 in external validation cohort. Good calibration curves were observed for the prediction probability in the internal validation and external validation cohort. The nomogram both performed well in terms of discrimination and calibration. Moreover, decision curve and clinical impact curve were also beneficial for COVID-19 pneumonia patients. CONCLUSION: Our nomogram can be used to predict COVID-19 pneumonia accurately and favourably.
https://www.medrxiv.org/content/10.1101/2020.04.03.20052068v1
- From the Wuhan group, an observational study of viral and clinical characteristic for prognoses of severe SARS-CoV-2 infection. Patients with severe COVID-19 have prolonged SARS-CoV-2 infection and delayed intermittent viral shedding. Older age, hyperlipemia, hypoproteinemia, corticosteroid usage, and prolonged SARS-CoV-2 IgM positive might be utilized as predicative

factors for the patients with poor recovery.
https://www.medrxiv.org/content/10.1101/2020.04.03.20051763v1

- Important data is now coming out of Italy. This is an interesting paper looking at ACE2 genetic variants and the susceptibility to SARS-CoV-2. It has recently been shown that 2019-nCov utilizes host receptors namely angiotensin converting enzyme 2 (ACE2) as host receptor and host proteases for cell surface binding and internalization. Thus, a predisposing genetic background can give reason for interindividual disease susceptibility and/or severity. Taking advantage of the Network of Italian Genomes (NIG), here we mined around 7000 exomes from 5 different Centers looking for ACE2 variants. A number of variants with a potential impact on protein stability were identified. Among these, three missense changes, p.Asn720Asp, p.Lys26Arg, p.Gly211Arg (MAF 0.002 to 0.015), which have never been reported in the Eastern Asia population, were predicted to interfere with protein cleavage and stabilization. Rare truncating variants likely interfering with the internalization process and one missense variant, p.Trp69Cys, predicted to interfere with 2019-nCov spike protein binding were also observed. These findings suggest that a predisposing genetic background may contribute to the observed inter-individual clinical variability associated with COVID-19. They allow an evidence-based risk assessment opening up the way to personalized preventive measures and therapeutic options.
https://www.medrxiv.org/content/10.1101/2020.04.03.20047977v1

DRUG DEVELOPMENT

- Though it hasn't entered human trials yet, this University of Pittsburg vaccine candidate has a really cool delivery technology!!! https://www.upmc.com/media/news/040220-falo-gambotto-sars-cov2-vaccine They are also using a tried and true method of expressing viral proteins to elicit the immune response.
- The components in Truvada, tenofovir and emtricitabine may be useful in SARS-CoV-2 therapy. We previously demonstrated that four nucleotide analogues (specifically, the active triphosphate forms of Sofosbuvir, Alovudine, AZT and Tenofovir alafenamide) inhibit the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp). Tenofovir and emtricitabine are the two components in DESCOVY and TRUVADA, the two FDA-approved medications for use as pre-exposure prophylaxis (PrEP) to prevent HIV infection. This is a preventative method in which individuals who are HIV negative (but at high-risk of contracting the virus) take the combination drug daily to reduce the chance of becoming infected with HIV. PrEP can stop HIV from replicating and spreading throughout the body. We report here that the triphosphates of tenofovir and emtricitabine, the two components in DESCOVY and TRUVADA, act as terminators for the SARS-CoV-2 RdRp catalyzed reaction. These results provide a molecular basis to evaluate the potential of DESCOVY and TRUVADA as PrEP for COVID-19.
https://www.biorxiv.org/content/10.1101/2020.04.03.022939v1
- Another reason we need to rethink the clinical trial paradigm for pandemics!! The SARS-CoV-2 major protease (Mpro) is validated target over highly pathogenic CoV. HIV protease inhibitors, like lopinavir (LPV), also inhibit the 2002 SARS-CoV Mpro. However, limited evidence exist whether other clinically approved antiretroviral protease inhibitors may bind more efficiently to this enzyme to block SARS-CoV-2 replication. Among these substances, atazanavir (ATV) has

documented bioavailability into the respiratory tract, motivating further evaluation on its ability to impair SARS-CoV-2 replication. Here, we describe that ATV docks stronger to SARS-CoV-2 Mpro active site than LPV, occupying the substrate cleft in the active side during the entire molecular dynamic analysis. ATV blocked Mpro activity in cell-free based assays at 10µM. In vitro assays with different cell types, Vero cells, human pulmonary epithelial cell line and human primary monocytes, confirmed that ATV, combined or not with RTV, inhibited SARS-CoV-2 replication. ***Moreover, these drugs performed better than chloroquine, recognized for its antiviral and anti-inflammatory activities, to reduce virus-induced IL-6 and TNF-α levels***. Our data highlights that ATV and ATV/RTV could be considered among the repurposed drugs undergoing clinical trials against COVID-19.
https://www.biorxiv.org/content/10.1101/2020.04.04.020925v1

- Herein, we report the remarkable sensitivity of SARS-CoV-2 to recombinant human interferons α and β (IFNα/β). Treatment with IFN-α at a concentration of 50 international units (IU) per milliliter drastically reduces viral titers by 3.4 log or over 4 log, respectively, in Vero cells. The EC50 of IFN-α and IFN-β treatment is 1.35 IU/ml and 0.76 IU/ml, respectively, in Vero cells. These results suggest that SARS-CoV-2 is more sensitive than many other human pathogenic viruses, including SARS-CoV. Overall, our results demonstrate the potent efficacy of human Type I IFN in suppressing SARS-CoV-2 infection, a finding which could inform future treatment options for COVID-19. [**note: I believe there are some trials going on with these drugs**]
https://www.biorxiv.org/content/10.1101/2020.04.02.022764v2
- Here's an in vitro screening paper identifying a large number of potential therapeutics. The robustness of the screen was assessed by the identification of drugs, such as Chloroquine derivatives and protease inhibitors, already in clinical trials. The hits were sorted according to their chemical composition and their known therapeutic effect, then EC50 and CC50 were determined for a subset of compounds. Several drugs, such as Azithromycine, Opipramol, Quinidine or **Omeprazol** present antiviral potency with 2<EC50<20 micromolar. By providing new information on molecules inhibiting SARS-CoV-2 replication in vitro, this study could contribute to the short-term repurposing of drugs against Covid-19. [**note: I wonder if those taking omemprazole have some kind of protection. Good observational study to do**]
https://www.biorxiv.org/content/10.1101/2020.04.03.023846v1

DIAGNOSTIC DEVELOPMENT

- An Italian group compares a quick serological test developed in Singapore to the RT-PCR screen. We verified in a consecutive series of 191 symptomatic patients the clinical information that new rapid serological colorimetric test qualitatively analyzing IgM/IgG expression can provide with respect to standard assay and with respect to clinical outcome of patients. Results Rapid serological test showed a sensitivity of 30% and a specificity of 89% with respect to the standard assay but, interestingly, these performances improve after 8 days of symptoms appearance. After 10 days of symptoms the predictive value of rapid serological test is higher than that of standard assay. When the behaviour of the two immunoglobulins was evaluated with respect to time length of symptoms appearance, no significant difference in immunoglobulins behaviour was shown. Conclusions The rapid serological test analyzed in the present study is candidate to provide information on immunoreaction of the subject to COVID-19 exposure.]**note: My**

favorite hobby horse is: Big Time Massive Serological Testing!!!]
https://www.medrxiv.org/content/10.1101/2020.04.03.20052183v1

- Great outside the box thinking from a Seattle group!! The abstract really does not do justice to this paper. It's worth downloading and reading. The SARS-CoV-2 pandemic demonstrates the need for accurate and convenient approaches to diagnose and therapeutically monitor respiratory viral infections. We demonstrated that self-sampling with foam swabs at home is well-tolerated and provides quantitative viral output concordant with flocked swabs. Nasal cytokine levels correlate and cluster according to immune cell of origin. Periods of stable viral loads are followed by rapid elimination, which could be coupled with cytokine expansion and contraction using mathematical models. Nasal foam swab self-sampling at home provides a precise, mechanistic readout of respiratory virus shedding and local immune responses. https://www.medrxiv.org/content/10.1101/2020.04.03.20051706v1 & and a different Washington group's results: https://www.medrxiv.org/content/10.1101/2020.04.01.20049478v1

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2020-04-07

For today's musical selection, a delightful performance of Beethoven's Piano Concerto #5 by the young Spanish pianist, Rosalia Gomez Lasheras: https://www.youtube.com/watch?v=q-hn8WH_-3U I came across this one yesterday when I was reading abstracts. Just fine, precision pianism.

Looks like today's notes will be brief. I didn't see many new abstracts on the sites I read. There is a new vaccine going into trials and the company had some experience in developing a MERS vaccine several years ago. For more on hydroxychloroquine see Derek Lowe's post (and the comments range from informed to demented so I will leave it for those of you who are interested to read them: https://blogs.sciencemag.org/pipeline/archives/2020/04/06/hydroxychloroquine-update-for-april-6 In a post-script note, Derek links to a Swedish newspaper report that some hospitals are scaling back or suspending use of the drug because of AEs. It's a brief article and you can use Google translate if you don't read Swedish.

I'm beginning to wonder if we will ever get any good clinical data on hydroxychloroquine with and without azithromycin (not to mention emerging anecdotal data that zinc is required for best treatment results). One hopes that there will be a lot of good observational data at the end of the day provided good case records are taken of Rx and OTC drugs that hospitalized patients are on. Of course this doesn't addressed all the non-hospitalized patients who 'might' be on a chemo-protective drug and are not counted (losartan for example).

Stay safe, mask up and wash hands.

Alan

MODELING

- Nothing new

NEWLY REGISTERED CLINICAL TRIALS

- Another vaccine trial is launched, this one from Inovio Pharmaceuticals. A Phase 1 trial on 40 healthy patients will look at safety and immunogenicity. This is a plasmid delivered DNA vaccine and more details on it are in the company's press release: http://ir.inovio.com/news-and-media/news/press-release-details/2020/INOVIO-Initiates-Phase-1-Clinical-Trial-Of-Its-COVID-19-Vaccine-and-Plans-First-Dose-Today/default.aspx NCT04336410

CLINICAL TRIAL RESULTS

- According to some reports, data on remdesivir may be forthcoming. It's worth noting that the drug is administered IV and not orally so it's use will not be in broad community settings.

DRUG DEVELOPMENT

- Nothing New

DIAGNOSTIC DEVELOPMENT

- No new FDA EUAs issued. However, there are press reports that companies are bringing self-validated tests to the market in the US. The ones that I have read about run on auto-analyzers and there is potential for scale up. Here is a statement from Ortho Diagnostics: https://www.orthoclinicaldiagnostics.com/en-us/home/covid-19-antibody-test-released-by-ortho-clinical-diagnostics and one from Eurofins: https://www.prnewswire.com/news-releases/eurofins-us-clinical-diagnostics-network-launches-coronavirus-covid-19-antibody-testing-301035358.html I think the critical issue right now concerns validation of serological assays.

