2020-05-18

Week nine of the newsletter; time flies when you are having fun (or at least it supposed to).

How have I managed to neglect the blues??? I did have Aretha in her Blues Brother's cameo but that didn't count. We need to take care of business right now. Who else to kick off a 'Blue Monday' than BB King. Here is 'The Thrill is Gone': https://www.youtube.com/watch?v=SgXSomPE_FY Nobody does it better.

More on the contretemps between the FDA and Seattle on viral testing from the New York Times. Surely, the FDA must adapt to how things must be done in a pandemic situation. They require Medication Guides to be distributed with certain drugs that may pose risks, why not a Pandemic Diagnostic Guide that addresses what the tests do and do not show. BE CREATIVE FDA!!

Here is a cautionary tale from the state of Utah regarding use of chloroquine. One wonders what these folks were thinking.

Early and encouraging data on the Moderna vaccine.

Nice story on a front line COVID-19 contact tracer. This will be of interest to the public health folks who read this newsletter and shows the challenges of doing the job correctly.

MODELING

- No new models, AMAZING and a relief for me!

NEWLY REGISTERED CLINICAL TRIALS

- The purpose of this study is to assess the efficacy and safety of prazosin to prevent cytokine storm syndrome and severe complications in hospitalized patients with Coronavirus disease 2019 (COVID-19). Catecholamines enhance inflammatory injury by augmenting the production of IL-6 and other cytokines through a self-amplifying feed-forward loop in immune cells that requires alpha-1 adrenergic receptor (α1-AR) signaling. The α1-AR antagonist prazosin prevents cytokine storm and markedly increased survival following inflammatory stimuli in preclinical models. In a retrospective study of outcomes in acute respiratory distress syndrome or pneumonia, patients who were taking α1-AR antagonists had significantly lower probability of needing invasive mechanical ventilation and dying in the hospital compared to non-users. Prazosin may blunt surges in catecholamines and self-amplifying cytokine production (including interleukin 6) and, as an early preemptive therapy in patients prior to disease progression, may prevent cytokine storm syndrome and severe complications of COVID-19. [**note: I've been following the path of this one on the OHDSI forum. The Johns Hopkins researchers did a deep data dive and found that α-adrenergic blockers appear to block cytokine storm though the most commonly used drug is tamsulosin.**] NCT04365257
- The primary objective of this study is to evaluate if the addition of zanubrutinib to supportive care increases the respiratory failure-free survival rate at Day 28 in participants hospitalized for Corona Virus Disease 2019 (COVID-19) and pulmonary distress. [**note: this is a tyrosine kinase**

inhibitor used for a specific lymphoma. Not sure what the rationale is for testing it against SARS-CoV-2] NCT04382586

CLINICAL TRIAL RESULTS

- I guess the clinicians took Sunday off.

DRUG DEVELOPMENT

- The COVID-2019 pandemic is the most severe acute public health threat of the twenty-first century. To properly address this crisis with both robust testing and novel treatments, we require a deep understanding of the life cycle of the causative agent, the SARS-CoV-2 coronavirus. Here, we examine the architecture and self-assembly properties of the SARS-CoV-2 nucleocapsid (N) protein, which binds viral RNA and assembles into a filament that is packaged into new virions. We determined a 1.4 angstrom resolution crystal structure of this protein's N2b domain, revealing a compact, intertwined dimer very similar to that of related coronaviruses SARS-CoV and MERS-CoV. Using size exclusion chromatography and multi-angle light scattering, we find that this domain forms a dimer in solution, and that addition of the C-terminal spacer B/N3 domain mediates tetramer formation. Using hydrogen-deuterium exchange mass spectrometry, we find evidence that at least part of this putatively disordered domain is structured, potentially forming an α-helix that either self-associates or docks against the N2b domain to mediate tetramer formation. Finally, we map the locations of over 4,400 individual amino acid substitutions in the N protein from ~17,000 SARS-CoV-2 genome sequences, and find that they are strongly clustered in the protein's N2a linker domain. The nearly 300 substitutions identified within the N1b and N2b domains cluster away from their functional RNA binding and dimerization interfaces. Overall, this work reveals the architecture and self-assembly properties of a key protein in the SARS-CoV-2 life cycle. As the N protein is a common target of patient antibodies, this work will also benefit ongoing efforts to develop robust and specific serological tests, and could also benefit the analysis of patient-derived antibodies. [**note: this is just a fascinating paper on how the virus assembles.**] https://connect.medrxiv.org/relate/content/181
- With currently over 4 million confirmed cases worldwide, including more than 300000 deaths, the current Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic has a major impact on the economy and health care system. Currently, a limited amount of prophylactic or therapeutic intervention options are available against SARS-CoV-2. In this study, we screened 400 compounds from the antimicrobial "Pandemic Response Box" library for inhibiting properties against SARS-CoV-2. We identified sixteen compounds that potently inhibited SARS-CoV-2 replication, of which five compounds displayed equal or even higher antiviral activity compared to Remdesivir. These results show that five compounds should be further investigated for their mode of action, safety and efficacy against SARS-CoV-2. [**note: I really don't like abstracts that leave out the "punch line." I read the paper and the drugs noted in the abstract are: chloroquine, NN-DNJ, PDNJ0803, 202 URMC-099-C, and Retro-2.1 have previously also been demonstrated to inhibit the replication of other 203 RNA-viruses such as Filoviruses, Flaviviruses and Picornaviruses. Other than chloroquine, I think the others are all experimental drugs and there may be little human data for them.**] https://www.biorxiv.org/content/10.1101/2020.05.17.100404v1

- Animal models are urgently needed to study the pathogenesis of COVID-19 and to screen candidate vaccines and treatments. Nonhuman primates (NHP) are considered the gold standard model for many infectious pathogens as they usually best reflect the human condition. Here, we show that African green monkeys support a high level of SARS-CoV-2 replication and develop pronounced respiratory disease that may be more substantial than reported for other NHP species including cynomolgus and rhesus macaques. In addition, SARS-CoV-2 was detected in mucosal samples of all animals including feces of several animals as late as 15 days after virus exposure. Importantly, we show that virus replication and respiratory disease can be produced in African green monkeys using a much lower and more natural dose of SARS-CoV-2 than has been employed in other NHP studies. [**note: PETA won't like this one; a new animal model for SARS-CoV-2 testing has been developed by these Texas researchers.**]
  https://www.biorxiv.org/content/10.1101/2020.05.17.100289v1
- Nrf2 is a transcription factor that regulates cellular redox balance and the expression of a wide array of genes involved in immunity and inflammation, including antiviral actions. Nrf2 activity declines with age, making the elderly more susceptible to oxidative stress-mediated diseases, which include type 2 diabetes, chronic inflammation, and viral infections. Published evidence suggests that Nrf2 activity may regulate important mechanisms affecting viral susceptibility and replication. We examined gene expression levels by GeneChip microarray and by RNA-seq assays. We found that the potent Nrf2 activating composition PB125 down regulates ACE2 and TMPRSS2 mRNA expression in human liver-derived HepG2 cells. ACE2 is a surface receptor and TMPRSS2 activates the spike protein for SARS-Cov-2 entry into host cells. Furthermore, in endotoxin-stimulated primary human pulmonary artery endothelial cells we report the marked down regulation by PB125 of 36 genes encoding cytokines. These include IL1-beta, IL6, TNF-alpha the cell adhesion molecules ICAM1, VCAM1, and E-selectin, and a group of IFN-gamma-induced genes. Many of these cytokines have been specifically identified in the cytokine storm observed in fatal cases of COVID-19, suggesting that Nrf2 activation may significantly decrease the intensity of the storm. [**note: interesting finding on the this transcription factor but it's not clear whether there is a pharmaceutical intervention that can be used.**]
  https://www.biorxiv.org/content/10.1101/2020.05.16.099788v1
- The pandemic of this virus has caused a high number of deaths in the world. In order to more efficiently combat this pandemic, it is necessary to develop a better understanding of how the virus infects host cells. Infection normally starts with the initial attachment of the virus to cell-surface glycans like heparan sulfate (HS) proteoglycans and sialic acid-containing oligosaccharides. In this study, we used glycan microarray technology to study the binding of the SARS-CoV-2 spike protein (S protein) to HS and sialic acid. Our results indicated that the S protein can bind to HS in a sulfation-dependent manner and the length of HS appears not to be a critical factor for the binding. No binding with sialic acid residues was detected. In addition, we applied sequence alignment and molecular docking to analyze and explain the observed binding results. Our results suggested that HS may stabilize the open conformation of the S protein to promote the subsequent binding of the S protein to the virus entry receptor ACE2. Overall, this work supports the potential importance of HS in SARS-CoV-2 infection and in the development of antiviral agents. [**note: more on the importance of glycan binding.**]
  https://www.biorxiv.org/content/10.1101/2020.05.17.100537v1

- We present a combinatorial machine learning method to evaluate and optimize peptide vaccine formulations, and we find for SARS-CoV-2 that it provides superior predicted display of viral epitopes by MHC class I and MHC class II molecules over populations when compared to other candidate vaccines. Our method is robust to idiosyncratic errors in the prediction of MHC peptide display and considers target population HLA haplotype frequencies during optimization. To minimize clinical development time our methods validate vaccines with multiple peptide presentation algorithms to increase the probability that a vaccine will be effective. We optimize an objective function that is based on the presentation likelihood of a diverse set of vaccine peptides conditioned on a target population HLA haplotype distribution and expected epitope drift. We produce separate peptide formulations for MHC class I loci (HLA-A, HLA-B, and HLA-C) and class II loci (HLA-DP, HLA-DQ, and HLA-DR) to permit signal sequence based cell compartment targeting using nucleic acid based vaccine platforms. Our SARS-CoV-2 MHC class I vaccine formulations provide 93.21% predicted population coverage with at least five vaccine peptide-HLA hits on average in an individual (≥ 1 peptide 99.91%) with all vaccine peptides perfectly conserved across 4,690 geographically sampled SARS-CoV-2 genomes. Our MHC class II vaccine formulations provide 90.17% predicted coverage with at least five vaccine peptide-HLA hits on average in an individual with all peptides having observed mutation probability ≤ 0.001. We evaluate 29 previously published peptide vaccine designs with our evaluation tool with the requirement of having at least five vaccine peptide-HLA hits per individual, and they have a predicted maximum of 58.51% MHC class I coverage and 71.65% MHC class II coverage given haplotype based analysis. We provide an open source implementation of our design methods (OptiVax), vaccine evaluation tool (EvalVax), as well as the data used in our design efforts. [**note: this is a useful paper regarding design of vaccine epitopes. It's also open source and available to all researchers.**] https://connect.medrxiv.org/relate/content/181
- Most antiviral agents are designed to target virus-specific proteins and mechanisms rather than the host cell proteins that are critically dysregulated following virus-mediated reprogramming of the host cell transcriptional state. To overcome these limitations, we propose that elucidation and pharmacologic targeting of host cell Master Regulator proteins--whose aberrant activities govern the reprogramed state of coronavirus-infected cells--presents unique opportunities to develop novel mechanism-based therapeutic approaches to antiviral therapy, either as monotherapy or as a complement to established treatments. Specifically, we propose that a small module of host cell Master Regulator proteins (ViroCheckpoint) is hijacked by the virus to support its efficient replication and release. Conventional methodologies are not well suited to elucidate these potentially targetable proteins. By using the VIPER network-based algorithm, we successfully interrogated 12h, 24h, and 48h signatures from Calu-3 lung adenocarcinoma cells infected with SARS-CoV, to elucidate the time-dependent reprogramming of host cells and associated Master Regulator proteins. We used the NYS CLIA-certified Darwin OncoTreat algorithm, with an existing database of RNASeq profiles following cell perturbation with 133 FDA-approved and 195 late-stage experimental compounds, to identify drugs capable of virtually abrogating the virus-induced Master Regulator signature. This approach to drug prioritization and repurposing can be trivially extended to other viral pathogens, including SARS-CoV-2, as soon as the relevant infection signature becomes available. [**note: another paper where the punch line is missing from the abstract. There are a bunch of compounds they identified as possible blockers of SARS-CoV-2 and they are from multiple therapeutic**

categories. It's well worth downloading and reading this paper.]
https://www.biorxiv.org/content/10.1101/2020.05.12.091256v1

DIAGNOSTIC DEVELOPMENT

- A still-debated percentage of patients develop coronavirus disease 2019 (COVID-19) after infection, whose symptoms include fever, cough, shortness of breath and fatigue. Acute and life-threatening respiratory symptoms are experienced by 10-20% of symptomatic patients, particularly those with underlying medical conditions that includes diabetes, COPD and pregnancy. One of the main challenges in the containment of COVID-19 is the identification and isolation of asymptomatic/pre-symptomatic individuals. As communities re-open, large numbers of people will need to be tested and contact-tracing of positive patients will be required to prevent additional waves of infections and enable the continuous monitoring of the viral loads COVID-19 positive patients. A number of molecular assays are currently in clinical use to detect SARS-CoV-2. Many of them can accurately test hundreds or even thousands of patients every day. However, there are presently no testing platforms that enable more than 10,000 tests per day. Here, we describe the foundation for the REcombinase Mediated BaRcoding and AmplificatioN Diagnostic Tool (REMBRANDT), a high-throughput Next Generation Sequencing-based approach for the simultaneous screening of over 100,000 samples per day. The REMBRANDT protocol includes direct two-barcoded amplification of SARS-CoV-2 and control amplicons using an isothermal reaction, and the downstream library preparation for Illumina sequencing and bioinformatics analysis. This protocol represents a potentially powerful approach for community screening, a major bottleneck for testing samples from a large patient population for COVID-19. [**note: any new method named after a Dutch painter is worthy of acknowledgement. This is another approach to bulk screening.**]
https://www.biorxiv.org/content/10.1101/2020.05.16.099747v1

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2020-05-19

Let's stay with the blues for at least another day. Another movie that featured an out of character blues number is 'Adventures in Babysitting' (which all my loyal readers should have watched more than once) where Elisabeth Shue and the three kids she is babysitting find themselves in a Chicago blues club. The only way out is…..yes, they gotta sing the blues: https://www.youtube.com/watch?v=552PLnE61TM 'Babysitting Blues' with the great Albert King on guitar and back up vocals. **Time for another contest!!! Which actor who gained notoriety playing an intense procedural detective in a Dick Wolf produced series plays the Thor-like character in this movie? First correct answer wins a nice prize!**

Oh dear! I'm sure that President Trump's decision that he made with his personal physician to take hydroxychloroquine will provoke more controversy. Here is what he said at the news conference, "I think it's good. I've heard a lot of good stories. And if it's not good, I'll tell you right. I'm not going to get hurt by it," he told reporters at the White House. "It's been around for 40 years — for malaria, for lupus, for other things. I take it. Front-line workers take it. A lot of doctors take it." Your loyal correspondent is right on top of this story!!! Part of this is true, there are some **clinical trials** to examine the impact of hydroxychloroquine in health care workers. Here are the trial sites in the US for prophylactic studies with hydroxychloroquine. The Duke study is the largest one. You can get more details by looking up the

trial number HERE as the dosages tend to vary. Baylor College of Medicine, 228 patients, NCT04333225; Hackensack, NJ, 45 patients, NCT04345653; Henry Ford hospital system, MI, 3000 patients placebo trial, NCT04341441; Montifiore, NY, 100 patients, NCT04350450; Louisiana State Med Center, 1700 placebo trial, NCT04363450; Duke University big placebo trial, 15,000, NCT04334148; GeoSentinal Foundation, NY (Cornell Med School), 374 placebo trial, NCT04352946; University of Minnesotta, 3500 placebo (not restricted to healthcare provideers), NCT04328467; There are at many more taking place in some other countries. Here is the most current FDA Fact Sheet on Hydroxychloroquine and here is the general FDA warning against the use of hydroxychloroquine outside of a hospital setting or clinical trial because of heart arrythmias.

Here is another opinion piece that will please our public health professionals. Contact tracing and isolation are what Hong Kong and South Korea practice and it works!!

Preliminary evidence is in on eight patients tested with the Moderna mRNA vaccine. Antibodies against SARS-CoV-2 were produced and no untoward adverse events were noted.

Are you having COVID-19 nightmares? I sure have been having a lot of bad dreams in the past two months. Here are some wise words on how to deal with this from today's Washington Post!!

STAT has a critique of the pharmaceutical market model that is partially justified. From the same site, here is an interesting opinion piece on how the pandemic will upend US healthcare.

Here is a fine piece on how serological testing can be used to manage the COVID-19 pandemic. One of the authors is Florian Krammer who developed one of the first reliable tests at Mt. Sinai School of Meciine.

Derek Lowe was especially busy yesterday with a critique of the Oxford vaccine, an in depth analysis of the Moderna vaccine results, and some other vaccine approaches. I have not had a chance to read these yet and would also suggest reading the comments to each piece. Often times experts weigh in on key issues.

The US Government is serious about bringing drug manufacturing back to the US. If the private sector won't do it, Uncle Sam will step into the breech.

After a very light reading day, there were a deluge of abstracts today. Lots of useless modeling papers along with some clinical results that are just too confounding. I've included the clinical papers but worry that folks are CV padding by trying to publish results on fewer than 100 patients. No rest for your weary curator this morning and I'm now heading out for a walk to clear my COVID-addled brain!!!