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2020-04-08

NO, NO, NO, I am not going to post any disco tunes!!! Don't wait for The Village People. However, here is ABBA singing for the Royal Family the night before the wedding of their son/daughter (I can't remember). Dancing Queen is one of my faves from that long last era:

https://www.youtube.com/watch?v=XN9lgrT8eVc (there will be no Elton John or Neil Diamond so don't hold your breath).

Here is a nice article on DIY mask making and what types of materials one ought to consider: https://www.nytimes.com/article/coronavirus-homemade-mask-material-DIY-face-mask-ppe.html Alas, my sewing skills are not up to this challenge.

Clinical trials are the best way to demonstrate safety and efficacy of drugs with the caveat that the full safety profile is not known at the time of approval given the number of those enrolled. https://www.city-journal.org/covid-19-clinical-trials-antimalarial-drugs is a concise description of the clinical trial process for the lay person. Most of you reading this won't find any surprises but you might want to forward the link on to friends who might not be as knowledgeable. Kudos to Dr. Ellen for writing this.

I'm still waiting for the deluge of serology test kits. Here is a statement from the FDA Commissioner: https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-serological-tests At the White House briefing yesterday, Dr. Birx stated that this continues to be a priority and people are working around the clock. I'll believe it when I see broad deployment of tests. Germany had a serology test a month ago and are using it for screening purposes.

There are some very interesting clinical trials listed below.

Stay Safe, Mask Up, Wash Hands!

Alan

MODELING

- No real developments. It's here, it's spreading, and we know how to control it.

NEWLY REGISTERED CLINICAL TRIALS

- No new trials

CLINICAL TRIAL RESULTS

- More observation data on BCG data and SARS-CoV-2. In the present study we compared the impact of COVID-19 in terms of case fatality rates (CFR) between countries with high disease burden and those with BCG revaccination policies presuming that revaccination practices would have provided added protection to the population against severe COVID-19. We found a significant difference in the CFR between the two groups of countries. Our data further supports the view that universal BCG vaccination has a protective effect on the course of COVID-19 probably preventing progression to severe disease and death. Clinical trials of BCG vaccine are urgently needed to establish its beneficial role in COVID-19 as suggested by the epidemiological data, especially in countries without a universal BCG vaccination policy. https://www.medrxiv.org/content/10.1101/2020.04.07.20053272v1
- Are these observational studies worth anything? I honestly don't know but here's another one looking at possible linkage of BCG vaccination and malaria prophylaxis. In this study we aimed

to analyse the relationship between malaria transmission and BCG vaccination with COVID-19 incidence in the world map. Materials and methods: We collected malaria cases data (World Health Organisation (WHO), 2018), worldwide COVID-19 cases and mortality data (European Centre for Disease Prevention and Control) and data on BCG vaccination. COVID-19 incidence and mortality was compared. Findings: *Data on 5316978938 persons from 166 countries were analysed.* [**note: really big data!!!** 😊] Malaria incidence rate was negatively correlated with COVID-19 incidence rate (correlation coefficient = -0.513, p<0.001). Malaria free countries had significantly higher number of COVID-19 cases compared to malaria endemic countries. In Europe and Americas, countries, which have higher BCG vaccination coverage, had significantly less mortality per thousand population compared to those with low BCG coverage (median 0.0002 (0-0.0005) vs 0.0029 (0.0002-0.0177), p=0.017). The case fatality ratio of COVID-19 was related nonlinearly to the malaria incidence. Conclusions: The results suggest the changing human immune system as we progress to eliminate parasitic diseases with time. Chloroquine exposure in malaria endemic zones might have a protective effect. https://www.medrxiv.org/content/10.1101/2020.04.03.20052563v1

- And more on BCG vaccination!! Prior work suggests that BCG vaccination reduces the risk of different infectious diseases. BCG vaccination may thus serve as a protective factor against COVID-19. Here, we drew on day-by-day reports of both confirmed cases and deaths and analyzed growth curves in countries that mandate BCG policies versus countries that do not. Linear mixed models revealed that the presence of mandated BCG policies was associated with a significant flattening of the exponential increase in both confirmed cases and deaths during the first 30-day period of country-wise outbreaks. This effect held after controlling for median age, gross domestic product per capita, population density, population size, geographic region, net migration rate, and various cultural dimensions (e.g., individualism and the tightness vs. looseness of social norms). Our analysis suggests that mandated BCG vaccination can be effective in the fight against COVID-19. https://www.medrxiv.org/content/10.1101/2020.04.05.20054163v1
- Here is an observational study from hard hit Iran on the clinical characteristic of infection in pregnant women. The current study is a systemic review and Meta-analysis to measure the risks and determine the presentations of COVID-19 in pregnant women and newborn. Methods: online data bases were searched on march 20. Heterogeneity of the included studies was assessed using the Cochran Q test and Higgins I2 statistic and expressed as percentage. All data were analyzed with 95% confidence intervals. Results: A total of 7 studies involving 50 participants with Positive test of COVID-19 were enrolled. Mean age of pregnant women was 30.57 years old and the Mean Gestational age was 36.9 weeks. Other variables such as Apgar score, birth weight, Sign and symptoms, Complications and Laboratory data were Analyzed. Conclusion: Our findings showed same clinical characteristics in pregnant women as in non-pregnant adults, with the main symptoms being cough and fever. No vertical transmission was seen and all patients delivered healthy neonates. Our findings would be of great help to the decision making process, regarding the management of pregnant women diagnosed with COVID-19. [**note: we need much more data from larger patient pools to confirm this. Still this is potentially a good sign**] https://www.medrxiv.org/content/10.1101/2020.04.05.20053983v1
- Here is a large cohort study on clinical and epidemiological characteristic of SARS-CoV-2 patients. A systematic review and pooled analysis was performed. Eligible studies were

identified from database and hand searches up to March 2, 2020. Data on clinical (including laboratory and radiological) and epidemiological (including demographic) characteristics of confirmed COVID-19 cases were extracted and combined by simple pooling. Results: Of 644 studies identified, 69 studies (**involving 48,926 patients**) were included in the analysis. The average age of the patients was 49.16 years. A total of 51.46% of the patients were men and 52.32% were non-smokers. Hypertension (50.82%) and diabetes (20.89%) were the most frequent comorbidities observed. The most common symptoms were fever (83.21%), cough (61.74%), and myalgia or fatigue (30.22%). Altered levels of blood and biochemical parameters were observed in a proportion of the patients. Most of the patients (78.50%) had bilateral lung involvements, and 5.86% showed no CT findings indicative of viral pneumonia. Acute respiratory distress syndrome (28.36%), acute cardiac injury (7.89%) and acute kidney injury (7.60%) were the most common complications recorded. Conclusions: Clinical and epidemiological characteristics of COVID-19 patients were mostly heterogeneous and non-specific. This is the most comprehensive report of the characteristics of COVID-19 patients to date. The information presented is important for improving our understanding of the spectrum and impact of this novel disease. https://www.medrxiv.org/content/10.1101/2020.04.02.20050989v1

- Here is a large UK observational study showing the linkage between loss of smell and viral infection. Design: Community survey. Setting and Participants: Subscribers of RADAR COVID-19, an app that was launched for use among the UK general population asking about COVID-19 symptoms. Main Exposure: Loss of smell and taste. Main Outcome Measures: COVID-19. Results: Between 24 and 29 March 2020, 1,573,103 individuals reported their symptoms via the app; 26% reported suffering from one or more symptoms of COVID-19. Of those, n=1702 reported having had a RT-PCR COVID-19 test and gave full report on symptoms including loss of smell and taste; 579 were positive and 1123 negative. In this subset, we find that loss of smell and taste were present in 59% of COVID-19 positive individuals compared to 18% of those negative to the test, yielding an odds ratio (OR) of COVID-19 diagnosis of OR[95%CI]=6.59[5.25; 8.27], P= 1.90x10-59 . We also find that a combination of loss of smell and taste, fever, persistent cough, fatigue, diarrhoea, abdominal pain and loss of appetite is predictive of COVID-19 positive test with sensitivity 0.54[0.44; 0.63], specificity 0.86[0.80; 0.90], ROC-AUC 0.77[0.72; 0.82] in the test set, and cross-validation ROC-AUC 0.75[0.72; 0.77]. When applied to the 410,598 individuals reporting symptoms but not formally tested, our model predicted that 13.06%[12.97%;13.15] of these might have been already infected by the virus. Conclusions and Relevance: Our study suggests that loss of taste and smell is a strong predictor of having been infected by the COVID-19 virus. Also, the combination of symptoms that could be used to identify and isolate individuals includes anosmia, fever, persistent cough, diarrhoea, fatigue, abdominal pain and loss of appetite. This is particularly relevant to healthcare and other key workers in constant contact with the public who have not yet been tested for COVID-19. https://www.medrxiv.org/content/10.1101/2020.04.05.20048421v1
- More data from China on predictive markers for severe disease progression. Here, we performed proteomic and metabolomic profiling of sera from 46 COVID-19 and 53 control individuals. We then trained a machine learning model using proteomic and metabolomic measurements from a training cohort of 18 non-severe and 13 severe patients. The model correctly classified severe patients with an accuracy of 93.5%, and was further validated using ten independent patients, seven of which were correctly classified. We identified molecular

changes in the sera of COVID-19 patients implicating dysregulation of macrophage, platelet degranulation and complement system pathways, and massive metabolic suppression. This study shows that it is possible to predict progression to severe COVID-19 disease using serum protein and metabolite biomarkers. Our data also uncovered molecular pathophysiology of COVID-19 with potential for developing anti-viral therapies.
https://www.medrxiv.org/content/10.1101/2020.04.07.20054585v1

DRUG DEVELOPMENT

- Looks like darunavir can be scratched from the list of possible drug candidates. Here's an in vitro study that shows no antiviral activity. Prezcobix/Rezolsta is a fixed-dose combination of 800 mg of the HIV protease inhibitor darunavir (DRV) and 150 mg cobicistat, a CYP3A4 inhibitor, which is indicated in combination with other antiretroviral agents for the treatment of HIV infection. There are currently no definitive data on the safety and efficacy of DRV/cobicistat for treatment of COVID-19. The in vitro antiviral activity of darunavir against a clinical isolate from a patient infected with SARS-CoV-2 was assessed. DRV showed no activity against SARS-CoV-2 at clinically relevant concentrations (EC^{50} >100 µM). Remdesivir, used as a positive control, showed potent antiviral activity (EC^{50} = 0.38 µM). Overall, the data do not support the use of DRV for treatment of COVID-19. https://www.medrxiv.org/content/10.1101/2020.04.03.20052548v1
- Here is another *in silico* study of possible therapeutics from the database of approved drugs. Sequainavir had the highest binding potential. They also tested darunavir which ranked second lowest amount the possible candidates and this one did not work as noted above. I haven't seen any comprehensive list of in vitro testing of repurposed drugs and likely one will come out retrospectively. https://www.medrxiv.org/content/10.1101/2020.04.05.20054254v1
- A Franco-American group suggests that the timing of antiviral treatment initiation is critical to reduce SARS-CoV-2 viral load. We modeled the viral dynamics of 13 untreated patients infected with SARS-CoV-2 to infer viral growth parameters and predict the effects of antiviral treatments. In order to reduce peak viral load by more than 2 logs, drug efficacy needs to be greater than 80% if treatment is administered after symptom onset; an efficacy of 50% could be sufficient if treatment is initiated before symptom onset. Given their pharmacokinetic/pharmacodynamic properties, current investigated drugs may be in a range of 20-70% efficacy. They may help control virus if administered very early, but may not have a major effect in severe patients. [**note: this has been my thinking and the focus on seriously ill patients has center on MAb and anti-cytokine storm therapy.**]
https://www.medrxiv.org/content/10.1101/2020.04.04.20047886v1