MODELING

- Objective To estimate the infection fatality rate of coronavirus disease 2019 (COVID-19) from data of seroprevalence studies. Methods Population studies with sample size of at least 500 and published as peer-reviewed papers or preprints as of May 12, 2020 were retrieved from PubMed, preprint servers, and communications with experts. Studies on blood donors were included, but studies on healthcare workers were excluded. The studies were assessed for design features and seroprevalence estimates. Infection fatality rate was estimated from each

study dividing the number of COVID-19 deaths at a relevant time point by the number of estimated people infected in each relevant region. Correction was also attempted accounting for the types of antibodies assessed. Results Twelve studies were identified with usable data to enter into calculations. Seroprevalence estimates ranged from 0.113% to 25.9% and adjusted seroprevalence estimates ranged from 0.309% to 33%. Infection fatality rates ranged from 0.03% to 0.50% and corrected values ranged from 0.02% to 0.40%. Conclusions The infection fatality rate of COVID-19 can vary substantially across different locations and this may reflect differences in population age structure and case-mix of infected and deceased patients as well as multiple other factors. Estimates of infection fatality rates inferred from seroprevalence studies tend to be much lower than original speculations made in the early days of the pandemic. [**note: this is a very important paper to READ! Ioannidis was one of the authors of the Santa Clara County serology study and has been consistent in his view that the IFR may be only marginally higher than seasonal flu. Obviously more data is needed from other studies to validate this.**] https://www.medrxiv.org/content/10.1101/2020.05.13.20101253v1

- The Milan metropolitan area in Northern Italy was among the most severely hit by the SARS-CoV-2 outbreak. The epidemiological trends of mild COVID-19 are however still unknown. The aim of this study was to examine the seroprevalence of SARS-CoV-2 infection in healthy asymptomatic adults, the risk factors, and laboratory correlates. Design: We conducted a cross-sectional study during the outbreak. Presence of anti-SARS-CoV-2 IgM/IgG antibodies against the Nucleocapsid protein was assessed by a lateral flow immunoassay. Setting: Blood center at a leading academic hospital serving as COVID-19 referral center. Participants: We considered a random sample of blood donors since the start of the outbreak (February 24th to April 8th 2020, n=789). Main outcome measures: The main outcome was the prevalence of IgG/IgM anti-SARS-CoV-2 antibodies . Results: The test had a 98.3% specificity and 100% sensitivity, and for IgG was validated in a subset by an independent ELISA against the Spike protein (N=34, P<0.001). At the start of the outbreak, the overall seroprevalence of SARS-CoV-2 was 4.6% (2.3 to 7.9; P<0.0001 vs. 120 historical controls). During the study period characterized by a gradual implementation of social distancing measures, there was a progressive increase in seroprevalence to 7.1% (4.4 to 10.8), due to a rise in IgG+ to 5% (2.8 to 8.2; P=0.004 for trend, adjusted weekly increase 2.7, SE 1.3%), but not of IgM+ (P=NS). At multivariate logistic regression analysis, seroconversion to IgG was more frequent in younger (P=0.043), while recent infections (IgM+) in older individuals (P=0.002). IgM+ was independently associated with higher triglycerides, eosinophils, and lymphocytes (P<0.05). Conclusions: SARS-CoV-2 infection was already circulating in Milan at the outbreak start. Social distancing may have been more effective in younger individuals, and by the end of April 4.4-10.8% of healthy adults had evidence of seroconversion. Asymptomatic infection may affect lipid profile and blood count. [**note: serology study from Milan**] https://www.medrxiv.org/content/10.1101/2020.05.11.20098442v1
- As COVID-19 is rapidly spreading across the globe, short-term modeling forecasts provide time-critical information for decisions on containment and mitigation strategies. A major challenge for short-term forecasts is the assessment of key epidemiological parameters and how they change when first interventions show an effect. By combining an established epidemiological model with Bayesian inference, we analyze the time dependence of the effective growth rate of new infections. Focusing on COVID-19 spread in Germany, we detect change points in the effective growth rate that correlate well with the times of publicly announced interventions.

Thereby, we can quantify the effect of interventions, and we can incorporate the corresponding change points into forecasts of future scenarios and case numbers. Our code is freely available and can be readily adapted to any country or region. [**note: interesting study from Germany on how to forecast new infections.**]
https://science.sciencemag.org/content/early/2020/05/14/science.abb9789

- Background. The unprecedented impact of the Covid-19 pandemics on modern society has ignited a "gold rush" for effective treatment and diagnostic strategies, with a significant diversion of economical, scientific and human resources towards dedicated clinical research. We aimed to describe trends in this rapidly changing landscape to inform adequate resource allocation. Methods. We developed informatic tools (Covid Trial Monitor) to analyze in real time growth rate, geographical distribution and characteristics of Covid-19 related trials. We defined structured semantic ontologies with controlled vocabularies to categorize trial interventions, study endpoints and study designs. Data and analyses are publicly available at https://bioinfo.ieo.it/shiny/app/CovidCT Results. We observe a clear prevalence of monocentric trials with highly heterogeneous endpoints and a significant disconnect between geographic distribution and disease prevalence, implying that most countries would need to recruit unrealistic percentages of their total prevalent cases to fulfill enrolment. Conclusions. This geographically and methodologically incoherent growth sheds doubts on the actual feasibility of locally reaching target sample sizes and the probability of most of these trials providing reliable and transferable result. We call for the harmonization of clinical trial design criteria for Covid19 and the increased use of larger master protocols incorporating elements of adaptive designs. Covid Trial Monitor identifies critical issues in current Covid19-related clinical research and represents a useful resource for researchers and policymakers to improve the quality and efficiency of related trials [**note: astute readers of this newsletter will already know that I have written about this exact issue in my *Clinical Trials in a Pandemic* paper!! Of course I'm just a mild manner drug regulatory guy whose cachet is limited!**
https://www.medrxiv.org/content/10.1101/2020.05.14.20101758v1
- Given a potential surge of COVID-19 cases in Germany, the federal government and the federal states have ordered a shutdown of businesses and social activities. An important purpose of the shutdown is to avoid overstretching intensive care unit (ICU) capacity (flattening the curve). The aim of this study was to determine the clinical and economic value of a successful shutdown. Methods: In the base case, the study compared a successful shutdown to a worst-case scenario with no ICU capacity left to treat COVID-19 patients. To this end, a decision model was developed using, e.g., information on age-specific fatality rates, ICU outcomes, and the herd protection threshold. The value of an additional life year was borrowed from new, innovative oncological drugs, as cancer reflects a condition with a similar morbidity and mortality burden in the general population in the short-term. Results: A successful shutdown is projected to yield an average gain between 0.02 and 0.08 life years (0.3 to 1.0 months) per capita in the German population. The corresponding economic value ranges between 2147 and 8056 euros per capita or, extrapolated to the total population, 5% to 19% of the gross domestic product in 2019. Nevertheless, if herd immunity is achieved through natural infection, even a shutdown that is successful in flattening the curve is expected to yield a loss of 0.40 life years per capita compared to the situation before the pandemic. Conclusion: A successful shutdown is forecasted to yield a considerable gain in life years in the German population. Nevertheless,

questions around the affordability and underfunding of other parts of the healthcare system emerge. [**note: I include this one as it attempts to model in some economic factors. I do have several readers of this newsletters who are economists! This one is for you!!!**] https://www.medrxiv.org/content/10.1101/2020.05.12.20098996v1

- The COVID-19 outbreak has clear clinical and economic impacts, but also affects behaviors e.g. through social distancing, and may increase stress and anxiety. However, while case numbers are tracked daily, we know little about the psychological effects of the outbreak on individuals in the moment. Here we examine the psychological and behavioral shifts over the initial stages of the outbreak in the United States in an observational longitudinal study. Through GPS phone data we find that homestay is increasing, while being at work dropped precipitously. Using regular real-time experiential surveys we observe an overall increase in stress and mood levels which is similar in size to the weekend vs. weekday differences. As there is a significant difference between weekday and weekend mood and stress levels, this is an important decrease in wellbeing. For some, especially those affected by job loss, the mental health impact is severe. [**note: How can I NOT include a paper with this title, "COVID-19 pandemic: every day feels like a weekday to most."**] https://www.medrxiv.org/content/10.1101/2020.05.11.20098228v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow!

CLINICAL TRIAL RESULTS

- Objective: To determine if Tocilizumab treatment in patients hospitalized with laboratory confirmed SARS-CoV-2 infection and subsequent COVID-19 disease provides short-term survival benefit. Design: Case-control, observational study that includes an observation period from arrival to discharge or inpatient death. Both Cox proportional hazards and average treatment effects models were used to determine survival and treatment benefits. Setting: Three Cone Health acute care hospitals including one COVID dedicated facility. Patients: Patients admitted with confirmed SARS-CoV-2 from March 16, 2020 through April 22, 2020. Exposure: Tocilizumab dosed at either 400 mg fixed dose or 8 mg/kg weight-based dose with maximum single dose of 800mg. Measurements and Main Results: Overall, 86 patients were admitted during the observation period with confirmed COVID-19 disease. Of these, 21 received Tocilizumab during the hospital stay. Both the Cox model and treatment effects models showed short-term survival benefit. There was an associated 75% reduction in the risk of inpatient death when treated (HR 0.25; 95% CI 0.07-0.90) in the Cox model. This association was confirmed in the treatment effects model where we found a 52.7% reduced risk of dying while hospitalized compared to those not treated (RR 0.472; 95% CI 0.449-0.497). In both models, we show short-term survival benefit in patients with severe COVID-19 illness. [**note: this is off label and not a clinical trial result of tocilizumab. Still it offers some information that the drug may be useful in treatment.**] https://www.medrxiv.org/content/10.1101/2020.05.14.20099234v1
- A hyperinflammatory state mediated by interleukin-6 (IL-6) has been proposed as a driver of severe disease. Use of the IL-6 receptor inhibitor tocilizumab for severe COVID-19 was first reported in China, where a case series described marked improvements in inflammatory markers, fever, oxygen requirement, and outcomes following its administration. Here, we provide the first description of a tocilizumab-treated cohort of patients with COVID-19 in the

United States. We describe 11 patients from a single academic medical center, nine (82%) of whom were critically ill requiring mechanical ventilation in an intensive care unit at the time of tocilizumab administration. C-reactive protein levels decreased in all patients following treatment (median 211.6 pre- vs. 19.7 mg/L 5 days post-tocilizumab [p=0.001]). When IL-6 levels were obtained before and after therapy, wide variation was seen in baseline levels; post-dose IL-6 concentrations were consistently increased. In contrast to prior reports, we did not observe significant clinical improvement in temperature or oxygen requirements in most patients. Two patients were discharged (18%), five remained in critical condition in the intensive care unit (46%), one was weaned off the ventilator to room air (9%), and three died (27%). Our findings suggest that tocilizumab should be used with caution in severe and critically ill patients and highlight the need for data from randomized controlled trials to determine its efficacy in the treatment of COVID-19. [**note: THIS IS WHY WE DO CLINICAL TRIALS. These researches only have a small patient experience and it seems not to show much benefit for tocilizumab.**] https://www.medrxiv.org/content/10.1101/2020.05.13.20100404v1

- Interleukin-6 signal blockade has shown preliminary beneficial effects in treating aberrant host inflammatory response against SARS-CoV-2 leading to severe respiratory distress. Objective: to describe the effect of off-label intravenous use of Sarilumab in patients with severe SARS-CoV-2-related pneumonia. Design: Observational clinical cohort study. Setting: Fondazione Policlinico Universitario A. Gemelli IRCCS as Italian Covid reference center. Participants: Patients with laboratory-confirmed SARS-CoV-2 infection and respiratory distress with PaO_2/FiO_2 ratio<300 treated with Sarilumab between March 23rd − April 4th, 2020. Date of final follow-up was April 18, 2020. Main outcomes and measures: We describe the clinical outcomes of 53 patients with SARS−CoV−2 severe pneumonia treated with intravenous Sarilumab in terms of pulmonary function improvement or Intensive Care Unit (ICU) admission rate in medical wards setting and of live discharge rate in ICU treated patients as well as in terms of safety. Each patient received Sarilumab 400 mg administered intravenously on day 1, with eventual additional infusion based on clinical judgement, and was followed for at least 14 days, unless previously discharged or dead. No gluco-corticosteroids were used at baseline. Results: Of the 53 SARS−CoV−2pos patients receiving Sarilumab, 39 (73.6%) were treated in medical wards (66.7% with a single infusion) while 14 (26.4%) in ICU (92.6% with a second infusion). The median PaO_2/FiO_2 of patients in the Medical Ward was 146(IQR:120−212) while the median PaO_2/FiO_2 of patients in ICU was 112(IQR:100−141.5), respectively. Within the medical wards, 7(17.9%) required ICU admission, 4 of whom were re−admitted to the ward within 5-8 days. At 19 days median follow−up, 89.7% of medical inpatients significantly improved (46.1% after 24 hours, 61.5% after 3 days), 70.6% were discharged from the hospital and 85.7% no longer needed oxygen therapy. Within patients receiving Sarilumab in ICU, 64.2% were discharged from ICU to the ward and 35.8% were still alive at the last follow-up. Overall mortality rate was 5.7% after Sarilumab administration: 1(2.5%) patient died in the Medical Ward whilst 2(14.2%) patients died in ICU, respectively. Conclusions and relevance: IL6−R inhibition appears to be a potential treatment strategy for severe SARS−CoV−2 pneumonia and intravenous Sarilumab seems a promising treatment approach showing, in the short term, an important clinical benefit and good safety. [**note: THIS IS WHY WE DO CLINICAL TRIALS??? I believe on sarilumab trial was stopped by a DSMB as it showed no efficacy. This large Italian team seems to show a different outcome. We are seeing so much confounding data that one wonders if any drug will be found to be an**

unequivocal solution to the pandemic.]
https://www.medrxiv.org/content/10.1101/2020.05.14.20094144v1

- Background: No consensus or evidence-based guideline currently exists for pharmacological therapy against Coronavirus Disease 2019 (COVID 19). While South Korea has been relatively successful in managing the pandemic, its management of confirmed cases and treatment outcomes have not been reported to date. Methods: A retrospective cohort study of the 358 laboratory-confirmed SARS CoV 2 or COVID 19 patients was conducted. Of these patients, 270 adult patients met inclusion criteria and were included in our analyses. The primary endpoints were time to viral clearance and clinical improvement. The mean duration to viral clearance and clinical improvements were displayed as bar-plots to visualize treatment responses. Results: Ninety-seven moderate COVID 19 patients were managed with hydroxychloroquine (HQ) plus antibiotics (n = 22), lopinavir/ritonavir (Lop/R) plus antibiotics (n = 35), or conservative treatment (n = 40). Time to viral clearance, as signified by negative conversion on PCR, after initiation of treatment was significantly shorter with HQ plus antibiotics compared to Lop/R plus antibiotics (hazard ratio [HR], 0.49; 95% confidence interval [95% CI], 0.28 to 0.87) or conservative treatments (HR, 0.44; 95% CI, 0.25 to 0.78). Hospital stay duration after treatment was also shortest for patients treated with HQ plus antibiotics compared to other treatment groups. Subgroup analysis revealed that mean duration to viral clearance was significantly reduced with adjunctive use of antibiotics compared to monotherapy (HR 0.81, 95% CI, 0.70 to 0.93). While both HQ and Lop/R showed side effects including nausea, vomiting, and elevation of liver transaminases, none were serious. Conclusion: This first report on pharmacological management of COVID 19 from South Korea revealed that HQ with antibiotics was associated with better clinical outcomes in terms of viral clearance, hospital stay, and cough symptom resolution compared to Lop/R with antibiotics or conservative treatment. The effect of Lop/R with antibiotics was not superior to conservative management. The adjunct use of the antibiotics may provide additional benefit in COVID 19 management but warrants further evaluation.[**note: THIS IS WHY WE DO CLINICAL TRIALS – PART 3. Should I even waste time reading papers where under 100 patients are treated without at control. I guess the HCQ/azithromycin folks will champion this research.**]
https://www.medrxiv.org/content/10.1101/2020.05.13.20094193v1
- We analyzed positive COVID-19 testing results counts within New York City ZIP Code Tabulation Areas (ZCTA) with Bayesian hierarchical Poisson spatial models using integrated nested Laplace approximations. Results. Spatial clustering accounted for approximately 32% of the variation in the data, with hot spots in all five boroughs. Spatial risk did not correspond precisely to population-based rates of positive tests. The strongest univariate association with positive testing rates was the proportion of residents in a ZIP Code Tabulation Area with Chronic Obstructive Pulmonary Disease (COPD). For every one unit increase in a scaled standardized measure of COPD in a community, there was an approximate 8-fold increase in the risk of a positive COVID-19 test in a ZCTA (Incidence Density Ratio = 8.2, 95% Credible Interval 3.7, 18.3). The next strongest association was with the proportion of Black and African American residents, for which there was a nearly five-fold increase in the risk of a positive COVID-19 test. (IDR = 4.8, 95% Cr I 2.4, 9.7). Increases in the proportion of residents older than 65, housing density and the proportion of residents with heart disease were each associated with an approximate doubling of risk. In a multivariable model including estimates for age, COPD, heart disease, housing

density and Black/African American race, the only variables that remained associated with positive COVID-19 testing with a probability greater than chance were the proportion of Black/African American residents and proportion of older persons. Conclusions. The population and spatial patterns of COVID-19 infections differ by race, age, physical environment and health status. Areas with large proportions of Black/African American residents are at markedly higher risk that is not fully explained by characteristics of the environment and pre-existing conditions in the population. [**note: UK data show a racial disparity but not to this extent.**] https://www.medrxiv.org/content/10.1101/2020.05.14.20101691v1