DIAGNOSTIC DEVELOPMENT

- Here is a nice paper from China discussing the optimization of a clinical serology test using the ration of IgM and IgG antibodies [**note: this is similar to past studies on this topic that I've seen**]. By reinterpreting the data in the article "Diagnostic Value of Combined Detection of Serum 2019 novel coronavirus IgM and IgG Antibodies in novel coronavirus in Infection", the positive likelihood ratio of IgM and IgG antibody in diagnosis of COVID-19 (nucleic acid positive

patients) was calculated, and the posterior probability of IgM and IgG antibodies and their tandem detection to diagnose was finally calculated. Results: The positive likelihood ratios of single IgM and IgG antibody were 18.50 and 12.65 respectively, and the posterior probabilities were 90.18% and 86.26% respectively. However, the posterior probability of the two antibodies tandem detection is 99.15%, which can give clinicians quantitative confidence in the diagnosis of COVID-19 from suspected cases. According to the results of this study, combining the advantages and disadvantages of nucleic acid detection and antibody detection, the clinical pathway for clinicians to diagnose COVID-19 is found. Conclusion: For suspected cases, IgM and IgG antibody tests should be firstly done at the same time. If the antibody tests are all positive, COVID-19 can be confirmed. If not, nucleic acid detection (one or more times) is performed, and in extreme cases, high-throughput viral genome sequencing is performed. https://www.medrxiv.org/content/10.1101/2020.04.07.20053660v1

- Here is a UK paper on estimating the false negative detection rate by RT-PCR. RT-PCR tests are highly specific and the probability of false positives is low, but false negatives can occur if the sample contains insufficient quantities of the virus to be successfully amplified and detected. The amount of virus in a swab is likely to vary between patients, sample location (nasal, throat or sputum) and through time as infection progresses. Here, we analyse publicly available data from patients who received multiple RT-PCR tests and were identified as SARS-CoV-2 positive at least once. We identify that the probability of a positive test decreases with time after symptom onset, with throat samples less likely to yield a positive result relative to nasal samples. Empirically derived distributions of the time between symptom onset and hospitalisation allowed us to comment on the likely false negative rates in cohorts of patients who present for testing at different clinical stages. We further estimate the expected numbers of false negative tests in a group of tested individuals and show how this is affected by the timing of the tests. Finally, we assessed the robustness of these estimates of false negative rates to the probability of false positive tests. This work has implications both for the identification of infected patients and for the discharge of convalescing patients who are potentially still infectious. https://www.medrxiv.org/content/10.1101/2020.04.05.20053355v1

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2020-04-09

Let's take another trip down Country lane. One of the finest singer/songwriters of our time is Kris Kristofferson (did you know he was both a Rhodes Scholar and an Army captain who passed ranger training?). His songs have been covered by singers of all genres. Here is 'Me and Bobby McGee' with Kris and group of stars from 2015: https://www.youtube.com/watch?v=5COkfKywjJA

I've seen more hydroxychloroquine trials registered than I can count with my old Abacus. I wonder when we will see some results. Check out the Canadian vaccine trial below, pretty cool *if* it works!

Here is a nice lay article on drug targets for SARS-CoV-2:
https://www.theatlantic.com/science/archive/2020/04/what-coronavirus-drug-will-look-like/609661/

Here is a nice warning letter for oral delivery of chlorine dioxide ("Miracle Mineral Solution"): https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-warns-seller-marketing-dangerous-chlorine-dioxide-products-claim You really need to read the press statement from FDA. Now I'm a big supporter of the use of bleach as the only stock in my portfolio that is positive on the year is Clorox. I have Clorox spray disinfectant and a two-quart bottle of Clorox bleach. I have never been tempted to dose myself orally; **nor should any of you reading this**.

Stay Safe, Mask Up, & Wash Hands

Alan

MODELING

- It's here, we know what to do, and it looks like social distancing is working.

NEWLY REGISTERED CLINICAL TRIALS

- Apeiron Biologics is launching a trial with recombinant human Angiotensin-converting Enzyme 2 as a treatment for patients with SARS-CoV-2. The presumption I it will block viral entry and decrease viral replication. Dosage is IV twice daily. NCT04335136
- Here is a Canadian vaccine trial by Symvivo Corporation. Protocol bacTRL-IL-Spike-1 will be the first-in-human study of bacTRL-Spike, and the first-in-human use of orally delivered bacTRL. Each oral dose of bacTRL-Spike contains bacterial medium with either 1 billion (Group 1A), 3 billion (Group 2A) or 10 billion (Group 3A) cfu of live Bifidobacterium longum, which has been genetically modified to express the spike protein from the SARS-CoV2 virus. Placebo will consist of bacterial medium without bacteria. Dosage is oral which is an interesting approach. [**note: I had not heard about this company before seeing this registered trial. More info is at the company's website: https://www.symvivo.com/ Eat yogurt and get vaccinated!!**] NCT04334980
- Here is a Dutch study looking at valsartan for presentation of ARDS in hospitalized patients with SARS-CoV-2. NCT04335786
- Here is an ultra-low cost approach to SARS-CoV-2 treatment. An English study proposes lipid formulated ibuprofen in the evaluation of severity and progression of lung injury. [**note: you cannot find acetaminophen on any pharmacy shelf in our area these days; will ibuprofen be next? We have more than enough naproxen which is also the subject of a CT**] NCT04334629
- Someone is looking at AEs!!! This retrospective study aims to perform a medication risk stratification using drug claims data and to simulate the impact of the addition of various repurposed drugs on the Medication Risk Score (MRS) in elderly people enrolled in PACE organizations. Our clinical tool would enable to identify potential multi-drug interactions and potentially reduce the risk of adverse drug events (ADE) developing in elderly patients infected with COVID-19. NCT04339634
- Isn't this what the fancy 'smart' watches do??? The same institution that brought us the scary outbreak model, Imperial College of London, aims to see if participant deterioration due to suspected coronavirus in a designated location (e.g. hotel) can be identified sooner by wearing

the sensor. If we can identify sick participants early, participants are more likely to have better outcomes; we believe that the sensor can help us do this. The sensor measures heart rate, respiratory rate and temperature every 2 minutes and this can be reviewed by the clinical team looking after the participants. [**note: I only have a smart phone, maybe I need to get a fit bit!**] NCT04337489

CLINICAL TRIAL RESULTS

- Again from the Wuhan group, a retrospective study of acute kidney injury (AKI) in SARS-CoV-2 patients. Results: A total of 287 patients, 55 with AKI and 232 without AKI, were included in the analysis. Compared to patients without AKI, AKI patients were older, predominantly male, and were more likely to present with hypoxia and have pre-existing hypertension and cerebrovascular disease. Moreover, AKI patients had higher levels of white blood cells, D-dimer, aspartate aminotransferase, total bilirubin, creatine kinase, lactate dehydrogenase, procalcitonin, C-reactive protein, a higher prevalence of hyperkalemia, lower lymphocyte counts, and higher chest computed tomographic scores. The incidence of stage 1 AKI was 14.3%, and the incidence of stage 2 or 3 AKI was 4.9%. Patients with AKI had substantially higher mortality. Conclusions: AKI is an important complication of COVID-19. Older age, male, multiple pre-existing comorbidities, lymphopenia, increased infection indicators, elevated D-dimer, and impaired heart and liver functions were the risk factors of AKI. AKI patients who progressed to stages 2 or 3 AKI had a higher mortality rate. Prevention of AKI and monitoring of kidney function is very important for COVID 19 patients. [**note: the OHDSI group is working on a schema for assessing AKI across medical records generally**]
https://www.medrxiv.org/content/10.1101/2020.04.06.20055194v1

DRUG DEVELOPMENT

- Merimepodib, an inhibitor of inosine-5'-monophosphae dehydrogenase, has *in vitro* activity against SARS-CoV-2. This drug has been around for several years and has been tested against a number of viruses. It is considered safe as more than 300 patients have been dosed in phase I and II clinical trials. We report here that MMPD suppresses SARS-CoV-2 replication in vitro. After overnight pretreatment of Vero cells with 10 μM of MMPD, viral titers were reduced by 4 logs of magnitude, while pretreatment for 4 hours resulted in a 3-log drop. The effect is dose-dependent, and concentrations as low as 3.3 μM significantly reduced viral titers when the cells were pretreated prior to infection. The results of this study provide evidence that MMPD may be a viable treatment option for COVID-19.
https://www.biorxiv.org/content/10.1101/2020.04.07.028589v1
- Here is an important paper from a Swiss/American group on the identification of a monoclonal antibody with potent neutralizing power against SARS-CoV-2. The paper notes that they plan on using this clinically but do not give any time estimation. Here we describe multiple monoclonal antibodies targeting SARS-CoV-2 S identified from memory B cells of a SARS survivor infected in 2003. One antibody, named S309, potently neutralizes SARS-CoV-2 and SARS-CoV pseudoviruses as well as authentic SARS-CoV-2 by engaging the S receptor-binding domain. Using cryo-electron

microscopy and binding assays, we show that S309 recognizes a glycan-containing epitope that is conserved within the sarbecovirus subgenus, without competing with receptor attachment. Antibody cocktails including S309 along with other antibodies identified here further enhanced SARS-CoV-2 neutralization and may limit the emergence of neutralization-escape mutants. These results pave the way for using S309 and S309-containing antibody cocktails for prophylaxis in individuals at high risk of exposure or as a post-exposure therapy to limit or treat severe disease. https://www.biorxiv.org/content/10.1101/2020.04.07.023903v1

- *In vitro* studies of potential inhibitors of SARS-CoV-2 are now coming out. Here, we report that nelfinavir, an HIV-1 protease inhibitor, potently inhibited replication of SARS-CoV-2. The effective concentrations for 50% and 90% inhibition (EC50 and EC90) of nelfinavir were 1.13 micro M and 1.76 micro M respectively, the lowest of the nine HIV-1 protease inhibitors including lopinavir. The trough and peak serum concentrations of nelfinavir were three to six times higher than EC50 of this drug. These results suggest that nelfinavir is a potential candidate drug for the treatment of COVID-19 and should be assessed in patients with COVID-19. [**note: It sure would be nice to have a multi-center clinical trial platform where drugs can quickly be put into trials and assessed. It's a better approach than recitation of anecdotes.**] https://www.biorxiv.org/content/10.1101/2020.04.06.026476v1

DIAGNOSTIC DEVELOPMENT

- Here is one interesting approach to large scale community testing for SARS-CoV-2. Here we propose LAMP-Seq, a barcoded Reverse-Transcription Loop-mediated Isothermal Amplification (RT-LAMP) protocol that could dramatically reduce the cost and complexity of population-scale testing. In this approach, individual samples are processed in a single heat step, producing barcoded amplicons that can be shipped to a sequencing center, pooled, and analyzed en masse. Using unique barcode combinations per sample from a compressed barcode space enables extensive pooling, significantly reducing cost and organizational efforts. Given the low cost and scalability of next-generation sequencing, we believe that this method can be affordably scaled to analyze millions of samples per day using existing sequencing infrastructure. https://www.biorxiv.org/content/10.1101/2020.04.06.025635v1
- And yet another scalable RT-PCR approach, We have developed a simplified qRT-PCR assay that removes the need for an RNA extraction process and can be run on a real-time thermal cycler. The assay uses custom primers and probes, and maintains diagnostic sensitivity within 98.0% compared to the assay run on a high-throughput, random-access automated platform, the Panther Fusion (Hologic). This assay can be used to increase capacity for COVID-19 testing for national programmes worldwide. https://www.biorxiv.org/content/10.1101/2020.04.06.028316v1
- Using flu and RSV clinical specimens, we have collected evidence that the RT-qPCR assay can be performed directly on patient sample material from a nasal swab immersed in virus transport medium (VTM) without an RNA extraction step. We have also used this approach to test for the direct detection of SARS-CoV-2 reference materials spiked in VTM. Our data, while preliminary, suggest that using a few microliters of these untreated samples still can lead to sensitive test results. If RNA extraction steps can be omitted without significantly affecting clinical sensitivity,

the turn-around time of COVID-19 tests and the backlog we currently experience can be reduced drastically. Next, we will confirm our findings using patient samples. https://www.biorxiv.org/content/10.1101/2020.04.06.028811v1

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2020-04-10

Today's music selection is a nice video performance of Schumann's Dichterliebe by the German soprano, Christine Schäfer: https://www.youtube.com/watch?v=L8d-d_-IF3k She is a wonderful artist, and gave the most stirring rendition of Schubert's Winterreise that I have ever seen in a live performance. I know Dichterliebe quite well having performed it in recital but this erotically charged version is well worth watching.

Pfizer issued a press release yesterday announcing they have identified a potent inhibitor of SARS-CoV-2 replication. There were no structural details and it is a novel compound so there is no human safety data available. Almost everything I've read to date has looked at repurposing approved or investigational drugs for which Phase 1 data is available.

Another good post from Derek Lowe on too much inflammation: https://blogs.sciencemag.org/pipeline/archives/2020/04/09/more-therapeutic-ideas-for-the-coronavirus-too-much-inflammation

Stay Safe, Mask Up, & Wash Hands (our governor just mandated the wearing of masks by all who go to pharmacies and grocery stores)

Alan

MODELING

- It's here; we know what to do; and we are doing it!

NEWLY REGISTERED CLINICAL TRIALS

- A new trial from Denver plans to learn more about the effects of a medicine called baricitinib on the progression of COVID-19 (coronavirus disease of 2019), the medical condition caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Baricitinib is FDA-approved for the treatment of rheumatoid arthritis, an autoimmune condition. This study intends to define the impact of baricitinib on the severity and progression of COVID-19. This drug might to lower the hyperinflammation caused by the virus, which would prevent damage to the lungs and possibly other organs. It's a small trial proposed for 30 patients. NCT04340232
- Here is a small trial to see if the DPP4 inhibitor linagliptin and help with diabetes control and reduce the severity of SARS-CoV-2 infection. It is commonly used to treat type 2 diabetes. NCT04341935
- This is a trial of something we can all use!!!! Due to the COVID-19 global health pandemic, many people are likely experiencing increased stress. Many obstetrics and gynecology patients are

additionally experiencing increased stress due to the healthcare changes the COVID-19 pandemic has caused including delayed or canceled elective surgeries, visitor restrictions, and telemedicine visits instead of in person clinic visits. Mindfulness meditation is a self-management strategy that can be utilized by anyone to assist with the management of stress. Mindfulness meditation mobile applications, such as the "Calm" app, can be used to help manage stress, especially during this uncertain time. The investigators propose a prospective randomized controlled trial evaluating perceived stress, anxiety, and sleep disturbance in the investigators outpatient OB/Gyn patients at Banner Women's Institute, with the use of a 30 day trial of the mindfulness meditation app, "Calm." All patients would ultimately receive a 30 day free trial of the mobile meditation app, however the intervention group would receive the 30-day free trial immediately and the control group would receive the 30-day free trial after the study period which is 30 days after enrollment. The investigators additionally want to evaluate the feasibility of using the mobile app, including looking at adherence to use of the app and patient satisfaction with use of the app. NCT04329533

- This trial is no *laughing matter* (could not resist stating it this way!). This study will enroll 470 healthcare professionals dedicated to care for patients with proven SARS-CoV-2 infection. Subjects will be randomized either in the observational (control) group or in the inhaled nitric oxide group. All personnel will observe measures on strict precaution in accordance with WHO and the CDC regulations. And it's from the Mass General Hospital who are the coordinating center!! NCT04312243

CLINICAL TRIAL RESULTS

- Maybe we don't have to worry about our pets (if one has them; we don't). It is now accepted that the wild fauna, probably bats, constitute the initial reservoir of the virus, but little is known about the role pets can play in the spread of the disease in human communities, knowing the ability of SARS-CoV-2 to infect some domestic animals. We tested 21 domestic pets (9 cats and 12 dogs) living in close contact with their owners (belonging to a veterinary community of 20 students) in which two students tested positive for COVID-19 and several others (n = 11/18) consecutively showed clinical signs (fever, cough, anosmia, etc.) compatible with COVID-19 infection. Although a few pets presented many clinical signs indicative for a coronavirus infection, no animal tested positive for SARS-CoV-2 by RT-PCR and no antibodies against SARS-CoV-2 were detectable in their blood using an immunoprecipitation assay. These original data can serve a better evaluation of the host range of SARS-CoV-2 in natural environment exposure conditions. https://www.biorxiv.org/content/10.1101/2020.04.07.029090v1
- Here is a Chinese paper that looks at single cell analysis of tocilizumab in severe SARS-CoV-2 patients. Tocilizumab treatment targeting interleukin 6 receptor has shown inspiring clinical results of severe COVID-19 patients.[**note: this is a Chinese paper that I have not seen so I don't know the size of the treatment arm or study design. I have read reports that clinical data from other trials should be coming out soon.**] However, the immune network with Tocilizumab treatment at single cell resolution has not been uncovered. Here, we profiled the single-cell transcriptomes of 13,289 peripheral blood mononuclear cells isolated at three longitudinal stages from two severe COVID-19 patients treated with Tocilizumab. We identified a severe stage-specific monocyte subpopulation and these cells centric immune cell interaction

network connected by the inflammatory cytokines and their receptors. The over-activated inflammatory immune response was attenuated after Tocilizumab treatment, yet immune cells including plasma B cells and CD8+ T cells still exhibited an intense humoral and cell-mediated anti-virus immune response in recovered COVID-19 patients. These results provided critical insights into the immunopathogenesis of severe COVID-19 and revealed fundamentals of effectiveness in Tocilizumab treatment.
https://www.biorxiv.org/content/10.1101/2020.04.08.029769v1

DRUG DEVELOPMENT

- More good work from China! Here is the strucutural basis for inhibition of the RNA-Dependent RNA Polymerase from SARS-CoV-2 by remdesivir. Now we need some positive clinical results!!! https://www.biorxiv.org/content/10.1101/2020.04.08.032763v1
- Here's a drug I haven't seen mentioned before, again from Chinese researchers. The main protease (Mpro) of COVID-19 virus is a key enzyme, which plays an essential role in viral replication and transcription, making it an ideal drug target. An FDA-approved antineoplastic drug,carmofur, has been identified as an inhibitor that targets COVID-19 virus Mpro. However, its inhibitory mechanism is unknown. Here, we report the 1.6-angstrom crystal structure of COVID-19 virus Mpro in complex with carmofur. The crystal structure shows that carmofur contains an electrophilic carbonyl reactive group, which covalently binds to C145, a member of the catalytic dyad. As a result, its fatty acid tail occupies the hydrophobic S2 subsite of Mpro whilst its 5-fluorouracil head is cleaved as product of the new covalent bond that has formed. Carmofur is active in a cell based antiviral assay with an EC50 of 24.87 μM. It is therefore a promising lead compound for the development of new antivirals to target COVID-19. https://www.biorxiv.org/content/10.1101/2020.04.09.033233v1
- I'm not sure this is really a good drug target but the paper is interesting in the concept of vulnerability of older males who have serious progression. we analyzed Genotype-Tissue Expression (GTEx) data to test whether lung aging is associated with transcriptional changes in human protein-coding genes that potentially interact with these viruses. We found decreased expression of the gene tribbles homolog 3 (TRIB3) during aging in male individuals, and its protein was predicted to interact with HCoVs nucleocapsid protein and RNA-dependent RNA polymerase. Using publicly available lung single-cell data, we found TRIB3 expressed mainly in alveolar epithelial cells that express SARS-CoV-2 receptor ACE2. Functional enrichment analysis of age-related genes, in common with SARS-CoV-induced perturbations, revealed genes associated with the mitotic cell cycle and surfactant metabolism. Given that TRIB3 was previously reported to decrease virus infection and replication, the decreased expression of TRIB3 in aged lungs may help explain why older male patients are related to more severe cases of the COVID-19. Thus, drugs that stimulate TRIB3 expression should be evaluated as a potential therapy for the disease. https://www.biorxiv.org/content/10.1101/2020.04.07.030767v1
- A UK/Qatar group looks at some repurposed chemotherapy drugs. A strategy for chemotherapy is to increase levels of endogenous reactive metabolites — such as reactive oxygen species and arginine-directed glycating agent, methylglyoxal — for selective toxicity to SARS-CoV-2. Sequence analysis of functional domains in the SARS-CoV-2 proteome showed 0.8 fold depletion

of cysteine residues and 4.9 fold enrichment of arginine residues, suggesting methylglyoxal modification may inactivate the virus. We discovered the peptide motif for MG modification: 3 – 5-fold enrichment of cationic residues preceding the target arginine. There was 5-fold enrichment of methylglyoxal modification sites in the SARS-CoV-2 proteome, compared to the human host - indicating selective toxicity of methylglyoxal to the virus. We found antitumor drugs, doxorubicin and paclitaxel, increase cellular methylglyoxal to virucidal levels. Taken together, these findings reveal a proteomic vulnerability of SARS-CoV-2 to methylglyoxal modification and provide a rationale for repurposing doxorubicin and paclitaxel for COVID-19 treatment. https://www.biorxiv.org/content/10.1101/2020.04.07.029488v1