- Most studies investigating racial and ethnic disparities to date have focused on hospitalized patients or have not characterized who received testing or those who tested positive for Covid-19. Objective: To compare patterns of testing and test results for coronavirus 2019 (Covid-19) and subsequent mortality by race and ethnicity in the largest integrated healthcare system in the United States. Design: Retrospective cohort study. Setting: United States Department of Veterans Affairs (VA). Participants: 5,834,543 individuals in care, among whom 62,098 were tested and 5,630 tested positive for Covid-19 between February 8 and May 4, 2020. Exposures: Self-reported race/ethnicity. Main outcome measures: We evaluated associations between race/ethnicity and receipt of Covid-19 testing, a positive test result, and 30-day mortality, accounting for a wide range of demographic and clinical risk factors including comorbid conditions, site of care, and urban versus rural residence. Results: Among all individuals in care, 74% were non-Hispanic white (white), 19% non-Hispanic black (black), and 7% Hispanic. Compared with white individuals, black and Hispanic individuals were more likely to be tested for Covid-19 (tests per 1000: white=9.0, [95% CI 8.9 to 9.1]; black=16.4, [16.2 to 16.7]; and Hispanic=12.2, [11.9 to 12.5]). While individuals from minority backgrounds were more likely to test positive (black vs white: OR 1.96, 95% CI 1.81 to 2.12; Hispanic vs white: OR 1.73, 95% CI 1.53 to 1.96), 30-day mortality did not differ by race/ethnicity (black vs white: OR 0.93, 95% CI 0.64 to 1.33; Hispanic vs white: OR 1.07, 95% CI 0.61 to 1.87). Conclusions: Black and Hispanic individuals are experiencing an excess burden of Covid-19 not entirely explained by underlying medical conditions or where they live or receive care. While there was no observed difference in mortality by race or ethnicity, our findings may underestimate risk in the broader US population as health disparities tend to be reduced in VA. [**VA data on racial and ethnic disparity.**] https://www.medrxiv.org/content/10.1101/2020.05.12.20099135v1
- On March 28, 2020, in response to the rapidly accelerating COVID-19 pandemic, U.S FDA issued emergency use authorization for hydroxychloroquine (HCQ) in hospitalized COVID-19 patients based on limited in-vitro and anecdotal clinical data. Analysis of the accumulated real-world data utilizing electronic medical records (EMR) could indicate HCQ therapy benefits as we await the results of clinical trials. However, any such analysis of retrospective observational data should account for variables such as demographics and comorbidities that could affect treatment strategies or outcomes. Therefore, we report the outcomes of HCQ treatment in a propensity-matched cohort of COVID-19 hospitalized patients. *Our analysis of a large retrospective cohort of hospitalized COVID-19 patients treated with HCQ did not show benefits in mortality or the need for mechanical ventilation when compared to a matched cohort of patients who did not receive HCQ.* [**note: over 3300 patients were included in this observational study. They excluded all patients who received treatment therapy other than HCQ including**

azithromycin combination therapy.]
https://www.medrxiv.org/content/10.1101/2020.05.12.20099028v1

- In April 2020, respiratory disease and increased mortality were observed in farmed mink on two farms in the Netherlands. In both farms, at least one worker had been found positive for SARS-CoV-2. Necropsies of the mink revealed interstitial pneumonia, and organ and swab samples tested positive for SARS-CoV-2 RNA by qPCR. Variations in viral genomes point at between-mink transmission on the farms and lack of infection link between the farms. Inhalable dust in the mink houses contained viral RNA, indicating possible exposure of workers. [**note: are people still buying mink coats???**] https://www.biorxiv.org/content/10.1101/2020.05.18.101493v1
- SARS-CoV-2, the pandemic coronavirus that causes COVID-19, has infected millions worldwide, causing unparalleled social and economic disruptions. COVID-19 results in higher pathogenicity and mortality in the elderly compared to children. Examining baseline SARS-CoV-2 cross-reactive coronavirus immunological responses, induced by circulating human coronaviruses, is critical to understand such divergent clinical outcomes. The cross-reactivity of coronavirus antibody responses of healthy children (n=89), adults (n=98), elderly (n=57), and COVID-19 patients (n=19) were analysed by systems serology. While moderate levels of cross-reactive SARS-CoV-2 IgG, IgM, and IgA were detected in healthy individuals, we identified serological signatures associated with SARS-CoV-2 antigen-specific Fcγ receptor binding, which accurately distinguished COVID-19 patients from healthy individuals and suggested that SARS-CoV-2 induces qualitative changes to antibody Fc upon infection, enhancing Fcγ receptor engagement. Vastly different serological signatures were observed between healthy children and elderly, with markedly higher cross-reactive SARS-CoV-2 IgA and IgG observed in elderly, whereas children displayed elevated SARS-CoV-2 IgM, including receptor binding domain-specific IgM with higher avidity. These results suggest that less-experienced humoral immunity associated with higher IgM, as observed in children, may have the potential to induce more potent antibodies upon SARS-CoV-2 infection. These key insights will inform COVID-19 vaccination strategies, improved serological diagnostics and therapeutics. [**note: serological differences between children and adults.**] https://www.medrxiv.org/content/10.1101/2020.05.11.20098459v1
- Initial reports indicate adequate performance of some serological-based SARS-CoV-2 assays. However, additional studies are required to facilitate interpretation of results, including how antibody levels impact immunity and disease course. Methods: In this study, a total of 968 subjects were tested for IgG antibodies reactive to SARS-CoV-2. We confirmed analytic specificity using 656 plasma samples from healthy donors, 49 sera from patients with rheumatic disease, and 90 specimens from individuals positive for PCR-based respiratory viral panel. One-hundred seventy-three cases of confirmed or suspected SARS-CoV-2 were tested for IgG. A subgroup of 37 SARS-CoV-2 PCR-positive cases was tested for nucleocapsid-specific IgM antibody using an in-house developed microarray method. Antibody levels were compared between disease severity groups. Results: All specificity specimens were negative for SARS-CoV-2 IgG antibodies (0/656, 0%). Cross reactivity was not detected in specimens with antinuclear antibodies and rheumatoid factor, or cases with previous diagnosis of viral infection including human coronavirus. Positive agreement of IgG with PCR was 83% of samples confirmed to be more than 14 days from symptom onset, with less than 100% sensitivity attributable to a case with severe immunosuppression. Virus-specific IgM was positive in a higher proportion of cases less than 3 days from symptom onset. No association was observed between mild and severe

disease course with respect to IgG and IgM levels. Conclusions: The studied SARS-CoV-2 IgG assay had 100% specificity and no adverse cross-reactivity. Index values of IgG and IgM antibodies did not predict disease severity in our patient population. [**note: a prize to anyone who has a plausible answer to what this study means.**]
https://www.medrxiv.org/content/10.1101/2020.05.15.20103580v1

DRUG DEVELOPMENT

- Repurposing clinically available drugs to treat the new coronavirus disease COVID-19 is an urgent need in these early stages of the SARS-CoV-2 pandemic, when very few treatment options are available. The iminosugar Miglustat is a well-characterized drug for the treatment of rare genetic lysosome storage diseases such as Gaucher and Niemann-Pick type C, and has also been described to be active against a variety of enveloped viruses. The activity of Miglustat is here demonstrated for SARS-CoV-2 at concentrations achievable in the plasma by current clinical regimens without cytotoxicity. The drug acts at the post-entry level and leads to a marked decrease of viral proteins and release of infectious virus. The mechanism resides in the inhibitory activity towards α-glucosidases that are involved in early stages of glycoprotein N-linked oligosaccharide processing in the endoplasmic reticulum, leading to a marked decrease of the viral Spike protein. The wealth of available data on the clinical use of Miglustat for the treatment of lysosomal storage disorders and the antiviral properties against SARS-CoV-2 make it an ideal candidate for drug repurposing. [**note: US FDA did not approve the drug, deeming there was insufficient efficacy data.**]
https://www.biorxiv.org/content/10.1101/2020.05.18.101691v1
- Antibody-based interventions against SARS-CoV-2 could limit morbidity, mortality, and possibly disrupt epidemic transmission. An anticipated correlate of such countermeasures is the level of neutralizing antibodies against the SARS-CoV-2 spike protein, yet there is no consensus as to which assay should be used for such measurements. Using an infectious molecular clone of vesicular stomatitis virus (VSV) that expresses eGFP as a marker of infection, we replaced the glycoprotein gene (G) with the spike protein of SARS-CoV-2 (VSV-eGFP-SARS-CoV-2) and developed a high-throughput imaging-based neutralization assay at biosafety level 2. We also developed a focus reduction neutralization test with a clinical isolate of SARS-CoV-2 at biosafety level 3. We compared the neutralizing activities of monoclonal and polyclonal antibody preparations, as well as ACE2-Fc soluble decoy protein in both assays and find an exceptionally high degree of concordance. The two assays will help define correlates of protection for antibody-based countermeasures including therapeutic antibodies, immune γ-globulin or plasma preparations, and vaccines against SARS-CoV-2. Replication-competent VSV-eGFP-SARS-CoV-2 provides a rapid assay for testing inhibitors of SARS-CoV-2 mediated entry that can be performed in 7.5 hours under reduced biosafety containment. [**note: a new assay that can be used under BL-2 conditions.**] https://www.biorxiv.org/content/10.1101/2020.05.18.102038v1

DIAGNOSTIC DEVELOPMENT

- We prospectively compared the efficacy of PCR detection of SARS-CoV-2 between paired nasopharyngeal and saliva samples in nine COVID-19 patients. SARS-CoV-2 was detected in saliva in 8 of 9 (89%) patients and in all 11 samples taken within 2 weeks after disease onset. Viral load was equivalent at earlier time points but declined in saliva than nasopharyngeal

samples. PCR negativity was also concordant in all 27 saliva samples from 24 patients between nasopharyngeal and saliva samples. These results suggest that saliva is a reliable noninvasive alternative to nasopharyngeal swabs and facilitate widespread PCR testing in the face of shortages of swabs and protective equipment without posing a risk to healthcare workers. [**note: data from Japan on the equivalence of saliva and nasal swabs for testing.**] https://www.medrxiv.org/content/10.1101/2020.05.13.20100206v1

- Numerous serologic immunoassays have been launched to detect antibodies to SARS-CoV-2, including rapid tests. Here, we validate use of a lateral flow immunoassay (LFI) intended for rapid screening and qualitative detection of anti-SARS-CoV-2 IgM and IgG in serum, plasma, and whole blood, and compare results with ELISA. We also seek to establish the value of LFI testing on blood obtained from a capillary blood sample. Methods: Samples collected by venous blood draw and capillary finger stick were obtained from patients with SARS-CoV-2 detected by RT-qPCR and control patients negative for SARS-CoV-2. Samples were tested with the 2019-nCoV IgG/IgM Detection Kit (Colloidal Gold) lateral flow immunoassay, and antibody calls were compared with results obtained by ELISA. Results: The Biolidics LFI kit shows clinical sensitivity of 92% at 7 days after PCR diagnosis of SARS-CoV-2 on venous blood. Test specificity was 92% for IgM and 100% for IgG. There was no significant difference in detecting IgM and IgG with Biolidics LFI and ELISA at D0 and D7 (p=1.00), except for detection of IgM at D7 (p=0.04). Finger stick whole blood of SARS-CoV-2 patients showed 93% sensitivity for antibody detection. Conclusions: Clinical performance of Biolidics 2019-nCoV IgG/IgM Detection Kit (Colloidal Gold) is comparable to ELISA and showed consistent results across different sample types. Furthermore, we show that capillary blood obtained by finger stick shows similar sensitivity for detecting anti-SARS-CoV-2 IgM and IgG antibodies as venous blood samples. This provides an opportunity for decentralized rapid testing in the community and may allow point-of-care and longitudinal self-testing for the presence of anti-SARS-CoV-2 antibodies. [**note: this is a US lab validation of a Singapore company Biolidics lateral flow serology test. I don't know if the company will be filing for an EUA with the FDA. There are some good tests out there and they should be employed ASAP here in the US.**] https://www.medrxiv.org/content/10.1101/2020.05.13.20098426v1

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2020-05-20

Let's shift gears and move on to some folk music. Here is a very young Joni Mitchell from England. Her voice was youthful and lyrical. Two of her well known songs of the day, 'Big Yellow Taxi' and my fave 'Both Sides Now': https://www.youtube.com/watch?v=GFB-d-8_bvY Alas, nobody got the quiz answer yesterday. Vincent d'Onofrio played the garage worker in the movie who the little girl thinks is Thor; he was an intense Detective Goren in 'Law and Order: Criminal Intent.' Better luck in the next contest; I have nice prizes I really want to give away to my loyal readers!!!

Here is another big shout out to the public health officers past and present who read this newsletter!! ProPublica's Caroline Chen covers the ins and outs of contact tracing in a nice story.

This is no surprise to those of us who have been around the block with clinical trials and expanded access of experimental drugs (whether or not they are approved for other indications). As the article in the NY Times notes, real clinical trials are suffering enrollment issues. At the end of the day I suspect the granting of the EUA to hydroxychloroquine by FDA will be viewed as a major mistake. Of course all of this was covered in my paper on Clinical Trials during a Pandemic.

Caution on interpreting the Moderna vaccine preliminary data, HERE and HERE.

Derek Lowe weighs in on the politics and real-world data of hydroxychloroquine. As with all of his posts, he is besieged with comments.

MODELING

- What a relief, no significant new modeling studies!!!

NEWLY REGISTERED CLINICAL TRIALS

- Here is a Stanford University trial. The primary objective of this study is to evaluate the safety and efficacy of intravenous (IV) infusion of ulinastatin compared to placebo with respect to time to recovery, disease severity, need for ventilator support, and mortality in patients with COVID 19. [**note: this is a urinary trypsin inhibitor and with a trade name of 'Miraclid' in Japan. Is this a miracle drug? This trial will let us know.**] NCT04393311
- These Egyptian researchers are trying out a herbal remedy. A randomized placebo controlled trial to assess the clinical outcome in COVID-19 Pneumonia following administration of Silymarin owing to its role as a p38 MAPK pathway inhibitor and its antiviral, anti-inflammatory and anti-oxidant effects NCT04394208

CLINICAL TRIAL RESULTS

- Background COVID-19 pneumonia is associated with significant mortality and has no approved antiviral therapy. Interferon beta1 has shown in vitro studies a potent inhibition of SARS-CoV and MERS-CoV. In an in vitro study, SARS-CoV-2 had more sensitivity to IFN-I pretreatment that SARS-CoV. A combination of IFN beta1b administered subcutaneously with other antiviral treatments has been recommended in several guidelines. However, clinical trial results for the treatment of COVID-19 are pending. We aimed to assess the efficiency of IFN beta1b in COVID19 comparing the in-hospital mortality between patients who received IFN beta1b and patients did not receive. Methods In this retrospective cohort study, we included hospitalized adults with COVID-19 between February 23th and April 4th, 2020, at the Central Defense Hospital (Madrid, Spain). Subcutaneous interferon beta-1b was recommended in moderate-severe pneumonia. The primary endpoint was in-hospital mortality. Univariate and multivariate analysis was performed to identify variables associated with in-hospital mortality. Findings We analyzed 256 patients (106 patients in interferon group and 150 patients in control group). At admission, patients who did not receive interferon beta1b presented a greater number of comorbidities. The overall mortality rate was 24.6% (63/256). Twenty-two patients (20.8%) in the interferon group died and 41 (27.3%) in the control group (p=0.229). In the multivariate analysis, the predictors of in-hospital mortality were age, severity of clinical picture at admission and

hydroxychloroquine treatment. Interpretation In hospitalized patients with COVID-19, interferon beta1b treatment was not associated to decrease in-hospital mortality. Further assessment of the earlier administration of this drug in randomized trials is recommended. [**note: negative results for interferon beta 1-b in COVID-19 pneumonia. Again, this is an observational study though with just over 100 patients on therapy it's larger than some I have read. TIWWDCT (my new acronym!!! This is Why We Do Clinical Trials. Maybe it will make it into the lexicon some day.)** https://www.medrxiv.org/content/10.1101/2020.05.15.20084293v1

- We studied the host transcriptional response to SARS-CoV-2 by performing metagenomic sequencing of upper airway samples in 238 patients with COVID-19, other viral or non-viral acute respiratory illnesses (ARIs). Compared to other viral ARIs, COVID-19 was characterized by a diminished innate immune response, with reduced expression of genes involved in toll-like receptor and interleukin signaling, chemokine binding, neutrophil degranulation and interactions with lymphoid cells. Patients with COVID-19 also exhibited significantly reduced proportions of neutrophils and macrophages, and increased proportions of goblet, dendritic and B-cells, compared to other viral ARIs. Using machine learning, we built 26-, 10- and 3-gene classifiers that differentiated COVID-19 from other acute respiratory illnesses with AUCs of 0.980, 0.950 and 0.871, respectively. Classifier performance was stable at low viral loads, suggesting utility in settings where direct detection of viral nucleic acid may be unsuccessful. Taken together, our results illuminate unique aspects of the host transcriptional response to SARS-CoV-2 in comparison to other respiratory viruses and demonstrate the feasibility of COVID-19 diagnostics based on patient gene expression. [**note: interesting stuff from UCSF researchers. At the end of the day there will be some very interesting genetic findings about SARS-CoV-2 infection.**] https://www.medrxiv.org/content/10.1101/2020.05.18.20105171v1
- Background The correlates of protection against SARS-CoV-2 and their longevity remain unclear. Studies in severely ill individuals have identified robust cellular and humoral immune responses against the virus. Asymptomatic infection with SARS-CoV-2 has also been described, but it is unknown whether this is sufficient to produce antibody responses. Methods We performed a cross-sectional study recruiting 554 health care workers from University Hospitals Birmingham NHS Foundation Trust who were at work and asymptomatic. Participants were tested for current infection with SARS-CoV-2 by nasopharyngeal swab for real-time polymerase chain reaction and for seroconversion by the measurement of anti-SARS-CoV-2 spike glycoprotein antibodies by enzyme linked immunosorbent assay. Results were interpreted in the context of previous, self-reported symptoms of illness consistent with COVID-19. Results The point prevalence of infection with SARS-CoV-2, determined by the detection of SARS-CoV-2 RNA on nasopharnygeal swab was 2.39% (n=13/544). Serum was available on 516 participants. The overall rate of seroconversion in the cohort was 24.4% (n=126/516). Individuals who had previously experienced a symptomatic illness consistent with COVID-19 had significantly greater seroconversion rates than those who had remained asymptomatic (37.5% vs 17.1%, $\chi 2$ =21.1034, p<0.0001). In the week preceding peak COVID-19-related mortality at UHBFT, seroconversion rates amongst those who were suffering from symptomatic illnesses peaked at 77.8%. Prior symptomatic illness generated quantitatively higher antibody responses than asymptomatic seroconversion. Seroconversion rates were highest amongst those working in housekeeping (34.5%), acute medicine (33.3%) and general internal medicine (30.3%) with lower rates observed in participants working in intensive care (14.8%) and emergency medicine