- Ig fragment from horse sera potently neutralizes SARS-CoV-2 in vitro. Interesting finding but I'm not sure about the applicability to humans because of possible neutralization by the immune system. https://www.biorxiv.org/content/10.1101/2020.04.07.029884v1
- Here is a cautionary tale regarding reliance on AI drug screening. You don't want compounds that test in vitro at levels that are cytotoxic. We predicted therapeutic candidates that could reverse the gene expression of coronavirus-infected host cells. Thirteen expression signatures computed from various experimental conditions and preclinical models could be reversed by those compounds known to be effective against SARS- or MERS-CoV, as well as the drug candidates recently shown to be effective against SARS-CoV-2. We selected ten novel candidates to further evaluate their in vitro efficacy against SARS-CoV-2 infection. Four compounds bortezomib, dactolisib, alvocidib and methotrexate inhibited the formation of virus infection-induced cytopathic effect in Vero E6 cells at 1 uM, yet such a concentration seems toxic to the cells as well. While the evaluation in other permissive cells and the prediction of toxicity are needed to optimize and minimize their antiviral activity and cytotoxicity, respectively, this computational approach has the potential to rapidly and rationally identify drug candidates against COVID-19.
  https://www.biorxiv.org/content/10.1101/2020.04.07.030734v1
- Here is an animal model that predicts the usefulness of JAK1 inhibitors. Cytokine storms are drivers of pathology and mortality in myriad viral infections affecting the human population. In SARS-CoV-2-infected patients, the strength of the cytokine storm has been associated with increased risk of acute respiratory distress syndrome, myocardial damage, and death. However, the therapeutic value of attenuating the cytokine storm in COVID-19 remains to be defined. Here, we report results obtained using a novel mouse model of lethal sterile anti-viral immune responses. Using a mouse model of Down syndrome (DS) with a segmental duplication of a genomic region encoding four of the six interferon receptor genes (Ifnrs), we demonstrate that these animals overexpress Ifnrs and are hypersensitive to IFN stimulation. When challenged with viral mimetics that activate Toll-like receptor signaling and IFN anti-viral responses, these animals overproduce key cytokines, show exacerbated liver pathology, rapidly lose weight, and die. Importantly, the lethal immune hypersensitivity, accompanying cytokine storm, and liver hyperinflammation are blocked by treatment with a JAK1-specific inhibitor. Therefore, these results point to JAK1 inhibition as a potential strategy for attenuating the cytokine storm and consequent organ failure during overdrive immune responses. *Additionally, these results indicate that people with DS, who carry an extra copy of the IFNR gene cluster encoded on chromosome 21, should be considered at high risk during the COVID-19 pandemic.* [**note: the**

population of Downs Syndrome individuals is not large and maybe even well designed observational studies would not pick this up.]

DIAGNOSTIC DEVELOPMENT

- Indiana University medical school is doing a trial to validate the use of a rapid, at home, point-of-care (POC) SARS-CoV-2 IgG antibody test in high risk healthcare workers. Additionally, we would like to evaluate the incidence of seroconversion in this high-risk population and to identify possible candidates for convalescent plasma donation for therapy/prophylaxis. The test uses a finger prick but no other details are in the CT description. NCT04334876
- Here is a French study looking to see if a simple salivary sample works as well as the more invasive nasal and throat swabs for diagnosis of SARS-CoV-2 virus. This test will be non-invasive, performed without any analytical device and will restore its qualitative result "infected versus non-infected" in less than 1 hour (30min objective). NCT04337424

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2020-04-11

One of the loyal readers request some Bach. Here goes and it's not what you expected, a nice (?) excerpt of the double concerto for two violins: https://www.youtube.com/watch?v=_EofKXc-EOo&t=302s Don't give up on it too early as there is some fine playing.

Lots of abstracts today!! I hope I get a day off tomorrow. I have provided a link to the remdesivir compassionate use trial showing some efficacy of the drug. Obviously, we need good data from a trial. In this regard, I've seen stories coming out that patients in France are refusing to take part in trials that involve hydroxychloroquine. We went around this block with the HIV drugs three decades ago, when compassionate use was broadly discussed. If you cannot do a proper clinical trial, you will (almost) never know if the drug works.

A big shout out to the OHDSI team for getting their observational safety study of hydroxychloroquine/azithromycin done in such a short period of time. The abstract and link are below. This was outstanding collaboration by individual researchers!!!

With all the buzz about diagnostic testing, ProPublica posted an on-line story yesterday about the accuracy of such tests: https://www.propublica.org/article/coronavirus-tests-are-being-fast-tracked-by-the-fda-but-its-unclear-how-accurate-they-are Here is a story about a test purchase gone bad: https://www.propublica.org/article/he-spent--500-000-to-buy-coronavirus-tests-health-officials-say-theyre-unreliable

Derek Lowe on small molecule screening - https://blogs.sciencemag.org/pipeline/archives/2020/04/10/more-on-screening-for-coronavirus-therapies

Stay Safe, Mask Up, & Wash Hands

A belated happy Pesach and a prospective happy Easter to all my loyal readers!!

Alan

MODELING

- It's here; we know what to do; and we are doing it!
- This paper looks very interesting to me in terms of 'real-time' prediction of hot spots. Of course more validation is required. I don't know if this fits In with what Google and Apple are trying to do with their smart phone apps. Containing outbreaks of infectious disease requires rapid identification of transmission hotspots, as the COVID-19 pandemic demonstrates. Focusing limited public health resources on transmission hotspots can contain spread, thus reducing morbidity and mortality, but rapid data on community-level disease dynamics is often unavailable. Here, we demonstrate an approach to identify anomalously elevated levels of influenza-like illness (ILI) in real-time, at the scale of US counties. Leveraging data from a geospatial network of thermometers encompassing more than one million users across the US, we identify anomalies by generating accurate, county-specific forecasts of seasonal ILI from a point prior to a potential outbreak and comparing real-time data to these expectations. Anomalies are strongly correlated with COVID-19 case counts and may provide an early-warning system to locate outbreak epicenters. [**note: more information is at the company's website - https://www.kinsahealth.co/**]
  https://www.medrxiv.org/content/10.1101/2020.04.06.20039909v1
- Here is a Spanish trial on Vitamin D (**see in the Drug Development section for more**). The new outbreak of the SARS-CoV-2 coronavirus is causing an important pandemic affecting a large number of people all-over the world. Vitamin D is a hormone precursor produced by our own body with the help of sunlight which has an important role on adaptive immunity and cellular differentiation, maturation and proliferation of several immune cells. Reduced levels of vitamin D in calves were positioned as the main cause of bovine coronavirus infection in the past. Therefore, it seems plausible that the use of vitamin D as a nutritional ergogenic aid could be a potential intervention to fight against COVID-19 infected patients which remain asymptomatic or which have non-severe and severe symptoms. This study aims to investigate whether the use of vitamin D as an immune modulator agent induces significant improvements of health status and outcomes in non-severe symptomatic patients infected with COVID-19 as well as preventing COVID-19 health deterioration. We hypothesize that vitamin D will significantly improve hard endpoints related to COVID-19 deleterious consequences compared with a usual care control group. NCT04334005

- Just so you all know how thorough I am in getting the important information out, here is a Pakistani trial looking at the effect of gargling agents in reducing intraoral viral load in SARS-CoV-2 patients. [**note: one recipient of this newsletter sent around an email two or so weeks ago saying Dr. Fauci recommended this. I could find no documentary evidence that his was true. However, one should look at the trial link on clinicaltrials.gov to see the protocol. They do not use Listerine!**] Pakistan is a resource restraint country, it's not possible to carry out coronavirus testing at mass scale. Owing to the aerosol producing nature of the dental profession, carrying out dental work on asymptomatic patients carrying coronavirus puts the entire dental team at a great risk of not only acquiring the infection but also transmitting it to the others. Identifying an antiviral gargle that could substantially reduce the colonies of COVID-19 residing in mouth and

oro-pharynx is likely to reduce the viral load. This topical therapy is speculated to substantially reduce the transmission of infection in micro-aerosol generated in the dental practice. Such topical anti-viral therapy could also help to improve the overall symptoms of the patient. NCT04341688

NEWLY REGISTERED CLINICAL TRIALS

- Here is a new trial from University of Alabama at Birmingham on the use of tranexamic acid (TXA) in outpatients recently diagnosed with SARS-CoV-2. A recent report in Physiological Reviews proposed that the endogenous protease plasmin acts on the COVID19 virus by cleaving a newly inserted furin site in the S protein portion of the virus resulting in increased infectivity and virulence. Patients with hypertension, diabetes, coronary artery disease, cerebrovascular illness, lung disease and kidney dysfunction commonly have elevated levels of plasmin/plasminogen and it was proposed that this may be the mechanism for poorer outcomes in patients with these co-morbidities. A logical treatment that might blunt this process would be the inhibition of the conversion of plasminogen to plasmin. There is an inexpensive, commonly used drug, tranexamic acid, (TXA), which suppresses this conversion and could be re-purposed for the treatment of COVID19. [**note: this would definitely be a game changer as it's cheap, safe, and readily available**] NCT04338074

CLINICAL TRIAL RESULTS

- This is more of a clinical finding and I'll leave it to the pulmonolgists to sort it out. We propose that the severity of the disease and many deaths are due to a local vascular problem due to activation of B1 receptors on endothelial cells in the lungs. SARS-CoV-2 enters the cell via ACE2, a cell membrane bound molecule with enzymatic activity that next to its role in RAS is needed to inactivate des-Arg^9 bradykinin, the potent ligand of the bradykinin receptor type 1 (B1). In contrast to bradykinin receptor 2 (B2), the B1 receptor on endothelial cells is upregulated by proinflammatory cytokines. Without ACE2 acting as a guardian to inactivate the ligands of B1, the lung environment is prone for local vascular leakage leading to angioedema. Angioedema is likely a feature already early in disease, and might explain the typical CT scans and the feeling of people that they drown. In some patients, this is followed by a clinical worsening of disease around day 9 due to the formation antibodies directed against the spike (S)-antigen of the corona-virus that binds to ACE2 that could contribute to disease by enhancement of local immune cell influx and proinflammatory cytokines leading to damage. In parallel, inflammation induces more B1 expression, and possibly via antibody-dependent enhancement of viral infection leading to continued ACE2 dysfunction in the lung because of persistence of the virus. In this viewpoint we propose that a bradykinin-dependent local lung angioedema via B1 and B2 receptors is an important feature of COVID-19, resulting in a very high number of ICU admissions. We propose that blocking the B1 and B2 receptors might have an ameliorating effect on disease caused by COVID-19. This kinin-dependent pulmonary edema is resistant to corticosteroids or adrenaline and should be targeted as long as the virus is present. In addition, this pathway might indirectly be responsive to anti-inflammatory agents or neutralizing

strategies for the anti-S-antibody induced effects, but by itself is likely to be insufficient to reverse all the pulmonary edema. Moreover, we provide a suggestion of how to ventilate in the ICU in the context of this hypothesis. https://www.preprints.org/manuscript/202004.0023/v1