(13.3%). Conclusions In a large cross-sectional seroprevalence study of health-care workers, we demonstrate that asymptomatic seroconversion occurs, however prior symptomatic illness is associated with quantitatively higher antibody responses. The identification that the potential for seroconversion in health-care workers can associate differentially with certain hospital departments may inform future infection control and occupational health practices. [**note: a look at asymptomatic infection in NHS healthcare workers shows seroconversion but at a lower rate than for those who had symptomatic illness.**]
https://www.medrxiv.org/content/10.1101/2020.05.18.20105197v1

- Background: Whether angiotensin-converting enzyme (ACE) Inhibitors and angiotensin receptor blockers (ARBs) mitigate or exacerbate SARS-CoV-2 infection remains uncertain. In a national study, we evaluated the association of ACE inhibitors and ARB with coronavirus disease-19 (COVID-19) hospitalization and mortality among individuals with hypertension. Methods: Among Medicare Advantage and commercially insured individuals, we identified 2,263 people with hypertension, receiving ≥1 antihypertensive agents, and who had a positive outpatient SARS-CoV-2 test (outpatient cohort). In a propensity score-matched analysis, we determined the association of ACE inhibitors and ARBs with the risk of hospitalization for COVID-19. In a second study of 7,933 individuals with hypertension who were hospitalized with COVID-19 (inpatient cohort), we tested the association of these medications with in-hospital mortality. We stratified all our assessments by insurance groups. Results: Among individuals in the outpatient and inpatient cohorts, 31.9% and 29.8%, respectively, used ACE inhibitors and 32.3% and 28.1% used ARBs. In the outpatient study, over a median 30.0 (19.0 - 40.0) days after testing positive, 12.7% were hospitalized for COVID-19. In propensity score-matched analyses, neither ACE inhibitors (HR, 0.77 [0.53, 1.13], P = 0.18), nor ARBs (HR, 0.88 [0.61, 1.26], P = 0.48), were significantly associated with risk of hospitalization. In analyses stratified by insurance group, ACE inhibitors, but not ARBs, were associated with a significant lower risk of hospitalization in the Medicare group (HR, 0.61 [0.41, 0.93], P = 0.02), but not the commercially insured group (HR: 2.14 [0.82, 5.60], P = 0.12; P-interaction 0.09). In the inpatient study, 14.2% died, 59.5% survived to discharge, and 26.3% had an ongoing hospitalization. In propensity score-matched analyses, neither use of ACE inhibitor (0.97 [0.81, 1.16]; P = 0.74) nor ARB (1.15 [0.95, 1.38]; P = 0.15) was associated with risk of in-hospital mortality, in total or in the stratified analyses. Conclusions: The use of ACE inhibitors and ARBs was not associated with the risk of hospitalization or mortality among those infected with SARS-CoV-2. However, there was a nearly 40% lower risk of hospitalization with the use of ACE inhibitors in the Medicare population. This finding merits a clinical trial to evaluate the potential role of ACE inhibitors in reducing the risk of hospitalization among older individuals, who are at an elevated risk of adverse outcomes with the infection. [**note: TIWWDCT!!! Interesting that in this observational study ACE inhibitors and not ARBs had more of a protective utility in one of the groups. One thing we know for sure: this class of drugs are safer than HCQ. I was going to make a snarky political comment but thought better of it (I'm sure all my readers know what I am thinking.)]**
https://www.medrxiv.org/content/10.1101/2020.05.17.20104943v1

DRUG DEVELOPMENT

- As a neuraminidase inhibitor, oseltamivir has effectively combated the pandemic influenza A and B, so it is a first-line commonly used antiviral drug, especially in primary hospitals. At the

same time, oseltamivir, as an over-the-counter drug, is also a popular antiviral drug. As healthcare workers fighting against coronavirus disease 2019 (COVID-19), we have found that many patients experiencing discomfort or considered to be infected with a virus take oseltamivir. From severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 to middle east respiratory syndrome coronavirus (MERS-CoV) in 2012, and now the current COVID-19 epidemic, there is not plenty of evidence showing that oseltamivir is effective against coronavirus. Still, there is also no sufficient evidence to refute its ineffectiveness. We cannot predict whether there will be a pandemic of respiratory coronavirus in the future, so we hope to initiate such research and preliminarily explore whether oseltamivir is effective for COVID-19, which can better guide healthcare workers in the selection of appropriate antiviral drugs in the face of coronavirus epidemics. If oseltamivir is effective, then a wide promotion of its application often can achieve a double effect with half the effort. If it is not effective, then considering the side effects of oseltamivir, it is not necessary to use unreasonable drugs that will not slow the progression of the disease but can cause adverse reactions. We found that oseltamivir is not suitable for fighting against COVID-19 through the method of computer aided drug design and in vitro study and retrospective case study. Meanwhile it was high-occurrence seasons for the influenza, COVID-19 should be highly suspected in patients who did not benefit from oseltamivir. We hope that the result of our study could be shared with the frontline physicians in fighting against COVID-19. [**note: this is not a surprise as oseltamivir is for a different class of viruses. The number of patients in the observational study is quite small however.**] https://www.medrxiv.org/content/10.1101/2020.05.15.20102392v1

- The coronavirus induced disease 19 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a worldwide threat to human lives, and neutralization antibodies present a great therapeutic potential in curing affected patients. We purified more than one thousand memory B cells specific to SARS-CoV-2 recombinant S1 or RBD antigens from 11 convalescent COVID-19 patients, and a total of 729 naturally paired heavy and light chain fragments were obtained by single B cell cloning technology. Among these, 178 recombinant monoclonal antibodies were tested positive for antigen binding, and 17 strong binders to S1 or RBD were identified with Kd(EC50) below 1 nM. Importantly, 12 antibodies could block pseudoviral entry into HEK293T cells overexpressing ACE2, with the best ones showing IC50 around 2-3 nM. From these 12 antibodies, we had tested two in authentic virus infection assay, and found one was able to effectively block live viral entry with IC50 around or below 15 nM. Interestingly, we also found a substantial portion of these antibodies crossreacting with the SARS-CoV spike protein. Altogether, our study provided potent neutralization antibodies as clinical therapeutics candidates for further development. [**note: more confirmation, this time from China, on the utility of monoclonal antibodies.**] https://www.biorxiv.org/content/10.1101/2020.05.19.104117v1
- Spike, Envelope and Membrane proteins from the SARS CoV-2 virus surface coat are important vaccine targets. We hereby report recombinant co-expression of the three proteins (Spike, Envelope and Membrane) in a engineered Saccharomyces cerevisiae platform (D-Crypt™) and their self-assembly as Virus-like particle (VLP). This design as a multi-antigenic VLP for SARS CoV-2 has the potential to be a scalable vaccine candidate. The VLP is confirmed by transmission electron microscopy (TEM) images of the SARS CoV-2, along with supportive HPLC, Dynamic Light Scattering (DLS) and allied analytical data. The images clearly outline the presence of a

Corona like morphology, and uniform size distribution. [**note: a good paper from and Indian group on the use of yeast to produce a SARS-CoV-2 vaccine candidate. Let's get this into trials!!**] https://www.biorxiv.org/content/10.1101/2020.05.18.099234v1

- The need for antiviral drugs is real and relevant. Broad spectrum antiviral drugs have a particular advantage when dealing with rapid disease outbreaks, such as the current COVID-19 pandemic. Since viruses are completely dependent on internal cell mechanisms, they must cross cell membranes during their lifecycle, creating a dependence on processes involving membrane dynamics. Thus, in this study we examined whether the synthesis of glycosphingolipids, biologically active components of cell membranes, can serve as an antiviral therapeutic target. We examined the antiviral effect of two specific inhibitors of GlucosylCeramide synthase (GCS); (i) Genz-123346, an analogue of the FDA-approved drug Cerdelga, (ii) GENZ-667161, an analogue of venglustat which is currently under phase III clinical trials. We found that both GCS inhibitors inhibit the replication of four different enveloped RNA viruses of different genus, organ-target and transmission route: (i) Neuroinvasive Sindbis virus (SVNI), (ii) West Nile virus (WNV), (iii) Influenza A virus, and (iv) SARS-CoV-2. Moreover, GCS inhibitors significantly increase the survival rate of SVNI-infected mice. Our data suggest that GCS inhibitors can potentially serve as a broad-spectrum antiviral therapy and should be further examined in preclinical and clinical trial. Analogues of the specific compounds tested have already been studied clinically, implying they can be fast-tracked for public use. With the current COVID-19 pandemic, this may be particularly relevant to SARS-CoV-2 infection. [**note: I have not seen this class of inhibitors come up in searches before. It's useful to throw everything at the virus to see what works and what doesn't. Any takers for a clinical trial on these compounds?**] https://www.biorxiv.org/content/10.1101/2020.05.18.103283v1

DIAGNOSTIC DEVELOPMENT

- Nothing new to report.

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2020-05-21

Let's stay with folk for another day. Two for the price of one! The lilting voice of Joan Baez is featured. First we have a live 1978 performance of 'the Night they Drove Old Dixie Down' from Norway: https://www.youtube.com/watch?v=XTNirVqTepE the second one is a song that I have always enjoyed, Leonard Cohen's 'Suzanne,' https://www.youtube.com/watch?v=39eFrEzZmYE from a French performance in 2000. Don't forget my book recommendation from earlier, David Hajdu's "Positively 4th Street: The Lives and Times of Joan Baez, Bob Dylan, Mimi Baez Fariña, and Richard Fariña."

As you know I am a big DIY fan. Imagine my surprise when THIS came across my newsfeed yesterday! I've always been fascinated by tiny house videos and didn't realize that Amazon has kits for sale. What a cool project this would be during pandemic times. You could even rent it out as a quarantine home. The price is right but I wonder if county zoning regulations will let me do this in my backyard. My older daughter is ready to help build one but my wife is quite skeptical of the project.

The Atlantic's Ed Yong with some more incisive reporting on the pandemic.

The CDC has changed its statement on transmission of SARS-CoV-2. Person to person spread is the chief concern and surface contact is a lessor concern.

Nice lay article with some good graphics on the differing types of COVID-19 vaccines from the NY Times.

Nice New England Journal of Medicine article on faster antiviral drug discovery.

Finally, a big data gathering experiment announced by CDC. The Centers for Disease Control and Prevention is embarking on an expansive study of the prevalence of novel coronavirus antibodies in people in 25 metropolitan areas, an effort to provide long-awaited insight into the way the virus is spreading and its presence in communities. The study, which plans to test 325,000 people by fall 2021, will build on an antibody study that has been underway in six of those cities since March, according to Michael Busch, who is overseeing the study and is director of the Vitalant Research Institute. CDC spokeswoman Kristen Nordlund confirmed plans to announce the study but declined to discuss details. [**note: of course this would be a lot easier to do if the country had a better EHR system where data could be better accessed. One really easy thing to do starting right now is to include a SARS-CoV-2 antibody test in every single diagnostic blood draw going forward. We plan to ask for this at our physicals next month.**]

From STAT, how coronavirus hijacks cells in unique ways.

There is an interesting Clinical Trial Result that might be worrying to President Trump.

MODELING

- Still more models but nothing of great importance that I saw.

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- Anti-inflammatory therapies such as IL-6 inhibition have been proposed for COVID-19 in a vacuum of evidence-based treatment. However, abrogating the inflammatory response in infectious diseases may impair a desired host response and predispose to secondary infection. To determine whether IL-6 inhibition is associated with an increased occurrence of secondary infections in patients admitted to the intensive care unit (ICU). We retrospectively reviewed the medical record of patients during an 8-week span and compared the incidence of secondary infection in patients who did and did not receive tocilizumab. The study was approved by the IRB. Additionally, we included representative histopathologic post-mortem findings from several COVID-19 cases that underwent autopsy at our institution. The study was conducted in a

cohorted COVID-19 ICU at a tertiary care university medical center. Patients 18 years of age or older admitted to the adult COVID-19 intensive care unit with COVID-19 were randomly selected. We reviewed the occurrence and nature of secondary infections and clinical outcomes in patients who did and did not receive tocilizumab. Measures were formulated prior to the study. For autopsy findings, we were interested in the lung pathology. 60 patients were selected of which 28 had received tocilizumab while 32 had not. Receiving tocilizumab was associated with a higher risk of secondary bacterial (64.3% vs. 31.3%, p=0.010) and fungal (7.1% vs. 0%, p=0.096) infections. 7 cases underwent autopsy. In 3 cases, tocilizumab had previously been given. All 3 patients demonstrated evidence of pneumonia on pathology. Of the 4 cases that had not been given tocilizumab, 2 showed evidence of aspiration pneumonia and 2 exhibited diffuse alveolar damage. Experimental therapies are currently being applied to COVID-19 outside of clinical trials. Anti-inflammatory therapies such as anti-IL-6 therapy have the potential to impair viral clearance, predispose to secondary infection, and cause harm. We seek to raise physician awareness of these issues and highlight the need to better understand the immune response in COVID-19. [**note: cautionary words from Chicago. Tocilizumab may lead to increased secondary infections.**] https://www.medrxiv.org/content/10.1101/2020.05.15.20103531v1

- **Oh No!!!!!** Background: Hydroxychloroquine is currently being tested as post-exposure prophylaxis against coronavirus disease 2019 (COVID-19) in several ongoing clinical trials. Objective: To compare the incidence of COVID-19 in Spanish patients with autoimmune rheumatic diseases treated with and without hydroxychloroquine. Methods: Retrospective electronic record review, from February 27th to April 16th, of patients with autoimmune inflammatory diseases followed at two academic tertiary care hospitals in Seville, Spain. The cumulative incidence of COVID-19, confirmed or suspected, was compared between patients with and without hydroxychloroquine as part of their treatment of autoimmune inflammatory diseases. Results: Among 722 included subjects, 290 (40%) were receiving hydroxychloroquine. During the seven-week study period, five (1.7% [95% CI: 0.5%-4.0%] cases of COVID-19 were registered among patients with hydroxychloroquine and five (1.2% [0.4%-2.7%]) (p=0.523) in without hydroxychloroquine. COVID-19 was confirmed by PCR in one (0.3%, 95% CI 0.008-1.9%) patient with hydroxychloroquine and two (0.5%, 95% CI 0.05%-1.6%) without hydroxychloroquine (p=1.0). One patient on hydroxychloroquine and two subjects without hydroxychloroquine were admitted to the hospital, none of them required to be transferred to the intensive care unit and no patient died during the episode. Conclusions: The incidence and severity of COVID-19 among patients with autoimmune rheumatic diseases with and without hydroxychloroquine was not significantly different. Hydroxychloroquine does not seem to be an appropriate therapy for post-exposure prophylaxis against COVID-19. [**note: we always knew that datamining to see how RA patients on HCQ might be doing if infected with SARS-CoA-2. OK, this is a small Spanish study and observational data from the US will help a lot but it looks like HCQ may not be protective. I guess I should email this bad news to President Trump.**] https://www.medrxiv.org/content/10.1101/2020.05.16.20104141v1
- COVID-19 induces progressive hypoxemic respiratory failure and acute respiratory distress syndrome, mostly due to a dysregulated inflammatory response. Since the first observations of COVID-19 patients, significant hypoalbuminemia was detected. This study aimed to investigate the hypothesis that hypoalbuminemia in COVID-19 patients is due to pulmonary capillary leakage and to test its correlation with indicators of respiratory function. Methods: 174 COVID-

19 patients, 92 admitted to the Intermediate Medicine ward (IMW), and 82 to the Intensive Care Unit (ICU) at Luigi Sacco Hospital in Milan were included in this study. Findings: Serum albumin concentration was decreased in the whole cohort, with ICU patients displaying lower values than IMW patients [20 (18-23) vs 28 (24-33) g/L, p<0.0001]. Lower albumin values were found in patients belonging to a more compromised group (lower PaO2 to FiO2 ratio and worst chest X-ray findings). In a subset of 26 patients, analysis of bronchoalveolar lavage fluid (BALF) highlighted high protein concentrations, which were correlated to Interleukin-8 and Interleukin-10 BALF concentration. The length of hospitalisation [20 (15-29) vs 8 (5-14) days, p<0.0001] and death rate (52.4% vs 21.7%, p<0.0001) were higher in ICU than in IMW patients, while a strict relation between hypoalbuminemia and 30 day-survival was detected in the whole cohort. Electron microscopy examinations of eight out of ten autopsy lung tissues showed diffuse loosening of interendothelial junctional complex. Interpretation: The degree of hypoalbuminemia can be considered as a useful severity marker in hospitalised COVID-19 patients. Pulmonary capillary leak syndrome secondary to the hyperinflammatory state plays a key role in the pathogenesis of COVID-19 respiratory dysfunction and should be regarded as a therapeutic target. [**note: more on the capillary leakage in the lungs from a Milan team.**] https://www.medrxiv.org/content/10.1101/2020.05.17.20104877v1

- Deciphering the dynamic changes of antibodies against SARS-CoV-2 is essential for understanding the immune response in COVID-19 patients. By comprehensively analyzing the laboratory findings of 1,850 patients, we describe the dynamic changes of the total antibody, spike protein (S)-, receptor-binding domain (RBD)-, and nucleoprotein (N)- specific IgM and IgG levels during SARS-CoV-2 infection and recovery. Our results indicate that the S-, RBD-, and N-specific IgG generation of severe/critical COVID-19 patients is one week later than mild/moderate cases, while the levels of these antibodies are 1.5-fold higher in severe/critical patients during hospitalization (P<0.01). The decrease of these IgG levels indicates the poor outcome of severe/critical patients. The RBD- and S-specific IgG levels are 2-fold higher in virus-free patients (P<0.05). Notably, we found that the patients who got re-infected had a low level of protective antibody on discharge. Therefore, our evidence proves that the dynamic changes of antibodies could provide an important reference for diagnosis, monitoring, and treatment, and shed new light on the precise management of COVID-19. [**note: good work from China analyzing antibody change over time following SARS-CoV-2 infection. It also may explain why some individuals get reinfected.**] https://www.medrxiv.org/content/10.1101/2020.05.18.20105155v1
- The rapid global spread of SARS-CoV-2 and resultant mortality and social disruption have highlighted the need to better understand coronavirus immunity to expedite vaccine development efforts. Multiple candidate vaccines, designed to elicit protective neutralising antibodies targeting the viral spike glycoprotein, are rapidly advancing to clinical trial. However, the immunogenic properties of the spike protein in humans are unresolved. To address this, we undertook an in-depth characterisation of humoral and cellular immunity against SARS-CoV-2 spike in humans following mild to moderate SARS-CoV-2 infection. We find serological antibody responses against spike are routinely elicited by infection and correlate with plasma neutralising activity and capacity to block ACE2/RBD interaction. Expanded populations of spike-specific memory B cells and circulating T follicular helper cells (cTFH) were detected within convalescent donors, while responses to the receptor binding domain (RBD) constitute a minor fraction. Using

regression analysis, we find high plasma neutralisation activity was associated with increased spike-specific antibody, but notably also with the relative distribution of spike-specific cTFH subsets. Thus both qualitative and quantitative features of B and T cell immunity to spike constitute informative biomarkers of the protective potential of novel SARS-CoV-2 vaccines. [**note: antibody formation, this time from Australia.**]
https://www.medrxiv.org/content/10.1101/2020.05.17.20104869v1

- The association between current tobacco smoking, the risk of developing COVID-19 and the severity of illness is an important information gap. Methods: UK users of the COVID Symptom Study app provided baseline data including demographics, anthropometrics, smoking status and medical conditions, were asked to log symptoms daily from 24th March 2020 to 23rd April 2020. Participants reporting that they did not feel physically normal were taken through a series of questions, including 14 potential COVID-19 symptoms and any hospital attendance. The main study outcome was the association between current smoking and the development of classic symptoms of COVID-19 during the pandemic defined as fever, new persistent cough and breathlessness. The number of concurrent COVID-19 symptoms was used as a proxy for severity. In addition, association of subcutaneous adipose tissue expression of ACE2, both the receptor for SARS-CoV-2 and a potential mediator of disease severity, with smoking status was assessed in a subset of 541 twins from the TwinsUK cohort. Results: Data were available on 2,401,982 participants, mean(SD) age 43.6(15.1) years, 63.3% female, overall smoking prevalence 11.0%. 834,437 (35%) participants reported being unwell and entered one or more symptoms. Current smokers were more likely to develop symptoms suggesting a diagnosis of COVID-19; classic symptoms adjusted OR[95%CI] 1.14[1.10 to 1.18]; >5 symptoms 1.29[1.26 to 1.31]; >10 symptoms 1.50[1.42 to 1.58]. Smoking was associated with reduced ACE2 expression in adipose tissue (Beta(SE)= -0.395(0.149); p=7.01x10-3). Interpretation: These data are consistent with smokers having an increased risk from COVID-19. [**note: phew!!! I'm glad to see that smoking may not be protective of SARS-CoV-2 infection. I was worried that earlier reports would prompt many people to take up this bad habit.**]
https://www.medrxiv.org/content/10.1101/2020.05.18.20105288v1
- Importance: Case series without control groups suggest that Covid-19 may cause ischemic stroke, but whether Covid-19 is associated with a higher risk of ischemic stroke than would be expected from a viral respiratory infection is uncertain. Objective: To compare the rate of ischemic stroke between patients with Covid-19 and patients with influenza, a respiratory viral illness previously linked to stroke. Design: A retrospective cohort study. Setting: Two academic hospitals in New York City. Participants: We included adult patients with emergency department visits or hospitalizations with Covid-19 from March 4, 2020 through May 2, 2020. Our comparison cohort included adult patients with emergency department visits or hospitalizations with influenza A or B from January 1, 2016 through May 31, 2018 (calendar years spanning moderate and severe influenza seasons). Exposures: Covid-19 infection confirmed by evidence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the nasopharynx by polymerase chain reaction, and laboratory-confirmed influenza A or B. Main Outcomes and Measures: A panel of neurologists adjudicated the primary outcome of acute ischemic stroke and its clinical characteristics, etiological mechanisms, and outcomes. We used logistic regression to compare the proportion of Covid-19 patients with ischemic stroke versus the proportion among patients with influenza. Results: Among 2,132 patients with emergency

department visits or hospitalizations with Covid-19, 31 patients (1.5%; 95% confidence interval [CI], 1.0%-2.1%) had an acute ischemic stroke. The median age of patients with stroke was 69 years (interquartile range, 66-78) and 58% were men. Stroke was the reason for hospital presentation in 8 (26%) cases. For our comparison cohort, we identified 1,516 patients with influenza, of whom 0.2% (95% CI, 0.0-0.6%) had an acute ischemic stroke. After adjustment for age, sex, and race, the likelihood of stroke was significantly higher with Covid-19 than with influenza infection (odds ratio, 7.5; 95% CI, 2.3-24.9). Conclusions and Relevance: Approximately 1.5% of patients with emergency department visits or hospitalizations with Covid-19 experienced ischemic stroke, a rate 7.5-fold higher than in patients with influenza. Future studies should investigate the thrombotic mechanisms in Covid-19 in order to determine optimal strategies to prevent disabling complications like ischemic stroke. [**note: from Cornell Med School, yet another potential problem associated with SARS-CoV-2. Chance of ischemic stroke is 7.5 times higher than with influenza patients!**]
https://www.medrxiv.org/content/10.1101/2020.05.18.20105494v1

- COVID-19 pandemic caused by SARS-CoV-2 infection is a public health emergency. COVID-19 typically exhibits respiratory illness. Unexpectedly, emerging clinical reports indicate that neurological symptoms continue to rise, suggesting detrimental effects of SARS-CoV-2 on the central nervous system (CNS). Here, we show that a Dusseldorf isolate of SARS-CoV-2 enters 3D human brain organoids within two days of exposure. Using COVID-19 convalescent serum, we identified that SARS-CoV-2 preferably targets soma of cortical neurons but not neural stem cells, the target cell type of ZIKA virus. Imaging cortical neurons of organoids reveal that SARS-CoV-2 exposure is associated with missorted Tau from axons to soma, hyperphosphorylation, and apparent neuronal death. Surprisingly, SARS-CoV-2 co-localizes specifically with Tau phosphorylated at Threonine-231 in the soma, indicative of early neurodegeneration-like effects. Our studies, therefore, provide initial insights into the impact of SARS-CoV-2 as a neurotropic virus and emphasize that brain organoids could model CNS pathologies of COVID-19. [**note: Oh Dear! Now we might have another effect of the virus to worry about. My mental acuity still seems to be OK but cabin fever also leads to bouts of delirium.**]
https://www.biorxiv.org/content/10.1101/2020.05.20.106575v1

DRUG DEVELOPMENT

- To curb the spread of SARS-CoV-2, the etiologic agent of the COVID-19 pandemic, we characterize the virucidal activity of long-acting Povidone Iodine (PVP-I) compositions developed using an *in-situ* gel forming technology. The PVP-I gel forming nasal spray (IVIEW-1503) and PVP-I gel forming ophthalmic eye drop (IVIEW-1201) rapidly inactivated SARS-CoV-2, inhibiting the viral infection of VERO76 cells. No toxicity was observed for the PVP-I formulations. Significant inactivation was noted with preincubation of the virus with these PVP-I formulations at the lowest concentrations tested. It has been demonstrated that both PVP-I formulations can inactivate SARS-CoV-2 virus efficiently in both a dose-dependent and a time-dependent manner. These results suggest IVIEW-1503 and IVIEW-1201 could be potential agents to reduce or prevent the transmission of the virus through the nasal cavity and the eye, respectively. Further studies are needed to clinically evaluate these formulations in early-stage COVID-19 patients. [**note: this is an obvious and pretty cool approach. From the manuscript, "By employing either the sustained release nasal spray or ophthalmic eye drop delivery technologies, we expect to**

reduce, treat, and eliminate SARS-CoV-2 in the nasal, sinus cavity, and in the eye. Additionally, the long-acting nasal spray can potentially be utilized as a prophylaxis for protecting against SARS-CoV-2 infection." I also imagine this may be a low cost approach.]
https://www.biorxiv.org/content/10.1101/2020.05.18.103184v1

- There is an urgent need for vaccines and therapeutics to prevent and treat COVID-19. Rapid SARS-CoV-2 countermeasure development is contingent on the availability of robust, scalable, and readily deployable surrogate viral assays to screen antiviral humoral responses, and define correlates of immune protection, and to down-select candidate antivirals. Here, we describe a highly infectious recombinant vesicular stomatitis virus bearing the SARS-CoV-2 spike glycoprotein S as its sole entry glycoprotein that closely resembles the authentic agent in its entry-related properties. We show that the neutralizing activities of a large panel of COVID-19 convalescent sera can be assessed in a high-throughput fluorescent reporter assay with rVSV-SARS-CoV-2 S and that neutralization of the rVSV and authentic SARS-CoV-2 by spike-specific antibodies in these antisera is highly correlated. Our findings underscore the utility of rVSV-SARS-CoV-2 S for the development of spike-specific vaccines and therapeutics and for mechanistic studies of viral entry and its inhibition. [**note: cool new approach to a possible vaccine candidate. I don't know how scalable this might be.**]
https://www.biorxiv.org/content/10.1101/2020.05.20.105247v1
- Due to the current COVID-19 pandemic, the rapid discovery of a safe and effective vaccine is an essential issue, consequently, this study aims to predict potential COVID-19 peptide-based vaccine utilizing the Nucleocapsid phosphoprotein (N) and Spike Glycoprotein (S) via the Immunoinformatics approach. To achieve this goal, several Immune Epitope Database (IEDB) tools, molecular docking, and safety prediction servers were used. According to the results, The Spike peptide peptides SQCVNLTTRTQLPPAYTNSFTRGVY is predicted to have the highest binding affinity to the B-Cells. The Spike peptide FTISVTTEI has the highest binding affinity to the MHC I HLA-B1503 allele. The Nucleocapsid peptides KTFPPTEPK and RWYFYYLGTGPEAGL have the highest binding affinity to the MHC I HLA-A0202 allele and the three MHC II alleles HLA-DPA1*01:03/DPB1*02:01, HLA-DQA1*01:02/DQB1-*06:02, HLA-DRB1, respectively. Furthermore, those peptides were predicted as non-toxic and non-allergen. Therefore, the combination of those peptides is predicted to stimulate better immunological responses with respectable safety. [**note: from a research group in Sudan!! Though it probably won't happen, it would be so cool if they came up with their own vaccine!!!!**]
https://www.biorxiv.org/content/10.1101/2020.05.20.106351v1
- The innate immune response is critical for protection against Coronaviruses. However, little is known about the interplay between the innate immune system and SARS-CoV-2. Here, we modeled SARS-CoV-2 infection using primary human airway epithelial (pHAE) cultures, which are maintained in an air-liquid interface. We found that SARS-CoV-2 infects and replicates in pHAE cultures and is directionally released on the apical, but not basolateral surface. Transcriptional profiling studies found that infected pHAE cultures had a molecular signature dominated by pro-inflammatory cytokines and chemokine induction, including IL-6, TNFα, CXCL8. We also identified NF-κB and ATF4 transcription factors as key drivers of this pro-inflammatory cytokine response. Surprisingly, we observed a complete lack of a type I or III IFN induction during SARS-CoV-2 infection. Pre-treatment or post-treatment with type I and III IFNs dramatically reduced virus replication in pHAE cultures and this corresponded with an upregulation of antiviral

effector genes. Our findings demonstrate that SARS-CoV-2 induces a strong pro-inflammatory cytokine response yet blocks the production of type I and III IFNs. Further, SARS-CoV-2 is sensitive to the effects of type I and III IFNs, demonstrating their potential utility as therapeutic options to treat COVID-19 patients. [**note: more data on interferon use *in vitro***] https://www.biorxiv.org/content/10.1101/2020.05.19.105437v1

- A collection of twelve organoselenium compounds, structural analogues of antioxidant drug ebselen were screened for inhibition of the papain-like protease (PL^{pro}) from the acute respiratory syndrome coronavirus 2 (SARS-CoV-2, CoV2). This cysteine protease, being responsible for the hydrolysis of peptide bonds between specific amino acids, plays a critical role in CoV2 replication and in assembly of new viral particles within human cells. The activity of the PL^{pro} CoV2 is essential for the progression of coronavirus disease 2019 (COVID-19) and it constitutes a key target for the development of anti-COVID-19 drugs. Here, we identified four strong inhibitors that bind favorably to the PL^{pro} CoV2 with the IC_{50} in the nanomolar range. [**note: these are variants of ebselen an experimental drug that is being explored for several different indications. I have no clue about the toxicity of this class of compounds.**] https://www.biorxiv.org/content/10.1101/2020.05.20.107052v2
- Thus far, there is no approved therapeutic drug, specifically targeting this emerging virus. Here we report the isolation and characterization of a panel of human neutralizing monoclonal antibodies targeting the SARS-CoV-2 receptor binding domain (RBD). These antibodies were selected from a phage display library constructed using peripheral circulatory lymphocytes collected from patients at the acute phase of the disease. These neutralizing antibodies are shown to recognize distinct epitopes on the viral spike RBD, therefore they represent a promising basis for the design of efficient combined post-exposure therapy for SARS-CoV-2 infection. [**note: Israeli research isolate a panel of mAbs. Lots of prepubs on mAbs but we need them to enter the clinic ASAP.**] https://www.biorxiv.org/content/10.1101/2020.05.20.106609v1
- An understanding of protective immunity to SARS-CoV-2 is critical for vaccine and public health strategies aimed at ending the global COVID-19 pandemic. A key unanswered question is whether infection with SARS-CoV-2 results in protective immunity against re-exposure. We developed a rhesus macaque model of SARS-CoV-2 infection and observed that macaques had high viral loads in the upper and lower respiratory tract, humoral and cellular immune responses, and pathologic evidence of viral pneumonia. Following initial viral clearance, animals were rechallenged with SARS-CoV-2 and showed 5 $\log_{10}$ reductions in median viral loads in bronchoalveolar lavage and nasal mucosa compared with primary infection. Anamnestic immune responses following rechallenge suggested that protection was mediated by immunologic control. These data show that SARS-CoV-2 infection induced protective immunity against re-exposure in nonhuman primates. [**note: Rhesus monkeys can be a useful non-human primate model.**] https://science.sciencemag.org/content/early/2020/05/19/science.abc4776 and following right on: The global COVID-19 pandemic caused by the SARS-CoV-2 virus has made the development of a vaccine a top biomedical priority. In this study, we developed a series of DNA vaccine candidates expressing different forms of the SARS-CoV-2 Spike (S) protein and evaluated them in 35 rhesus macaques. Vaccinated animals developed humoral and cellular immune responses, including neutralizing antibody titers comparable to those found in convalescent humans and macaques infected with SARS-CoV-2. Following vaccination, all

animals were challenged with SARS-CoV-2, and the vaccine encoding the full-length S protein resulted in >3.1 and >3.7 $\log_{10}$ reductions in median viral loads in bronchoalveolar lavage and nasal mucosa, respectively, as compared with sham controls. Vaccine-elicited neutralizing antibody titers correlated with protective efficacy, suggesting an immune correlate of protection. These data demonstrate vaccine protection against SARS-CoV-2 in nonhuman primates. [**note: a prototype DNA vaccine. There is a Janssen Pharma co-author but I don't know if this is the prototype they are working with.**]
https://science.sciencemag.org/content/early/2020/05/19/science.abc6284

DIAGNOSTIC DEVELOPMENT

- To allow every laboratory or hospital access to an inhouse assay, we developed two low cost SARSCoV2 detection assay protocols using inhouse primers and reagents equipment on hand in most biology or diagnostic laboratories a SYBR Green based RTPCR and PCR assays. RNA extraction has also become a major bottleneck due to limited supplies and the required labor. Thus, we validated alternative RNA extraction protocols. Methods SARSCoV2 genome sequences deposited into the GISAID database were retrieved to design and synthesize inhouse primers. Forty patient samples were collected by nasopharyngeal swab, coded, and used to develop and validate the assay protocols. Both assays used TRIzol and heat-processing techniques to extract RNA from patient samples and to inactivate the virus; thus, testing was conducted in a conventional biosafety level 2 laboratory. Results The sensitivity and specificity of the primers were evaluated using samples previously confirmed positive for SARSCoV2. The positive amplicons were sequenced to confirm the results. The assay protocols were developed, and the specificity of each PCR product was confirmed using melting curve analyses. The most accurate heat processing technique for primers with short amplicon lengths was 95C for 15 mins. Of 40 samples, both the SYBR Green based quantitative RTPCR assay and the PCR assay detected SARSCoV2 target genes in 28 samples, with no false positive or false-negative results. These findings were concordant with those of the diagnostic laboratory that tested the same samples using a Rotor Gene PCR cycler with an Altona Diagnostics SARSCoV2 kit (R2=0.889). Conclusions These approaches are reliable, repeatable, specific, sensitive, simple, and low cost tools for the detection of SARSCoV2 in a conventional biosafety level 2 laboratory, offering alternative approaches when commercial kits are unavailable or cost ineffective. [**note: a big shout out to this Saudi team for coming up with a lower cost approach to diagnostic testing.**]
https://www.medrxiv.org/content/10.1101/2020.05.18.20105510v1
- The gold standard for COVID-19 diagnosis is detection of viral RNA in a reverse transcription PCR test. Due to global limitations in testing capacity, effective prioritization of individuals for testing is essential. Here, we devised a model that estimates the probability of an individual to test positive for COVID-19 based on answers to 9 simple questions regarding age, gender, presence of prior medical conditions, general feeling, and the symptoms fever, cough, shortness of breath, sore throat and loss of taste or smell, all of which have been associated with COVID-19 infection. Our model was devised from a subsample of a national symptom survey that was answered over 2 million times in Israel over the past 2 months and a targeted survey distributed to all residents of several cities in Israel. Overall, 43,752 adults were included, from which 498 self-reported as being COVID-19 positive. The model provides statistically significant predictions on held-out individuals and achieves a positive predictive value (PPV) of 46.3% at a 10%

sensitivity. As our tool can be used online and without the need of exposure to suspected patients, it may have worldwide utility in combating COVID-19 by better directing the limited testing resources through prioritization of individuals for testing, thereby increasing the rate at which positive individuals can be identified and isolated. [**note: these Israeli researchers developed a model to figure out who should be tested when there are limitations of diagnostic tools. Loss of taste and smell are important and I need to get back to work on the COVID-19 Scent Strip™ ASAP**]
https://www.medrxiv.org/content/10.1101/2020.05.18.20105569v1