- Two weeks to a pre-print. Congrats to the OHDSI team that has been referenced before in this newsletter (worldwide list of authors from 24 institutions!). Here is an observational study of the safety of hydroxychloroquine alone and in combination with azithromycin. We studied the safety of hydroxychloroquine, alone and in combination with azithromycin. Methods: New user cohort studies were conducted including 16 severe adverse events (SAEs). Rheumatoid arthritis patients aged 18+ and initiating hydroxychloroquine were compared to those initiating sulfasalazine and followed up over 30 days. Self-controlled case series (SCCS) were conducted to further establish safety in wider populations. Separately, SAEs associated with hydroxychloroquine-azithromycin (compared to hydroxychloroquine-amoxicillin) were studied. Data comprised 14 sources of claims data or electronic medical records from Germany, Japan, Netherlands, Spain, UK, and USA. Propensity score stratification and calibration using negative control outcomes were used to address confounding. Cox models were fitted to estimate calibrated hazard ratios (CalHRs) according to drug use. Estimates were pooled where I2<40%. Results: Overall, 956,374 and 310,350 users of hydroxychloroquine and sulfasalazine, and 323,122 and 351,956 users of hydroxychloroquine-azithromycin and hydroxychloroquine-amoxicillin were included. No excess risk of SAEs was identified when 30-day hydroxychloroquine and sulfasalazine use were compared. SCCS confirmed these findings. However, when azithromycin was added to hydroxychloroquine, we observed an increased risk of 30-day cardiovascular mortality (CalHR2.19 [1.22-3.94]), chest pain/angina (CalHR 1.15 [95% CI 1.05-1.26]), and heart failure (CalHR 1.22 [95% CI 1.02-1.45]) Conclusions: Short-term hydroxychloroquine treatment is safe, but addition of azithromycin may induce heart failure and cardiovascular mortality, potentially due to synergistic effects on QT length. We call for caution if such combination is to be used in the management of Covid-19. https://www.medrxiv.org/content/10.1101/2020.04.08.20054551v1

- Data has been published from a compassionate use study of remdesivir. Of the 61 patients who received at least one dose of remdesivir, data from 8 could not be analyzed (including 7 patients with no post-treatment data and 1 with a dosing error). Of the 53 patients whose data were analyzed, 22 were in the United States, 22 in Europe or Canada, and 9 in Japan. At baseline, 30 patients (57%) were receiving mechanical ventilation and 4 (8%) were receiving extracorporeal membrane oxygenation. During a median follow-up of 18 days, 36 patients (68%) had an improvement in oxygen-support class, including 17 of 30 patients (57%) receiving mechanical ventilation who were extubated. A total of 25 patients (47%) were discharged, and 7 patients (13%) died; mortality was 18% (6 of 34) among patients receiving invasive ventilation and 5% (1 of 19) among those not receiving invasive ventilation. Conclusions In this cohort of patients hospitalized for severe Covid-19 who were treated with compassionate-use remdesivir, clinical improvement was observed in 36 of 53 patients (68%). Measurement of efficacy will require ongoing randomized, placebo-controlled trials of remdesivir therapy. (Funded by Gilead Sciences.) https://www.nejm.org/doi/full/10.1056/NEJMoa2007016 (**don't know if this is behind the New England Journal paywall**)
- More good stuff from Wuhan, blood glucose levels which are easy to measure may be predictive of poor clinical outcome. We included all patient admitted to Wuhan Union Hospital and treated by the supportive medical team of Beijing Tongren Hospital as of March 20, 2020.

Indicators of injuries for multiple organs, including the heart, kidney and liver, and glucose homeostasis were specifically analyzed for predicting primary outcomes (an intensive care unit (ICU) or death). RESULTS The data of 120 patients with a severity equal to or greater than Moderate, discharged or died were extracted. After excluding patients with history of diabetes, chronic heart, kidney, and liver disease, 69 patients were included in the final analysis. There were 26 cases with primary outcomes including 16 deaths. Univariable analysis indicated that fasting blood glucose (FBG), lactate dehydrogenase (LDH), hydroxybutyrate dehydrogenase (HBDH), creatine kinase and creatinine were associated with primary outcomes and death. Among patients with primary outcomes, although FBG levels were much higher on admission, they dramatically decreased subsequently, while in deceased patients they increased continuously. Multivariable Cox regression indicated that FBG≥7mmol/L was the only independent predictor for death (HR = 3.75, 95% CI 1.26-11.15). Cluster analysis found more proximities of FBG (at the time of admission) with LDH, HDDH or Creatinine (after 2-4 days of hospitalization) (r=0.43, 0.43 and 0.50, respectively, P<0.01 for all). Conclusions Blood glucose is a representative of the clustered indicators of multi-organ injury and earlier predictor for poor outcomes and death in the COVID patients. As it is easy to perform for clinical practices and self-monitoring, glucose testing will be much helpful for predicting poor outcomes to facilitate appropriate intensive care.
https://www.medrxiv.org/content/10.1101/2020.04.08.20058040v1

- Yes, it's a small number of patients but Interferon-α2b shows some promise. We describe here the clinical course of COVID-19 in a cohort of confirmed cases in Wuhan, China, treated with the repurposed potential experimental therapeutics IFN-α2b, arbidol or a combination of IFN-α2b plus arbidol. Methods 77 adults with confirmed COVID-19 were treated with either nebulized IFN-α2b (5mU,b.i.d.), arbidol (200mg dispersible tablet, t.i.d.) or a combination of IFN-α2b plus arbidol. Serial SARS-CoV-2 testing along with hematological measurements, including cell counts and blood biochemistry, serum cytokine levels, temperature and blood oxygen saturation levels were recorded for each patient during their hospital stay. Findings Treatment with IFN-α2b with or without arbidol significantly reduced the duration of detectable virus in the upper respiratory tract and in parallel reduced duration of elevated blood levels for the inflammatory markers IL-6 and CRP. Interpretation IFN-α2b should be investigated as therapy in COVID-19 cases.
https://www.medrxiv.org/content/10.1101/2020.04.06.20042580v1
- Here is an interesting study of human ACE2 receptor polymorphisms with a conjecture that SARS-CoV-2 susceptibility may be variable in a subset of the human population. The SARS-CoV-2 S-protein has acquired mutations that increase its affinity to human ACE2 by ~10-15-fold compared to SARS-CoV S-protein, making it highly infectious. In this study, we assessed if ACE2 polymorphisms might alter host susceptibility to SARS-CoV-2 by affecting the ACE2 S-protein interaction. Our comprehensive analysis of several large genomic datasets that included over 290,000 samples representing >400 population groups identified multiple ACE2 protein-altering variants, some of which mapped to the S-protein-interacting ACE2 surface. Using recently reported structural data and a recent S-protein-interacting synthetic mutant map of ACE2, we have identified natural ACE2 variants that are predicted to alter the virus-host interaction and thereby potentially alter host susceptibility. In particular, human ACE2 variants S19P, I21V, E23K, K26R, T27A, N64K, T92I, Q102P and H378R are predicted to increase susceptibility. The T92I variant, part of a consensus NxS/T N-glycosylation motif, confirmed the role of N90 glycosylation

in immunity from non-human CoVs. Other ACE2 variants K31R, N33I, H34R, E35K, E37K, D38V, Y50F, N51S, M62V, K68E, F72V, Y83H, G326E, G352V, D355N, Q388L and D509Y are putative protective variants predicted to show decreased binding to SARS-CoV-2 S-protein. Overall, ACE2 variants are rare, consistent with the lack of selection pressure given the recent history of SARS-CoV epidemics, however, are likely to play an important role in altering susceptibility to CoVs. https://www.biorxiv.org/content/10.1101/2020.04.07.024752v1

- This is a really, really, really, complicated clinical paper (did I overuse the word 'really?'). I include it as I'm an allergy sufferer and have been thinking about the role seasonal allergies might play in SARS-CoV-2 infection. Most of the researchers here are from the National Jewish Hospital in Denver where a lot of good pulmonary research is done. Here we explore the role of genetics and co-expression networks in regulating these genes in the airway, through the analysis of nasal airway transcriptome data from 695 children. We identify expression quantitative trait loci (eQTL) for both ACE2 and TMPRSS2, that vary in frequency across world populations. Importantly, we find TMPRSS2 is part of a mucus secretory network, highly upregulated by T2 inflammation through the action of interleukin-13, and that interferon response to respiratory viruses highly upregulates ACE2 expression. Finally, we define airway responses to coronavirus infections in children, finding that these infections upregulate IL6 while also stimulating a more pronounced cytotoxic immune response relative to other respiratory viruses. Our results reveal mechanisms likely influencing SARS-CoV-2 infectivity and COVID-19 clinical outcomes. From the conclusion in the paper, "*Together, these studies provisionally suggest that T2 inflammation may predispose individuals to experience better COVID-19 outcomes through a decrease in airway levels of ACE2 that override any countervailing effect from increased expression of TMPRSS2. However, both in vitro experiments examining IL-13 effects on SARS-CoV-2 infection and empirical data on COVID-19 outcomes among T2-high and T2-low patients will certainly be needed to determine whether this common airway inflammatory endotype ultimately protects against or exacerbates COVID-19 illness."* [**note: maybe my upper respiratory inflammation is protecting me against this virus!**] https://www.biorxiv.org/content/10.1101/2020.04.09.034454v1
- Here is a follow on study from the finding in China about blood type and clinical progression. It comes from NY Presbyterian hospital. A recent study of patients in China discovered an association between ABO blood type and SARS-CoV-2 infection status by comparing COVID-19 patients with the general population. Whether blood type is associated with increased COVID-19 morbidity or mortality remains unknown. We used observational healthcare data on 1559 individuals tested for SARS-CoV-2 (682 COV+) with known blood type in the New York Presbyterian (NYP) hospital system to assess the association between ABO+Rh blood type and SARS-CoV-2 infection status, intubation, and death. We found a higher proportion of blood group A and a lower proportion of blood group O among COV+ patients compared to COV-, though in both cases the result is significant only in Rh positive blood types. We show that the effect of blood type is not explained by risk factors we considered (age, sex, hypertension, diabetes mellitus, overweight status, and chronic cardiovascular and lung disorders). In a meta-analysis of NYP data with previously-reported data from China, we find enrichment for A and B and depletion of O blood groups among COVID-19 patients compared to the general population. Our data do not provide strong evidence of associations between blood group and intubation or death among COVID-19 patients. In this preliminary observational study of data currently being

collected during the outbreak, we find new evidence of associations between B, AB, and Rh blood groups and COVID-19 and further evidence of recently-discovered associations between A and O blood groups and COVID-19. [**note: is it too early for those of us O+ folks to breathe a little easier?**] https://www.medrxiv.org/content/10.1101/2020.04.08.20058073v1