- BACKGROUND: In the Covid-19 pandemic, highly selective serological testing is essential to define exposure to SARS-CoV-2 virus. Many tests have been developed, yet with variable speed to first result, and of unknown quality, particularly when considering the prediction of neutralizing capacity. OBJECTIVES/METHODS: The LIAISON® SARS-CoV-2 S1/S2 IgG assay was designed to measure antibodies against the SARS-CoV-2 native S1/S2 proteins in a standardized automated chemiluminescent assay. Clinical and analytical performance of the test were validated in an observational study using residual samples (>1500) with positive or negative Covid-19 diagnosis. RESULTS: The LIAISON® SARS-CoV-2 S1/S2 IgG assay proved highly selective and specific, and offers semiquantitative measures of serum or plasma levels of anti-S1/S2 IgG with neutralizing activity. The diagnostic sensitivity was 91.3% and 95.7% at >5 or ≥ 15 days from diagnosis respectively, and 100% when assessed against a neutralizing assay. The specificity ranged between 97% and 98.5%. The average imprecision of the assay was <5 % coefficient of variation. Assay performance at 2 different cut-offs was evaluated to optimize predictive values in settings with different % disease prevalence. CONCLUSIONS: The automated LIAISON® SARS-CoV-2 S1/S2 IgG assay brings efficient, sensitive, specific, and precise serological testing to the laboratory, with the capacity to test large amounts of samples per day: first results are available within 35 minutes with a throughput of 170 tests/hour. The test also provides a semiquantitative measure to identify samples with neutralizing antibodies, useful for a large scale screening of convalescent plasma for safe therapeutic use. [**note: from hard hit Italy, a way to automate serology testing for SARS-CoV-2 antibodies.**]
https://www.biorxiv.org/content/10.1101/2020.05.19.105445v1

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2020-05-22

I cannot think of any vocal quartet that puts a smile on my face as the Manhattan Transfer. The arrangements and blend in voices is just sublime. Here is 'Boy from New York City':
https://www.youtube.com/watch?v=k2fN36wq-zc

Another critique, this time from The Atlantic, about the CDC statistics on testing. Even we novices know that serology and RT-PCR measure different things. Test data should be separated into the two categories and not blended together.

One of my favorite economic blogs is Bill McBride's 'Calculated Risk' (it turns out he was four years behind me in the same school district in San Diego!). Bill regularly posts economics indicators that I follow and he pointed me to this very nice website TestAndTrace which has very good information on

how to set up test and trace systems. Let's hope policy makers at the state and local levels take time to read and implement this.

Speaking of track and trace, at least two localities are implementing programs!! Anne Arundel county in Maryland and Paterson, New Jersey both took action to get the job done!!! Lessons more areas can and should follow.

From the beginning I have harped on the big failure in imagination. The New York Times has a useful reminder of this in regard to university and other small testing labs making a quick pivot to do SARS-CoV-2 testing only to see the labs under utilized.

Derek Lowe on the unique coronavirus immune response.

Some cautionary news on vaccines from STAT and a nice discussion of what interventions work best in a pandemic.

More confounding information on HCQ today. It's good to see one of the gifts from the Three Magi enter clinical trials.

MODELING

- Neuroscience and psychology agree that dreaming helps to cope with negative emotions and learn from experience. The current global threat related to the COVID-19 pandemic led to widespread social isolation. How does this situation affect dreams? Is dreaming during the pandemic related to mental suffering? Does the act of observing dreams help to mitigate mental suffering? To address these questions, we applied natural language processing tools to study 210 dream reports (n=67, Pandemic group n=42, plus control group balanced for age, sex and education) either before the Covid-19 outbreak or during March-April, 2020, following the pandemic announcement by the WHO and quarantine was imposed in Brazil. Post-announcement dreams showed a higher proportion of words related to anger and sadness, and higher average semantic similarities to the terms contamination and cleanness, which tended to increase over time. These features were associated with mental suffering related to social isolation, as they explained 39% of the variance in PANSS negative subscale (p=0.0092). Besides, dream observation was positively self-evaluated and dream similarities to contamination and cleanness revealed different impacts of the dream observation effect. These results corroborate the hypothesis that pandemic dreams reflect mental suffering, fear of contagion, and important changes in daily habits. [**note: I can't let the modeling topic lie fallow as COVID-19 nightmares are afflicting me!!! What I wouldn't give for a decent night of sleep and perchance NOT to dream. Alas poor Yorick, I knew him too well (apologies to William S.**]
  https://www.medrxiv.org/content/10.1101/2020.05.19.20107078v1

NEWLY REGISTERED CLINICAL TRIALS

- Yesterday I linked to a Povidone-Iodine preparation in the drug development section. I should have double checked on the Clinical Trial Data Base. There are five trials registered: three in the US (Stanford, NYU, University of Kentucky), France and the UK.
- This is a complicated three drug trial in the UK. TACTIC-E will assess the efficacy of the novel immunomodulatory agent EDP1815 and a combination of the approved cardiovascular drugs dapagliflozin and ambrisentan as potential treatments for COVID-19 disease against Standard of Care alone. These agents target the dysregulated immune response that drive the severe lung, and other organ, damage frequently seen during COVID-19 infection, with an aim to promote a positive vascular response to reduce end-organ damage. EDP1815 is an orally administered pharmaceutical preparation of a single strain of Prevotella histicola isolated from the duodenum of a human donor. EDP1815 is currently in phase 2 clinical development and has European and US approval to initiate a multinational psoriasis study. NCT04393246
- The purpose of this adaptive trial is to determine the clinical efficacy of Ifenprodil in the treatment of patients infected with COVID-19. This Protocol is largely based on the recommendations of the WHO R&D Blueprint Clinical Trials Expert Group COVID-19 Therapeutic Trial Synopsis, and associated Master Protocol. NP-120 (Ifenprodil) is an N-methyl-D-Aspartate (NDMA) inhibitor that is specific for the NR2B subunit of the NMDA Receptor. The NMDA receptor, and specifically the NR2B subunit, is involved in glutamate signaling, and is expressed on both neutrophils and T cells. In the case of neutrophils, activation of the NMDA receptor can (1) result in expression of CD11b which targets neutrophils via ICAM-1 to areas of inflammation, and (2) trigger the autocrine release of glutamate. In the case of T-cells, activation of T cells via glutamate can cause (1) T cell proliferation and, (2) the release of cytokines. The activation of T cells and cytokine release can be blocked in vitro by the addition of Ifenprodil. As such it could be a potent anti-inflammatory agent. NCT04382924
- Here is an interesting Israeli trial. ArtemiC is a medical spray comprised of Artemisinin (6 mg/ml), Curcumin (20 mg/ml), Frankincense (=Boswellia) (15 mg/ml) and vitamin C (60 mg/ml) in micellar formulation for spray administration. Leading among these considerations are well established immuno-modulatory activities of the active ingredients as established in vitro and in vivo and published over the years. These activities as apparent, for example, in diminishing activity of TNF alpha and IL-6 levels are acknowledged to be relevant to the pathophysiology processes involved in the progressive form of COVID-19. The active agents have in addition prominent anti-oxidant, anti-inflammatory as well as anti-aggregant and anti-microbial activities. [**note: I hope there was a good reason for not adding mhyrr to this mixture.]** NCT04382040
- The most severe manifestations of COVID-19 include respiratory failure, coagulation problems, and death. Inflammation and blood clotting are believed to play an important role in these manifestations. Research in humans has shown that dipyridamole can reduce blood clotting. This research study is being conducted to learn whether 14 days of treatment with dipyridamole will reduce excessive blood clotting in COVID-19. NCT04391179

CLINICAL TRIAL RESULTS

- Background. The COVID pandemic has had a major impact on healthcare in hospitals, including the diagnosis and treatment of infections. Hospital-acquired infective endocarditis (HAIE) is a severe complication of medical procedures that has shown a progressive increase in recent

years. Objectives. to determine whether the incidence of HAIE during the first two months of the epidemic (March-April 2020) was higher than previously observed and to describe the clinical characteristics of these cases. The probability of studied event (HAIE) during the studied period was calculate by Poisson distribution. Results. Four cases of HAIE were diagnosed in our institution during the study period. The incidence of HAIE during the study period was 2/patient-month and 0.25/patient-month during the previous 5 years (p=0.024). Two cases appeared during admission for COVID-19 with pulmonary involvement treated with metilprednisolone and tocilizumab. The other two cases were admitted to the hospital during the epidemic. All cases underwent central venous and urinary catheterization during admission. The etiology of HAIE was Enterococcus faecalis (2 cases), Staphylococcus aureus and Candida albicans (one case each). A source of infection was identified in three cases (central venous catheter, peripheral venous catheter, sternal wound infection, respectively). One patient was operated on. There were no fatalities during the first 30 days of follow-up. Conclusion. The incidence of HAIE during COVID-19 pandemic in our institution was higher than usual. In order to reduce the risk of this serious infection, optimal catheter care, appropriate use of corticosteroids and interleukin antagonists and early treatment of every local infection should be prioritized during coronavirus outbreaks. [**note: a small number but a cautionary tale nonetheless about ancillary problems while treating SARS-CoV-2 patients in hospitals.**]
https://www.medrxiv.org/content/10.1101/2020.05.17.20101063v1

- Background: The coronavirus disease 2019 (COVID-19) pandemic has affected over millions of individuals and caused hundreds of thousands of deaths worldwide. It can be difficult to accurately predict mortality among COVID-19 patients presenting with a spectrum of complications, hindering the prognostication and management of the disease. Methods: We applied machine learning techniques to clinical data from a large cohort of 5,051 COVID-19 patients treated at the Mount Sinai Health System in New York City, the global COVID-19 epicenter, to predict mortality. Predictors were designed to classify patients into Deceased or Alive mortality classes and were evaluated in terms of the area under the receiver operating characteristic (ROC) curve (AUC score). Findings: Using a development cohort (n=3,841) and a systematic machine learning framework, we identified a COVID-19 mortality predictor that demonstrated high accuracy (AUC=0.91) when applied to test sets of retrospective (n= 961) and prospective (n=249) patients. This mortality predictor was based on five clinical features: age, minimum O2 saturation during encounter, type of patient encounter (inpatient vs. various types of outpatient and telehealth encounters), *hydroxychloroquine use*, and maximum body temperature. Interpretation: An accurate and parsimonious COVID-19 mortality predictor based on five features may have utility in clinical settings to guide the management and prognostication of patients affected by this disease. [**note: this is a decent size data set from Mt. Sinai. The interesting thing is the effect of HCQ treatment. They note that it was standard of care so I'm not sure how they parsed out the treatment effect. I took a look at the paper and didn't want to spend too much time looking at the tables and statistical approach. I'm sure this will be seized upon by the champions of HCQ as evidence of efficacy.**]
https://www.medrxiv.org/content/10.1101/2020.05.19.20103036v1
- Importance: Patients in long-term care facilities (LTCF) are at a high-risk of contracting COVID-19 due to advanced age and multiple comorbidities. Without effective treatments, outbreaks in such facilities will become commonplace and will result in severe morbidity and mortality. The

effectiveness of doxycycline (DOXY) and hydroxychloroquine (HCQ) combination therapy in high risk COVID-19 patients in long-term care facilities is not yet understood. Objective: The goal of this analysis is to describe outcomes after use of DOXY-HCQ combination in high-risk COVID-19 patients in LTCF. Design: Case-series analysis. Setting: Three (3) LTCFs in New York. Participants: From March 19 to March 30, 2020, fifty-four (54) patients, residents of three (3) LTCFs in New York and diagnosed (confirmed or presumed) with COVID-19, were included in this analysis. Exposure: All patients who were diagnosed (confirmed or presumed) with COVID-19 received DOXY-HCQ combination therapy along with standard of care. Main Outcomes and Measures: Patients characteristics, clinical recovery, radiological improvements, medication side-effects, hospital transfer, and death were assessed as outcome measures. Results: A series of fifty-four (54) high-risk patients, who developed a sudden onset of fever, cough, and shortness of breath (SOB) and were diagnosed or presumed to have COVID-19, were started with a combination of DOXY-HCQ and 85% (n=46) patients showed clinical recovery defined as: resolution of fever and SOB, or a return to baseline setting if patients are ventilator-dependent. A total of 11% (n=6) patients were transferred to acute care hospitals due to clinical deterioration and 6% (n=3) patients died in the facilities. Naive Indirect Comparison suggests these data were significantly better outcomes than the data reported in MMWR (reported on March 26, 2020) from a long-term care facility in King County, Washington where 57% patients were hospitalized, and 22% patients died. Conclusion: The clinical experience of this case series indicates DOXY-HCQ treatment in high-risk COVID-19 patients is associated with a reduction in clinical recovery, decreased transfer to hospital and decreased mortality were observed after treatment with DOXY-HCQ. [**note: another small patient number with HCQ but this time with doxycycline. TIWWDCT!! I guess we will have to wait for the clinical trial results to sort out all of this. There are a couple of doxycycline only trials going on.**]
https://www.medrxiv.org/content/10.1101/2020.05.18.20066902v1

- Background: Covid-19 pandemic by the new coronavirus SARS-Cov-2 has produced devastating effects on the health care system, affecting also cancer patient care. Data about COVID-19 infection in cancer patients are scarce, and they point out a higher risk of complications due to the viral infection in this population. Moreover, cancer treatments could increase viral complications, specially those treatments based on the use of immunotherapy with checkpoints antibodies. There are no clinical data about the safety of immune check point antibodies in cancer patients when they become infected by SARS-CoV-2. As checkpoint inhibitors, mainly anti PD-1 and anti CTLA-4 antibodies, are an effective treatment for most melanoma patients, avoiding their use during the pandemic could lead to a decrease in the chances of curing melanoma. Methods: In Spain we have started a national registry of melanoma patients infected by SARS-Cov-2 since April 1st, 2020. A retrospective analysis of patients included in the Spanish registery has been performed weekly since the activation of the study. Interim analysis shows unexpected findings about cancer treatment safety in SARS-Cov-2 infected melanoma patients, so a rapid communication to the scientific community is mandatory Results: Fifty patients have been included as of May 17th, 2020. Median age is 69 years (range 6 to 94 years), 27 (54%) patients are males and 36 (70%) patients have stage IV melanoma. Twenty-two (44%) patients were on active anticancer treatment with anti PD-1 antibodies, 16 (32%) patients were on treatment with BRAF plus MEK inhibitors and 12 (24%) patients were not on active cancer treatment. COVID-19 episode has been resolved in 43 cases, including 30 (70%) patients cured,

four (9%) patients that have died due to melanoma progression, and nine (21%) patients that have died from COVID-19. Mortality rates from COVID-19 according to melanoma treatment type were 16%, 15% and 36% for patients on immunotherapy, targeted drugs, and for those that were not undergoing active cancer treatment, respectively. Conclusion: These preliminary findings show that the risk of death in those patients undergoing treatment with anti PD-1 antibodies does not exceed the global risk of death in this population. These results could be relevant in order to select melanoma therapy during the COVID-19 pandemic [**note: interesting and important data from Spain. Co-morbidities are an important feature of mortality following infection with SARS-CoV-2. Even though the number is small (and this is to be expected in the case of melanoma treatment), one treatment therapy appears not to impact mortality.**] https://www.medrxiv.org/content/10.1101/2020.05.19.20106971v1

DRUG DEVELOPMENT

- Background: Recent reports on the use of hydroxychloroquine (HCQ) alone, or combined with azithromycin (AZM) in the management of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have raised cardiac safety concerns. Currently, there is limited mechanistic data evaluating cardiac safety with HCQ and AZM therapy. Methods: Using comprehensive In Vitro ProArrhythmia Assay (CiPA) Schema IC50 paradigms, we examined the cardiac electrophysiological effects of HCQ and HCQ/AZM. Molecular modelling explored HCQ and AZM binding properties to hERG. Langendorff-perfused guinea-pig hearts were electrically and optically mapped by multi-electrode array and voltage (RH237) and Ca2+ (Rhod-2 AM) dyes. Human action potential and ion current reconstructions were performed in silico. Results: HCQ blocked IKr and IK1 with IC50 concentrations (10+-0.6 and 34+-5.0 microM) within the therapeutic range observed clinically. HCQ also blocked INa and ICaL but at higher IC50, whilst Ito and IKs were unaffected. Contrastingly, AZM produced minor inhibition of INa, ICaL, IKs, and IKr,, with no effect on IK1 and Ito. HCQ + AZM combined inhibited IKr and IK1 with IC50s of 7.7 +- 0.8 microM and 30.4 +- 3.0 microM, but spared INa, ICaL and Ito,. Molecular modelling confirmed potential HCQ binding to hERG. Cardiac mapping and ECG studies in isolated hearts demonstrated that HCQ slowed heart rate and ventricular conduction with associated prolongation of PR, QRS and QT intervals. Optical mapping demonstrated, and prolonged, more heterogeneous, action potential durations and intracellular Ca2+ transients. These effects were accentuated with combined HCQ+AZM treatment, which elicited electrical alternans, re-entrant circuits and wave breaks. Reconstruction in a human in-silico model demonstrated that this is attributable to the integrated action of HCQ and AZM reducing IKr, IKs and IK1. Conclusions: *These data provide an electrophysiological basis for recent FDA guidelines cautioning against combined HCQ/AZM administration for the treatment of Covid-19 on the grounds of potential cardiac safety.* We would strongly recommend monitoring of electrocardiographic QT interval with the use of this combination of medications. [**note: *in vitro* modeling shows the potential mechanism for HCQ/azithromycin arrythmias.**]
  https://www.biorxiv.org/content/10.1101/2020.05.21.108605v2
- Recently emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the pathogen responsible for the ongoing coronavirus disease 2019 (COVID-19) pandemic. Currently, there is no vaccine available for preventing SARS-CoV-2 infection. Like closely related severe acute respiratory syndrome coronavirus (SARS-CoV), SARS-CoV-2 also uses its receptor-

binding domain (RBD) on the spike (S) protein to engage the host receptor, human angiotensin-converting enzyme 2 (ACE2), facilitating subsequent viral entry. Here we report the immunogenicity and vaccine potential of SARS-CoV-2 RBD (SARS2-RBD)-based recombinant proteins. Immunization with SARS2-RBD recombinant proteins potently induced a multi-functional antibody response in mice. The resulting antisera could efficiently block the interaction between SARS2-RBD and ACE2, inhibit S-mediated cell-cell fusion, and neutralize both SARS-CoV-2 pseudovirus entry and authentic SARS-CoV-2 infection. In addition, the anti-RBD sera also exhibited cross binding, ACE2-blockade, and neutralization effects towards SARS-CoV. More importantly, we found that the anti-RBD sera did not promote antibody-dependent enhancement of either SARS-CoV-2 pseudovirus entry or authentic virus infection of Fc receptor-bearing cells. These findings provide a solid foundation for developing RBD-based subunit vaccines for SARS-CoV2. [**note: another prototype vaccine candidate from China.**] https://www.biorxiv.org/content/10.1101/2020.05.21.107565v1

- SARS-CoV-2 is a new type of coronavirus capable of rapid transmission and causing severe clinical symptoms; much of which has unknown biological etiology. It has prompted researchers to rapidly mobilize their efforts towards identifying and developing anti-viral therapeutics and vaccines. Discovering and understanding the virus' pathways of infection, host-protein interactions, and cytopathic effects will greatly aid in the design of new therapeutics to treat COVID-19. While it is known that chloroquine and hydroxychloroquine, extensively explored as clinical agents for COVID-19, have multiple cellular effects including inhibiting autophagy, there are also dose-limiting toxicities in patients that make clearly establishing their potential mechanisms-of-action problematic. Therefore, we evaluated a range of other autophagy modulators to identify an alternative autophagy-based drug repurposing opportunity. In this work, we found that 6 of these compounds blocked the cytopathic effect of SARS-CoV-2 in Vero-E6 cells with EC50 values ranging from 2.0 to 13 μM and selectivity indices ranging from 1.5 to >10-fold. Immunofluorescence staining for LC3B and LysoTracker dye staining assays in several cell lines indicated their potency and efficacy for inhibiting autophagy correlated with the measurements in the SARS-CoV-2 cytopathic effect assay. Our data suggest that autophagy pathways could be targeted to combat SARS-CoV-2 infections and become an important component of drug combination therapies to improve the treatment outcomes for COVID-19. [**note: you have to read the paper to discover which six compounds they are talking about. In addition to the antimalarials, the experimental drug ROC-325 and the tricyclic antidepressant clomipramine are mentioned (I don't know if they screened for chlorpromazine which has come up in some other papers and is now in clinical trials. The hypothesis laid out in the paper is interesting but it's an indirect antiviral mechanism.**] https://www.biorxiv.org/content/10.1101/2020.05.16.091520v1

DIAGNOSTIC DEVELOPMENT

- The SARS-CoV-2 beta coronavirus is spreading globally with unprecedented consequences for modern societies. The early detection of infected individuals is a pre-requisite for all strategies aiming to contain the virus. Currently, purification of RNA from patient samples followed by RT-PCR is the gold standard to assess the presence of this single-strand RNA virus. However, these procedures are time consuming, require continuous supply of specialized reagents, and are prohibitively expensive in resource-poor settings. Here, we report an improved nucleic-acid-

based approach to detect SARS-CoV-2, which alleviates the need to purify RNA, reduces handling steps, minimizes costs, and allows evaluation by non-specialized equipment. The use of unprocessed swap samples and the ability to detect as little as three viral genome equivalents is enabled by employing a heat-stable RNA- and DNA-dependent DNA polymerase, which performs the double task of stringent reverse transcription of RNA at elevated temperatures as well as PCR amplification of a SARS-CoV-2 specific target gene. As results are obtained within 2 hours and can be read-out by a hand-held LED-screen, this novel protocol will be of particular importance for large-scale virus surveillance in economically constrained settings. [**note: good work from a German company with a new approach to PCR amplification. A lot of good diagnostic work is coming out this pandemic that will improve diagnostic capabilities.**]
https://www.medrxiv.org/content/10.1101/2020.05.19.20103150v1

- Background: The serologic response of individuals with mild forms of SARS-CoV-2 infection is poorly characterized. Methods: Hospital staff who had recovered from mild forms of PCR-confirmed SARS-CoV-2 infection were tested for anti-SARS-CoV-2 antibodies using three assays: a LuLISA targeting the N protein (99% specificity), a rapid immunodiagnostic test (99.4% specificity), and a S- Flow assay (>99.5% specificity). The neutralizing activity of the sera was tested with a pseudovirus-based assay. Results: Of 162 hospital staff who participated in the investigation, 160 reported SARS-CoV- 2 infection that had not required hospital admission and were included in these analyses. The median time from symptom onset to blood sample collection was 24 days (IQR: 21-28, range 13-39). The LuLISA N assay detected antibodies in 129 (80.6%) of the samples, with better performance for samples collected after 28 days (45/48 = 93.8%); the rapid immunodiagnostic test in 153 (95.6%); and the S-Flow assay in 159 (99.4%), failing to detect antibodies in one sample collected 18 days after symptom onset (none of the other tests detected antibodies in that patient). Neutralizing antibodies (NAbs) were detected in 79%, 92% and 98% of samples collected 13-20, 21-27 and 28-41 days after symptom onset, respectively (P=0.02). Conclusion: Antibodies against SARS-CoV-2 were detected in virtually all hospital staff after 13 days from the COVID-19 symptom onset. This finding supports the use of serologic testing for the diagnosis of individuals who have recovered from SARS-CoV-2 infection. The neutralizing activity of the antibodies increased overtime. Future studies will help assess the persistence of the humoral response and its associated neutralization capacity in recovered patients. [**note: this an interesting clinical and diagnostic result from a look at French healthcare workers who were infected with SARS-CoV-2 antibodies. It shows the usefulness and timing of serological testing.**]
https://www.medrxiv.org/content/10.1101/2020.05.19.20101832v1
- As assay details and primer sequences become widely known, many laboratories could perform diagnostic tests using methods such as RT-PCR or isothermal RT-LAMP amplification. A key advantage of RT-LAMP based approaches compared to RT-PCR is that RT-LAMP is known to be robust in detecting targets from unprocessed samples. In addition, RT-LAMP assays are performed at a constant temperature enabling speed, simplicity, and point-of-use testing. Here, we provide the details of an RT-LAMP isothermal assay for the detection of SARS-CoV-2 virus with performance comparable to currently approved tests using RT-PCR. We characterize the assay by introducing swabs in virus spiked synthetic nasal fluids, moving the swab to viral transport medium (VTM), and using a volume of that VTM for performing the amplification without an RNA extraction kit. The assay has a Limit-of-Detection (LOD) of 50 RNA copies/uL in

the VTM solution within 20 minutes, and LOD of 5000 RNA copies/uL in the nasal solution. Additionally, we show the utility of this assay for real-time point-of-use testing by demonstrating detection of SARS-CoV-2 virus in less than 40 minutes using an additively manufactured cartridge and a smartphone-based reader. Finally, we explore the speed and cost advantages by comparing the required resources and workflows with RT-PCR. This work could accelerate the development and availability of SARS-CoV-2 diagnostics by proving alternatives to conventional laboratory benchtop tests. [**note: good thinking from this Univ of Illinois group. Let's get testing deployed!!**] https://www.biorxiv.org/content/10.1101/2020.05.21.108381v1

- Abstract. Background: Due to the huge demand for SARS-Cov-2 determination, alternatives to the standard qtPCR tests are potentially useful for increasing the number of samples screened. Our aim was to develop a direct fluorescent PCR capillary-electrophoresis detection of the viral genome. We validated this approach on several SARS-Cov-2 positive and negative samples. Study design: We isolated the naso-pharingeal RNA from 20 positive and 10 negative samples. The cDNA was synthesised and two fragments of the SARS-Cov-2 were amplified. One of the primers for each pair was 5-end fluorochrome labelled. The amplifications were subjected to capillary electrophoresis in ABI3130 sequencers to visualize the fluorescent peaks. Results: The two SARS-Cov-2 fragments were successfully amplified in the positive samples, while the negative samples did not render fluorescent peaks. Conclusion: We describe and alternative method to identify the SARS-Cov-2 genome that could be scaled to the analysis of approximately 100 samples in less than 5 hours. By combining a standard PCR with capillary electrophoresis our approach would overcome the limits imposed to many labs by the qtPCR (lack of reactive and real-time PCR equipment) and increase the testing capacity. [**note: cool work from Spain! I did a fair amount of electrophoresis studies back in my lab days.**] https://www.biorxiv.org/content/10.1101/2020.05.16.099242v1

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2020-05-23

I read a fascinating article on the Russo-German pianist Igor Levit in The New Yorker. He started doing house recitals over Twitter right after the lock down was imposed in Germany (he lives in the Mitte section of Berlin; I had my eyes on a very nice flat in that area following a visit to Berlin several years ago but it wasn't that amenable as a weekend getaway). He was invited to do a reprise of one of the concerts at the President's house, Schloss Bellevue. Here is Beethoven's Waldstein Sonata: https://www.youtube.com/watch?v=lC8DBTfJI90&t=7s (don't be alarmed by the drop out in the first movement, it's a glitch in the recording) and a double treat, his NPR Tiny Desk Concert: https://www.youtube.com/watch?v=HSyJNWyRZ8Y

(repost of the 2nd email I sent out yesterday just for archive purposes) Loyal Readers: this one could not wait until tomorrow. The Lancet just published a very large registry study on the use of HCQ in 671 hospitals from six continents. Money quote: "We were unable to confirm a benefit of hydroxychloroquine or chloroquine, when used alone or with a macrolide, on in-hospital outcomes for COVID-19. Each of these drug regimens was associated with decreased in-hospital survival and an increased frequency of ventricular arrhythmias when used for treatment of COVID-19" The Washington

Post has a story on this with some good interviews though some will caution that the Post only publishes "fake" news.

The always estimable Derek Lowe weighs in on the new study.

A fair number of papers regarding clinical results.

MODELING

- Nothing jumps out as particularly new or interesting.

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- Background: Dipeptidyl peptidase-4 inhibitor (DPP-4i) and renin-angiotensin system (RAS) blockade are reported to affect the clinical course of coronavirus disease 2019 (COVID-19) in patients with diabetes mellitus (DM). However, the effectiveness of these drugs in large populations is unclear. Subjects and Methods: As of May 2020, data analysis was conducted on all subjects who could confirm their history of claims related to COVID-19 in the National Health Review and Assessment Service database in Korea. Using the COVID-19 and claims data of the past 5 years, we compared the short-term prognosis of COVID-19 infection according to the use of DPP-4i and RAS blockade. Results: Totally, data of 67850 subjects were accessible. Of these, 5080 were confirmed COVID-19. Among these, 832 subjects with DM were selected for analysis in this study. Among the subjects, 263 (31.6%) and 327 (39.3%) were DPP-4i and RAS blockade users, respectively. Thirty-four subjects (4.09%) received intensive care or died. The adjusted odds ratio for severe treatment among DPP-4i users was 0.362 [95% confidence interval (CI), 0.135-0.971], and that for RAS blockade users was 0.599 (95% CI, 0.251-1.431). No synergy was observed for subjects using both drugs. Conclusion: This population-based study suggests that DPP-4i is significantly associated with a better clinical outcome of patients with COVID-19. However, the effect of RAS blockade is not significant. [**note: This is a pretty large observational study in South Korea. I provided a link to the list of drugs that fall into the DPP-4i category and some of these are in clinical trials. This is an intriguing finding.**] https://www.medrxiv.org/content/10.1101/2020.05.20.20108555v1
- This retrospective case-control study was aimed at identifying potential independent predictors of severe/lethal COVID-19, including the treatment with Angiotensin-Converting Enzyme inhibitors (ACEi) and/or Angiotensin II Receptor Blockers (ARBs). Methods and Results: All adults with SARS-CoV-2 infection in two Italian provinces were followed for a median of 24 days. ARBs and/or ACEi treatments, and hypertension, diabetes, cancer, COPD, renal and major cardiovascular diseases (CVD) were extracted from clinical charts and electronic health records, up to two years before infection. The sample consisted of 1603 subjects (mean age 58.0y; 47.3% males): 454 (28.3%) had severe symptoms, 192 (12.0%) very severe or lethal disease (154 deaths; mean age 79.3 years; 70.8% hypertensive, 42.2% with CVD). The youngest deceased person aged 44 years. Among hypertensive subjects (n=543), the proportion of those treated

with ARBs or ACEi were 88.4%, 78.7% and 80.6% among patients with mild, severe and very severe/lethal disease, respectively. At multivariate analysis, no association was observed between therapy and disease severity (Adjusted OR for very severe/lethal COVID-19: 0.87; 95% CI: 0.50-1.49). Significant predictors of severe disease were older age (with AORs largely increasing after 70 years of age), male gender (AOR: 1.76; 1.40-2.23), diabetes (AOR: 1.52; 1.05-2.18), CVD (AOR: 1.88; 1.32-2.70) and COPD (1.88; 1.11-3.20). Only gender, age and diabetes also predicted very severe/lethal disease. Conclusion: No association was found between COVID-19 severity and treatment with ARBs and/or ACEi, supporting the recommendation to continue medication for all patients unless otherwise advised by their physicians. [**note: data from Italy showing no association with ARBs or ACE inhibitors and poor progression. It still doesn't address the issue whether there is some protection afforded by the drugs.**]
https://www.medrxiv.org/content/10.1101/2020.05.21.20109082v1

- Background: In this pandemia, it is essential for rheumatologist and patients to know the relationship between COVID-19 and inflammatory rheumatic diseases (IRD). We want to assess the role of targeted synthetic or biologic disease modifying antirheumatic drugs (ts/bDMARDs) and other variables in the development of moderate-severe COVID-19 disease in IRD. Methods: An observational longitudinal study was conducted (1stMar to 15thApr 2020). All patients from the rheumatology outpatient clinic from a hospital in Madrid with a medical diagnosis of IRD were included. Main outcome: hospital admission related to COVID-19. Independent variable: ts/bDMARDs. Covariates: sociodemographic, comorbidities, type of IRD diagnosis, glucocorticoids, NSAIDs and conventional synthetic DMARDs (csDMARDs). Incidence rate (IR) of hospital admission related to COVID-19, was expressed per 1,000 patients-month. Cox multivariate regression analysis was run to examine the influence of ts/bDMARDs and other covariates on IR. Results: 3,591 IRD patients were included (5,896 patients-month). Concerning csDMARDs, methotrexate was the most used followed by antimalarials. 802 patients were on ts/bDMARDs, mainly anti-TNF agents, and rituximab. Hospital admissions related to COVID-19 occurred in 54 patients (1.36%) with an IR of 9.15 [95%CI: 7-11.9]. In the multivariate analysis, older, male gender, presence of comorbidities and specific systemic autoimmune conditions (Sjoegren, polychondritis, Raynaud and mixed connective tissue disease) had more risk of hospital admissions regardless other factors. Exposition to ts/bDMARDs did not achieve statistical signification. Use of glucocorticoids, NSAIDs, and csDMARDs dropped from the final model. Conclusion: This study provides additional evidence in IRD patients regarding susceptibility to moderate-severe infection related to COVID-19. [**note: from Madrid, a study of rheumatology patients on a variety of different drug regimens. There was some interesting results on tocilizumag, abatacept, and baricitinib but the patient numbers are too small to draw any firm conclusions. TIWWDCT!**]
https://www.medrxiv.org/content/10.1101/2020.05.21.20108696v1
- Background: The impact of remdesivir on length of stay of hospitalization, high-risk state, and death stratified by the severity of COVID-19 at enrollment is controversial. Methods: We applied a simulated two-arm controlled study design to the data on compassionate use of remdesivir as a secondary analysis. Dynamics of risk states and death from COVID-19 patients defined by the six-point disease severity recommended by the WHO R&D and the time to discharge from hospital were used to evaluate the efficacy of remdesivir treatment compared with standard care. Results: Stratified by the risk state at enrollment, low-risk patients exhibited the highest

efficacy of remdesivir in reducing subsequent progression to high-risk state by 67% (relative risk (RR)=0.33,95% CI: 0.30-0.35) and further to death by 55% (RR=0.45, 95%CI: 0.39-0.50). For the medium-risk patients, less but still statistically significant efficacy results were noted in reducing progression to high-risk state by 52% (RR=0.48, 95% CI: 0.45-0.51) and further to death by 40% (RR=0.60, 95% CI:0.54-0.66). High-risk state patients treated with remdesivir led to a 25% statistically significant reduction in death (RR=0.75, 95% CI: 0.69-0.82). Regarding the outcome of discharge, remdesivir treatment was most effective for medium-risk patients at enrollment (RR: 1.41, 95% CI: 1.35-1.47) followed by high- (RR=1.34, 95% CI: 1.27-1.42) and low-risk patients (RR=1.28, 95% CI: 1.25-1.31). Conclusion: Our results with a simulated two-arm controlled study have provided a new insight into the precision treatment of remdesivir for COVID-19 patients based on risk-stratified efficacy. [**note: these authors get an 'F' in abstract writing! They should have mentioned the number of patients in the remdesivir group as that would have saved me some time. There were only 53 patients here and of course that is close to meaningless when one tries to do slice and dice statistics. I guess this is the wild world of preprints! TIWWDCT!!!**] https://www.medrxiv.org/content/10.1101/2020.05.17.20104711v1