- More data from New York City (I'll keep posting these abstracts for a while longer but at some point wait until one of the medical journals publishes a good summary of case findings). Primary outcomes were hospitalization and critical illness (intensive care, mechanical ventilation, hospice and/or death). We conducted multivariable logistic regression to identify risk factors for adverse outcomes, and maximum information gain decision tree classifications to identify key splitters. Results: Among 4,103 Covid-19 patients, 1,999 (48.7%) were hospitalized, of whom 981/1,999 (49.1%) have been discharged home, and 292/1,999 (14.6%) have died or were discharged to hospice. Of 445 patients requiring mechanical ventilation, 162/445 (36.4%) have died. Strongest hospitalization risks were age ≥75 years (OR 66.8, 95% CI, 44.7-102.6), age 65-74 (OR 10.9, 95% CI, 8.35-14.34), BMI>40 (OR 6.2, 95% CI, 4.2-9.3), and heart failure (OR 4.3 95% CI, 1.9-11.2). Strongest critical illness risks were admission oxygen saturation <88% (OR 6.99, 95% CI 4.5-11.0), d-dimer>2500 (OR 6.9, 95% CI, 3.2-15.2), ferritin >2500 (OR 6.9, 95% CI, 3.2-15.2), and C-reactive protein (CRP) >200 (OR 5.78, 95% CI, 2.6-13.8). In the decision tree for admission, the most important features were age >65 and obesity; for critical illness, the most important was SpO2<88, followed by procalcitonin >0.5, troponin <0.1 (protective), age >64 and CRP>200. Conclusions: Age and comorbidities are powerful predictors of hospitalization; however, admission oxygen impairment and markers of inflammation are most strongly associated with critical illness. https://www.medrxiv.org/content/10.1101/2020.04.08.20057794v1

DRUG DEVELOPMENT

- I guess this one belongs in this category and if this holds up, increases therapeutic options. In this study, we assessed currently marketed intravenous immunoglobulin (IVIG) products for antibodies against human common coronaviruses that may cross-react with the SARS-CoV-2 virus. Methods: Gamunex©-C and Flebogamma© DIF (Grifols) IVIG were tested against several betacoronaviruses antigens using ELISA techniques: HCoV (undetermined antigen), HCoV-HKU1 (N protein), SARS-CoV (culture lysate), MERS-CoV (N protein; S1 protein/RBD; S protein), and SARS-CoV-2 (S1 protein). Results: Both IVIG products showed consistent reactivity to components of the tested viruses. Positive cross-reactivity was seen in SARS-CoV, MERS-CoV, and SARS-CoV-2. For SARS-CoV-2, positive reactivity was observed at IVIG concentrations ranging from 100 µg/mL with Gamunex-C to 1 mg/mL with Flebogamma 5% DIF. Conclusion: Gamunex-C and Flebogamma DIF IVIG contain antibodies reacting against SARS-CoV-2 antigens. These preparations may be useful for immediate treatment of COVID-19 disease. https://www.biorxiv.org/content/10.1101/2020.04.07.029017v1
- In addition to us all getting our BCG shots, it may be that the MMR vaccine offers some protection as well. Perhaps this is why young people may not present with serious clinical symptoms. Herein, we investigated the potential of childhood vaccination, specifically against measles, mumps and rubella (MMR), to identify if this could potentially confer acquired protection over SARS-CoV-2. We identified sequence homology between the fusion proteins of SARS-CoV-2 and measles and mumps viruses. Moreover, we also identified a 29% amino acid

sequence homology between the Macro (ADP-ribose-1-phosphatase) domains of SARS-CoV-2 and rubella virus. The rubella Macro domain has surface-exposed conserved residues and is present in the attenuated rubella virus in MMR. Hence, we hypothesize that MMR could protect against poor outcome in COVID-19 infection. As an initial test of this hypothesis, we identified that 1) age groups that most likely lack of MMR vaccine-induced immunity had the poorest outcome in COVID-19, and 2) COVID-19 disease burden correlates with rubella antibody titres, potentially induced by SARS-CoV2 homologous sequences. We therefore propose that vaccination of at risk age groups with an MMR vaccination merits further consideration as a time-appropriate and safe intervention.
https://www.medrxiv.org/content/10.1101/2020.04.10.20053207v1

- Here is a linkage study from China regarding the patients on ACEI/ARB and progression to severe pneumonia. Nonspecific antiviral therapy did not prevent clinical progression to severe pneumonia, although fewer hypertensive patients on angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers (ACEI/ARB) therapy developed severe pneumonia in contrast with those on non-ACEI/ARB antihypertensive therapy (1 of 16 [6.3%] patients and 16 of 49 [32.7%] patients, respectively [difference, 26.4%; 95% CI, 1.5% to 41.3%]). Multivariate logistic regression analysis showed that hypertension without receiving ACEI/ARB therapy was an independent risk factor (odds ratio [OR], 2.07; 95% CI, 1.07 to 4.00) for developing severe pneumonia irrespective of age. Besides, none of patients treated with chloroquine developed severe pneumonia, though without significance (difference, 12.0%; 95% CI, -3.5% to 30.0%) by propensity score matching. CONCLUSIONS AND RELEVANCE Hypertensive patients on ACEI or ARB may be protective from severe pneumonia in COVID-19 and hence these therapies should not be ceased unless there is a strong indication or further epidemiological evidence. Though none of the current antiviral and immunoregulation therapy showed benefit in preventing COVID-19 progression, chloroquine deserved further investigation.
https://www.medrxiv.org/content/10.1101/2020.04.08.20057539v1
- An observational study of the role of Vitamin D as relating to cytokine storm. A potential association between severe Vit D deficiency and age-specific case fatality (CFR) was investigated. Reported medical characteristics of 793 COVID-19 patients were used to evaluate the intensity of cytokine storm in severe COVID-19 using C-reactive protein (CRP) levels. Medical data reported from a national study of 3,848 participants in 2007-2008 was used to investigate the association between Vit D status and CRP. Odds ratio and risk factors from these conditions were used to predict the potential impact of Vit D on the reduction of cytokine storm and severe COVID-19. Findings: Age-specific CFR in Italy, Spain, and France (70 yo ≤ age < 80 yo) was substantially higher (>1.9 times) than other countries (Germany, South Korea, China); for the elderly (age ≥70 yo), Italy and Spain present the highest CFR (>1.7 times that of other countries). The age-specific ratio of confirmed cases in Italy, Spain, and France has also been substantially higher than in other countries. A more severe deficiency of Vit D (mean 25-hydroxyvitamin D (25OHD) concentration <0.25 ng/L) is reported in Italy and Spain compared to other countries. Our analysis of the reported clinical data (25OHD, CRP) from multiple studies suggests that elimination of severe Vit D deficiency reduces the risk of high CRP levels (odds ratio of 2) which may be used as a surrogate marker of cytokine storm which was estimated to a potential reduction in severe COVID-19 cases of up to 15%. Interpretation: The substantially higher age-specific CFR and the age-specific ratio of confirmed cases in Italy and Spain (countries with low

mean 25OHD level) suggest a potential link between severe Vit D deficiency and severe COVID-19, which can lead to a higher CFR. No direct link between the performance of health care systems, the age distribution of the nation, or Vitamin A deficiency and the CFR of COVID-19 were observed. Our analysis of the published data on the status of Vit D and CRP levels (in the US) and laboratory data (CRP levels) reported from 792 patients in China suggests that a proper supplementation of Vit D across populations may reduce the number of severe COVID-19 cases by up to 15 percentage points by lowering the risk factors related to cytokine storm. Our analysis did not eliminate the possibility of the circulation of different sub-genera of COVID-19 across the globe or other factors. [**note: another thing to add to your hydroxychloroquine therapy; your are taking your malaria medicines, aren't you?**]
https://www.medrxiv.org/content/10.1101/2020.04.08.20058578v1

- Making the hydroxychloroquine studies muddier, here is an observational study from Quebec looking at pneumonia prevention in at-risk population. It is reasonable to expect that if HCQ can prevent or reduce the adverse effects of influenza, it may also reduce the effects of COVID-19 in humans. The objective of this study was to test whether HCQ can prevent or reduce the risk and severity of influenza. Methods. This is an observational cohort study using medico-administrative data from Quebec. Patients included had at least one emergency department (ED) visit in 2012 or 2013, with a prior diagnosis of chronic conditions, and were admissible to the public drug insurance plan. Two sub-cohorts were considered depending on reasons for ED visit: other than influenza or pneumonia (primary prevention) and influenza or pneumonia (secondary prevention). Results. In the primary prevention analysis (n=417,353), patients taking HCQ (n=3,659) had an increased risk of hospitalization for pneumonia in the following year compared to those who did not (5.2% vs. 2.9%; adjusted OR=1.25, p=0.0079). In the secondary prevention analysis (n=27,152), patients taking HCQ (n=392), compared to those who did not had a modest and non-significant increased risk of hospitalization for pneumonia after 30 days (25.8% vs. 22.6%; adjusted OR=1.14, p=0.3177). Interpretation: Based on the assumption that HCQ has similar effects on the COVID-19 as those observed on influenza, we can infer that it will not have positive effects on COVID-19. We should therefore act cautiously before initiating prospective interventional studies on the use of HCQ to reduce adverse effects of COVID-19. [**note: stop taking your malaria medicine!**]
https://www.medrxiv.org/content/10.1101/2020.04.08.20057893v1

DIAGNOSTIC DEVELOPMENT

- From the Canary Islands of Spain, Based on the urgent need for high-throughput COVID-19 screening, we tested the performance of three alternative, simple and affordable protocols to rapidly detect SARS-CoV-2, overcoming the long and tedious RNA extraction step. Although with an average increase of 6.1 (±1.6) cycles compared to standard tests with RNA extracts, we show that RT-qPCR yielded consistent results in nasopharyngeal swab samples that were subject to a direct 70°C incubation for 10 min. Our findings provide viable options to overcome any supply chain issue and help to increase the throughput of diagnostic tests by using any qPCR device, thereby complementing standard COVID-19 testing.
https://www.medrxiv.org/content/10.1101/2020.04.08.20058495v1

- Here is a scalable approach to testing from a small California company. Herein, we describe a novel molecular diagnostic for COVID-19 based on Sanger sequencing. This assay uses the addition of a frame-shifted spike-in, a modified PCR master mix, and custom Sanger sequencing data analysis to detect and quantify SARS-CoV-2 RNA at a limit of detection comparable to existing qPCR-based assays, at 10-20 genome copy equivalents. Crucially, our assay was able to detect SARS-CoV-2 RNA from viral particles suspended in transport media that was directly added to the PCR master mix, suggesting that RNA extraction can be skipped entirely without any degradation of test performance. Since Sanger sequencing instruments are widespread in clinical laboratories and commonly have built-in liquid handling automation to support up to 3840 samples per instrument per day, *the widespread adoption of qSanger COVID-19 diagnostics can unlock more than 1,000,000 tests per day in the US*. [**note: wow! See the company website for more info - https://billiontoone.com/**]
  https://www.biorxiv.org/content/10.1101/2020.04.07.029199v1
- Here is a nice Danish paper that evaluates nine commercial SARS-CoV-2 immunoassays. This study evaluated the sensitivity and specificity of nine commercially available serological tests. These included three enzyme-linked immunosorbent assays (ELISAs) and six point-of-care (POC) lateral flow tests. The assays were validated using serum samples from: i) SARS-CoV-2 PCR-positive patients with a documented first day of disease; ii) archived sera obtained from healthy individuals before the emergence of SARS-CoV-2 in China; iii) sera from patients with acute viral respiratory tract infections caused by other coronaviruses or non-coronaviruses; and iv) sera from patients positive for dengue virus, cytomegalovirus and Epstein Barr virus. The results showed 100% specificity for the Wantai SARS-CoV-2 Total Antibody ELISA, 93% for the Euroimmun IgA ELISA, and 96% for the Euroimmun IgG ELISA with sensitivities of 90%, 90%, and 65%, respectively. The overall performance of the POC tests according to manufacturer were in the rank order of AutoBio Diagnostics > Dynamiker Biotechnology = CTK Biotech > Artron Laboratories > Acro Biotech ≥ Hangzhou Alltest Biotech. Overall, these findings will facilitate selection of serological assays for the detection SARS-CoV-2-specific antibodies towards diagnosis as well as sero-epidemiological and vaccine development studies. [**note: we need to really get this validation work done so a test(s) can be deployed**]
  https://www.medrxiv.org/content/10.1101/2020.04.09.20056325v1

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2020-04-12

A lot of years ago I got my start in choral singing at All Souls Episcopal Church in San Diego. One of my good friends invited me to join the youth choir. I asked my mother about this as a good Jewish son ought to do. She said it was fine but at some point they will pass crackers around. "You don't want to take one," she admonished. I was with the choir for about four years singing at all kinds of services. Easter morning we always performed Handel's Hallelujah chorus from The Messiah. Here is a wonderful version for these pandemic times: https://www.youtube.com/watch?v=_PQYVbd1Gwg This was some feat and quite stirring.

It was a slow day on the pre-print search. I would direct attention to the Brazil high/low dose chloroquine safety report shown below. Will we ever get decent data out of all these trials???

MODELING

- The Mt. Sinai group have been doing some very good research. New York City (NYC) has emerged as one of the epicenters of the current SARS-CoV2 pandemic. To identify the early events underlying the rapid spread of the virus in the NYC metropolitan area, we sequenced the virus causing COVID19 in patients seeking care at one of the hospitals of the Mount Sinai Health System. Phylogenetic analysis of 84 distinct SARS-CoV2 genomes indicates multiple, independent but isolated introductions mainly from Europe and other parts of the United States. Moreover, we find evidence for community transmission of SARS-CoV2 as suggested by clusters of related viruses found in patients living in different neighborhoods of the city. https://www.medrxiv.org/content/10.1101/2020.04.08.20056929v1

NEWLY REGISTERED CLINICAL TRIALS

- CanSino is moving into Phase 2 trials with their adenovirus vaccine. This is a phase II, randomised, double-blinded and placebo-controlled clinical trial in healthy adults above 18 years of age. This clinical trial is designed to evaluate the immunogenicity and safety of Ad5-nCoV which encodes for a full-length spike (S) protein of SARS-CoV-2. 500 subjects will be enrolled, 250 subjects in middle-dose vaccine group, 125 subjects in low-dose and placebo group, respectively. Immunogenicity will be tested on days 0, 14, 28 and 6 months after vaccination NCT04341389

CLINICAL TRIAL RESULTS

- YIKES!!! In from Brazil, a safety study of high and low dose chloroquine for treatment of SARS-CoV-2. Eligible participants were allocated to receive orally or via nasogastric tube high dose CQ (600mg CQ twice daily for 10 days or total dose 12g); or low dose CQ (450mg for 5 days, twice daily only on the first day, or total dose 2.7g). In addition, *all patients received ceftriaxone and azithromycin.* [**note: this obviously leads to study interpretation problems**] This study was registered with ClinicalTrials.gov, number NCT04323527. Findings Out of a pre-defined 440 patients sample size, 81 patients were enrolled. The high dose CQ arm presented more QTc>500ms (25%), and a trend toward higher lethality (17%) than the lower dosage. Fatality rate was 13.5% (95%CI=6.9-23.0%), overlapping with the CI of historical data from similar patients not using CQ (95%CI=14.5-19.2%). In 14 patients with paired samples, respiratory secretion at day 4 was negative in only one patient. Interpretation Preliminary findings suggest that the higher CQ dosage (10-day regimen) should not be recommended for COVID-19 treatment because of its potential safety hazards. Such results forced us to prematurely halt patient recruitment to this arm. [**note: The paper notes that there was little demonstration of efficacy in this trial relative to the observed mortality level of the disease in non-treated populations. There was no placebo arm in this study.**] https://www.medrxiv.org/content/10.1101/2020.04.07.20056424v1
- Maybe we should all be on ACE inhibitors!!! This UK group found that counter thinking ACE inhibitors was associated with less severe disease. We evaluated this hypothesis in an early

cohort of 205 acute inpatients with COVID-19 at King's College Hospital and Princess Royal University Hospital, London, UK with the primary endpoint being death or transfer to a critical care unit for organ support within 7-days of symptom onset. Findings: 53 patients out of 205 patients reached the primary endpoint. Contrary to the hypothesis, treatment with ACE-inhibitors was associated with a reduced risk of rapidly deteriorating severe disease. There was a lower rate of death or transfer to a critical care unit within 7 days in patients on an ACE-inhibitor OR 0.29 (CI 0.10-0.75, p<0.01), adjusting for age, gender, comorbidities (hypertension, diabetes mellitus, ischaemic heart disease and heart failure). Interpretation: Although a small sample size, we do not see evidence for ACE-inhibitors increasing the short-term severity of COVID-19 disease and patients on treatment with ACE-inhibitors should continue these drugs during their COVID-19 illness. A potential beneficial effect needs to be explored as more data becomes available. [**note: this is why we need MORE observational research across databases from all over!!!**] https://www.medrxiv.org/content/10.1101/2020.04.07.20056788v1

- Here is another small scale retrospective study on SARS-CoV-2 infection in pregnant women. Methods In this retrospective cohort study, we enrolled 31 pregnant women and 35 non-pregnant women from Jan 28 to Feb 28, 2020 to evaluate the effects of SARS-CoV-2 infection during pregnancy. Inflammatory indices were used to assess the severity of COVID-19. Evidence of vertical transmission was determined by laboratory confirmation of SARS-CoV-2 in amniotic fluid, placenta, neonatal throat and anal swab and breastmilk samples. Findings Compared with non-pregnant women, pregnant women had a significantly lower proportion of fever (54.8% vs. 87.5%, p= 0.006), a shorter average interval from onset to hospitalization, and a higher proportion of severe or critical COVID-19 (32.3% vs. 11.4%, p=0.039). Neutrophil-to-lymphocyte ratio (NLR) and systematic immune-inflammation-based prognostic index (SII) were significantly higher on admission in severe/critical pneumonia group than moderate pneumonia group. We could not detect the presence of SARS-CoV-2 by RT-PCR in amniotic fluid, placenta, neonatal throat and anal swab and breastmilk samples. Conclusions The clinical symptoms of COVID-19 in pregnant women were insidious and atypical, compared with those in non-pregnant patients. SII and NLR could be a useful marker to evaluate the severity of COVID-19. *There was no evidence of vertical transmission during pregnancy with SARS-CoV-2 infection.* https://www.medrxiv.org/content/10.1101/2020.04.07.20053744v1
- Maybe we should not be lining up for BCG injections after all! A recent publication, linked nation based universal Bacillus Calmette-Guerin (BCG) vaccination to potential protection against morbidity and mortality from SARS-CoV-2, and received much attention in public media, even before its peer review. We wished to validate the findings by examining the association between daily rates of COVID-19 case fatality (i.e. Death Per Case /Days of the endemic [dpc/d]) and the presence of universal BCG vaccination before 1980, or the year of the establishment of universal vaccination. *There was no significant association in either analysis*. In this work we emphasize caution amidst the publication surge on COVID-19, and highlight the political/economical-, arbitrary selection-, and fear/anxiety related biases, which may obscure scientific rigor. https://www.medrxiv.org/content/10.1101/2020.04.09.20056903v1

DRUG DEVELOPMENT

- Here is an important finding from a US/Chinese group on a possible route to a vaccine. The SARS-coronavirus 2 (SARS-CoV-2) spike (S) protein mediates entry of SARS-CoV-2 into cells expressing the angiotensin-converting enzyme 2 (ACE2). The S protein engages ACE2 through its receptor-binding domain (RBD), an independently folded 197-amino acid fragment of the 1273-amino acid S-protein protomer. Antibodies to the RBD domain of SARS-CoV (SARS-CoV-1), a closely related coronavirus which emerged in 2002-2003, have been shown to potently neutralize SARS-CoV-1 S-protein-mediated entry, and the presence of anti-RBD antibodies correlates with neutralization in SARS-CoV-2 convalescent sera. Here we show that immunization with the SARS-CoV-2 RBD elicits a robust neutralizing antibody response in rodents, comparable to 100 μg/ml of ACE2-Ig, a potent SARS-CoV-2 entry inhibitor. Importantly, anti-sera from immunized animals did not mediate antibody-dependent enhancement (ADE) of S-protein-mediated entry under conditions in which Zika virus ADE was readily observed. These data suggest that an RBD-based vaccine for SARS-CoV-2 could be safe and effective.
https://www.biorxiv.org/content/10.1101/2020.04.10.036418v1
- Nature always manages to come up with interesting compounds (lest we not forget the modern antibiotic age came from microorganism found out in the soil!!). Here is a Chinese study looking at a traditional medicine from *scutellaria baicalensis*. The main protease of SARS-CoV-2, 3C-like protease (3CLpro), is highly conserved across coronaviruses and is essential for the maturation process of viral polyprotein. Scutellariae radix (Huangqin in Chinese), the root of Scutellaria baicalensis has been widely used in traditional Chinese medicine to treat viral infection related symptoms. The extracts of S. baicalensis have exhibited broad spectrum antiviral activities. We studied the anti-SARS-CoV-2 activity of S. baicalensis and its ingredient compounds. We found that the ethanol extract of S. baicalensis inhibits SARS-CoV-2 3CLpro activity in vitro and the replication of SARS-CoV-2 in Vero cells with an EC50 of 0.74 μg/ml. Among the major components of S. baicalensis, baicalein strongly inhibits SARS-CoV-2 3CLpro activity with an IC50 of 0.39 μM. We further identified four baicalein analogue compounds from other herbs that inhibit SARS-CoV-2 3CLpro activity at microM concentration. Our study demonstrates that the extract of S. baicalensis has effective anti-SARS-CoV-2 activity and baicalein and analogue compounds are strong SARS-CoV-2 3CLpro inhibitors. [**note: for you chemists in the audience, the structure and some further information is here: https://en.wikipedia.org/wiki/Baicalin There have been other flavonoids that have inhibitory activity of Mpro**]
https://www.biorxiv.org/content/10.1101/2020.04.10.035824v1

DIAGNOSTIC DEVELOPMENT

- No news on this front is not good news.