- Background Since December 2019, Coronavirus Disease 2019 (COVID-19) has become a global pandemic, causing mass morbidity and mortality. Prior studies in other respiratory infections suggest that convalescent plasma transfusion may offer benefit to some patients. Here, the outcomes of thirty-nine hospitalized patients with severe to life-threatening COVID-19 who received convalescent plasma transfusion were compared against a cohort of retrospectively matched controls. Methods Plasma recipients were selected based on supplemental oxygen needs at the time of enrollment and the time elapsed since the onset of symptoms. Recipients were transfused with convalescent plasma from donors with a SARS-CoV-2 (severe acute respiratory disease coronavirus 2) anti-spike antibody titer of ≥1:320 dilution. Matched control patients were retrospectively identified within the electronic health record database. Supplemental oxygen requirements and survival were compared between plasma recipients and controls. Results Convalescent plasma recipients were more likely than control patients to remain the same or have improvements in their supplemental oxygen requirements by post-transfusion day 14, with an odds ratio of 0.86 (95% CI: 0.75~0.98; p=0.028). Plasma recipients also demonstrated improved survival, compared to control patients (log-rank test: p=0.039). In a covariates-adjusted Cox model, convalescent plasma transfusion improved survival for non-intubated patients (hazard ratio 0.19 (95% CI: 0.05 ~0.72); p=0.015), but not for intubated patients (1.24 (0.33~4.67); p=0.752). Conclusions Convalescent plasma transfusion is a potentially efficacious treatment option for patients hospitalized with COVID-19; however, these data suggest that non-intubated patients may benefit more than those requiring mechanical ventilation. [**note: the Mt. Sinai group continues to impress!! I think this is the first study on convalescent plasma that I have seen. It looks progression to intubation is bad news so antibody treatment needs to be done early on. We need mAbs.**] https://www.medrxiv.org/content/10.1101/2020.05.20.20102236v1
- In severe COVID-19 pulmonary failure, hypoxia is mainly related to pulmonary vasodilation with altered hypoxic pulmonary vasoconstriction (HPV). Besides prone positioning, other non-ventilatory strategies may reduce the intrapulmonary shunt. This study has investigated almitrine, a pharmacological option to improve oxygenation. Patients and Method. A case control series of 17 confirmed COVID-19 mechanically ventilated patients in prone or supine

positioning was collected: 10 patients received two doses of almitrine (4 and 12 mcg/kg/min) at 30-45 min interval each, and were compared to 7 control COVID-matched patients conventionally treated. The end-point was the reduction of intra-pulmonary shunt increasing the PaO2 and ScvO2. Results Patients were male (59%) with median (25th, 75th percentiles) age of 70 (54-78) years and a BMI of 29 (23-34). At stable mechanical ventilatory settings, PaO2 (mmHg) at FiO2 1 (135 (85, 195) to 214 (121, 275); p = 0.06) tended to increase with almitrine. This difference was significant when the best PaO2 between the 2 doses was used : 215 (123,294) vs baseline (p = 0.01). A concomitant increase in ScvO2 occurred ((73 (72, 76) to 82 (80, 87); p = 0.02). Eight over 10 almitrine-treated patients increased their PaO2, with no clear dose-effect. During the same time, the controls did not change PaO2. In conclusion, in early COVID-19 with severe hypoxemia, almitrine infusion is associated with improved oxygenation in prone or supine positioning. This pharmacological intervention may offer an alternative and/or an additional effect to proning and might delay or avoid more demanding modalities such as ECMO. [**note: I think there is a trial going on with almitrine.**]
https://www.medrxiv.org/content/10.1101/2020.05.18.20105502v1

- objectives: to assess the prevalence of COVID-19 (PCR-test) in residents and staff of a nursing home. To examine the presence of IgM and IgG antibodies in the sample and the relation between PCR and antibody test results. design: cross-sectional and (retrospective) cohort study setting: a nursing home for the elderly Bessemerberg in Lanaken (Belgium) with up to 130 beds. Lanaken is situated in the Belgian province with the highest COVID-19 prevalence. participants: residents (N=108) and staff members (N=93) of the nursing home outcomes: PCR, IgM and IgG results: the prevalence of COVID-19, based on PCR test was 34% (N=40) for residents and 13% (N=11) for staff members, respectively. Of the residents, 13% showed positive IgM results and 15% positive IgG results. In 17% of the residents, at least one of the antibodies was positive. In total 13% of the staff members had positive IgM and 16% had a positive IgG. In 20% of the staff members at least one of these antibody tests was positive. In PCR positive residents, the percentage of IgM positive, IgG positive, and at least one of both was 28%, 34%, and 41%. In PCR positive staff, we found 30%, 60%, and 60%. Additional antibody tests were performed in nine residents between day 11 and 14 after the positive PCR test. Of those, 7 (78%) tested positive on at least one antibody. When retesting three weeks later, all remaining residents also tested positive. conclusions: Recently it was reported that in Belgium antibodies are present in 3-4% of the general population. Although, the prevalence in our residents is higher, the number is largely insufficient for herd immunity. In staff members of the regional hospital the prevalence of antibodies was 6%. The higher prevalence in nursing home staff (21%) may be related to the complete absence of good quality protection in the first weeks of the outbreak. [**note: data from a Belgian nursing home.**]
https://www.medrxiv.org/content/10.1101/2020.05.18.20105874v1
- COVID19 pandemic has so far caused over three hundred thousand deaths worldwide, primarily due to complications from SARS-CoV-2-associated acute respiratory distress syndrome (ARDS). While an ARDS-driven hyperinflammatory phenotype is associated with higher mortality in non-COVID patients, there is little information on how cytokines and chemokines expressions correlate with clinical outcomes in COVID19 patients. We prospectively enrolled a cohort of 41 patients with acute respiratory distress syndrome on mechanical ventilation. Patients blood was obtained at enrollment and outcome measures were liberation from mechanical ventilation and

hospital-free days. We determined the expression levels of 44 circulating cytokines/chemokines and found 13 of them associated with worse outcomes. After correcting for multiple comparisons/false discovery rate, only one chemokine (CCL19) remained significantly associated with outcomes (p=0.009). Although not described in association with COVID19, this chemokine was previously found elevated in an animal model of SARS-CoV. Moreover, CCL19 seems to be relevant for bronchus-associated lymphoid tissue (BALT) maintenance and for lung immunity to influenza virus. While this finding requires corroboration, CCL19 determination could facilitate early identification COVID19-ARDS patients at higher risk of death and be novel target for immunotherapy in this setting. [**note: a new marker for disease progression.**]
https://www.medrxiv.org/content/10.1101/2020.05.21.20051300v2

- We are currently facing a frightening increase in COVID-19 patients admitted to the ICU. Aiming at screening for secondary pneumonia, we collected the data of our first twelve ICU patients who underwent bronchoalveolar lavage (BAL). Surprisingly, four were detected with Pneumocystis jirovecii (Pj) DNA and RNA, resulting in Pj prevalence of 17%. Pj is a ubiquitous ascomycetes fungus that thrives at the surface of type-I pneumocytes, specifically in human alveoli, leading to pneumocystosis in immunocompromised patients. Interestingly, none of our patients was immunocompromised per se before admission, while all presented the recognized risk factors for life-threatening COVID-19 infection. Observing such high prevalence in COVID-infected patients was unexpected. Almost all patients developed ARDS and received high-dose steroids to prevent worsening, as suggested by reports from China. In Pj-positive patients requiring steroids, prophylaxis was given to avoid the risk of pneumocystosis and increased lung inflammation that may compromise the outcome. We are strongly convinced that testing deep lung specimens for Pj in severe COVID-19 patients should be recommended and Pj-positive patients treated with steroids, and given anti-Pj prophylaxis. This message is important, given the high mortality rate of COVID-19 patients in the ICU. [**note: from France a new concern about opportunistic infection with Pneumocystis jirovecii!**]
https://www.medrxiv.org/content/10.1101/2020.05.18.20105296v1
- BACKGROUND Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) and its associated clinical syndrome COVID-19 are causing overwhelming morbidity and mortality around the globe, disproportionately affecting New York City. A comprehensive, integrative autopsy series that advances the mechanistic discussion surrounding this disease process is still lacking. METHODS Autopsies were performed at the Mount Sinai Hospital on 67 COVID-19 positive patients and data from the clinical records were obtained from the Mount Sinai Data Warehouse. The experimental design included a comprehensive microscopic examination carried out by a team of expert pathologists, along with transmission electron microscopy, immunohistochemistry, RNA in situ hybridization, as well as immunology and serology assays. RESULTS Laboratory results of our COVID-19 cohort show elevated inflammatory markers, abnormal coagulation values, and elevated cytokines IL-6, IL-8 and TNFα. Autopsies revealed large pulmonary emboli in four cases. We report microthrombi in multiple organ systems including the brain, as well as conspicuous hemophagocytosis and a secondary hemophagocytic lymphohistiocytosis-like syndrome in many of our patients. We provide electron microscopic, immunofluorescent and immunohistochemical evidence of the presence of the virus and the ACE2 receptor in our samples. CONCLUSIONS We report a comprehensive autopsy series of 67 COVID-19 positive patients revealing that this disease, so far conceptualized as a primarily

respiratory viral illness, also causes endothelial dysfunction, a hypercoagulable state, and an imbalance of both the innate and adaptive immune responses. Novel findings reported here include an endothelial phenotype of ACE2 in selected organs, which correlates with clotting abnormalities and thrombotic microangiopathy, addressing the prominent coagulopathy and neuropsychiatric symptoms. Another original observation is that of macrophage activation syndrome, with hemophagocytosis and a hemophagocytic lymphohistiocytosis-like disorder, underlying the microangiopathy and excessive cytokine release. We discuss the involvement of critical regulatory pathways. [**note: more from Mt. Sinai, this time pathology data.**] https://www.medrxiv.org/content/10.1101/2020.05.18.20099960v1

DRUG DEVELOPMENT

- Pseudotyped particles have significant importance and use in virology as tools for studying the biology of highly pathogenic viruses in a lower biosafety environment. The biological, chemical, and serological studies of the recently emerged SARS-CoV-2 will be greatly aided by the development and optimization of a suitable pseudotyping system. Here, we pseudotyped the SARS-CoV-2 Spike glycoprotein (SPG) on a retroviral (MMLV) as well as a third generation lentiviral (pLV) vector and tested the transduction efficiency in several mammalian cell lines expressing SARS-CoV-2 receptor hACE2. While MMLV pseudotyped the vesicular stomatitis virus G glycoprotein (VSV-G) efficiently, it could not pseudotype SPG. In contrast, pLV pseudotyped both glycoproteins efficiently; however, much higher titers of pLV-G particles were produced. Among all the tested mammalian cells, 293Ts expressing hACE2 were most efficiently transduced using the pLV-S system. The pLV-S particles were efficiently neutralized by diluted serum (>:640) from a recently recovered COVID-19 patient who showed high SARS-CoV-2 specific IgM and IgG levels. In summary, pLV-S pseudotyped virus provides a valid screening tool for the presence of anti SARS-CoV-2 specific neutralizing antibodies in convalescent patient serum. [**note: The study here describes a pseudotyping system which mimics the surface properties of SARS-CoV-2 and can be used in lower biosafety level laboratory for the purpose of vaccine studies, drug inhibition studies, and serological screening to determine the status of herd immunity.**] https://www.medrxiv.org/content/10.1101/2020.05.21.20108951v1
- Understanding and preventing the emergence of novel viruses requires an accurate and comprehensive understanding of their genomes. One under-investigated class of functional genomic elements is overlapping genes (OLGs), which allow a single stretch of nucleotides to encode two distinct proteins in different reading frames. Viral OLGs are common and have been associated with the origins of pandemics, but are still widely overlooked. We investigate de novo OLG candidates in SARS-CoV-2 and identify a new gene here named ORF3c. ORF3c has been documented elsewhere but is unnamed, unannotated, or conflated with ORF3b of other SARS-related betacoronaviruses (sarbecoviruses). In fact, ORF3c is not homologous to ORF3b, as the two genes occupy different genomic positions and reading frames. We find that ORF3c exhibits clear evidence of translation from ribosome profiling and important immunological properties. We then conduct an evolutionary analysis of ORF3c at three levels: between-species, between-host, and within-host. Specifically, 21 representative sarbecovirus genomes show ORF3c is also present in some pangolin-CoVs but not more closely related bat-CoVs; 3,978 SARS-CoV-2 genomes reveal ORF3c gained a new stop codon (G25563U) that rose drastically in frequency during the current COVID-19 pandemic; and 401 deeply sequenced samples of SARS-

CoV-2 demonstrate the recurrence of this mutation in multiple hosts. Surprisingly, the newly gained ORF3c stop codon hitchhiked early with haplotype 241U/3037U/14408U/23403G (Spike-D614G), which appears to drive the European pandemic spread. Our results liken ORF3c to other important viral accessory genes recombined, lost, split, or truncated before or during outbreaks, including ORF3b and ORF8 in sarbecoviruses. OLGs deserve considerably more attention, as their rapid evolution may be more important than is currently appreciated in the emergence of zoonotic viruses. [**note: this is another example of the Rumsfeld Paradigm. We probably still do not know everything there is to know about the virus. This particular finding may or may not be useful in drug design.**] https://www.biorxiv.org/content/10.1101/2020.05.21.109280v1

- Viruses are the major aetiological agents of acute and chronic severe human diseases that place a tremendous burden on global public health and economy; however, for most viruses, effective prophylactics and therapeutics are lacking, in particular, broad-spectrum antiviral agents. Herein, we identified 2 secreted bacterial lipases from a *Chromobacterium* bacterium, named *Chromobacterium* antiviral effector-1 (*Cb*AE-1) and *Cb*AE-2, with a broad-spectrum virucidal activity against dengue virus (DENV), Zika virus (ZIKV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), human immunodeficiency virus (HIV) and herpes simplex virus (HSV). The *Cb*AEs potently blocked viral infection in the extracellular milieu through their lipase activity. Mechanistic studies showed that this lipase activity directly disrupted the viral envelope structure, thus inactivating infectivity. A mutation of *Cb*AE-1 in its lipase motif fully abrogated the virucidal ability. Furthermore, *Cb*AE-2 presented low toxicity *in vivo* and *in vitro*, highlighting its potential as a broad-spectrum antiviral drug. [**note: as my doctoral dissertation was on lipid altering enzymes, this paper intrigued me. However, making a claim that certain lipases may possess virucidal activity seems a step too far. There is the big question of drug delivery and an even bigger one of what it might do to host membrane systems. Here is a good trivia point, one of the chapters of my thesis was on the bee venom peptide melittin that has potent detergent properties. That should also be a potent anti-viral compound but will wreak havoc with other membranes as well.**]
  https://www.biorxiv.org/content/10.1101/2020.05.22.109900v1
- The COVID-19 pandemic is a major threat to global health for which there are only limited medical countermeasures, and we lack a thorough understanding of mechanisms of humoral immunity. From a panel of monoclonal antibodies (mAbs) targeting the spike (S) glycoprotein isolated from the B cells of infected subjects, we identified several mAbs that exhibited potent neutralizing activity with IC50 values as low as 0.9 or 15 ng/mL in pseudovirus or wild-type (wt) SARS-CoV-2 neutralization tests, respectively. The most potent mAbs fully block the receptor-binding domain of S (SRBD) from interacting with human ACE2. Competition-binding, structural, and functional studies allowed clustering of the mAbs into defined classes recognizing distinct epitopes within major antigenic sites on the SRBD. Electron microscopy studies revealed that these mAbs recognize distinct conformational states of trimeric S protein. Potent neutralizing mAbs recognizing unique sites, COV2-2196 and COV2-2130, bound simultaneously to S and synergistically neutralized authentic SARS-CoV-2 virus. In two murine models of SARS-CoV-2 infection, passive transfer of either COV2-2916 or COV2-2130 alone or a combination of both mAbs protected mice from severe weight loss and reduced viral burden and inflammation in the lung. These results identify protective epitopes on the SRBD and provide a structure-based framework for rational vaccine design and the selection of robust immunotherapeutic cocktails.

[**note: lots of papers coming out now about isolation of mAbs. We need to get these into the clinic and start producing stuff at scale. Let's figure out how they can be formulated into autoinjectors for home use as a pseudo-vaccine!!! This is where the Fast Grants project out to be putting some money.**] https://www.biorxiv.org/content/10.1101/2020.05.22.111005v1

- Background: There is an urgent need of active treatment for coronavirus disease 2019 (Covid-19). Although efficacy have not been proven, lopinavir/ritonavir 400 mg/100 mg twice daily has been proposed as a treatment of moderate to severe Covid-19. Previously published cohorts showed Covid-19 is associated with major inflammation. To date, no data are available regarding lopinavir/ritonavir plasma concentration and its safety in Covid-19 patients. Methods: Real-world Covid-19 experience based on a retrospective cohort study Results: On the cohort of 31 patients treated by lopinavir/ritonavir for Covid-19, *we observed very high lopinavir plasma concentrations, increased of 4.6-fold (IQR 2.9-6.4), with regards to average plasma concentrations in HIV treatment. All except two patients were above the upper limit of the concentration ranges of HIV treatment*. In this cohort, about one over four to five patients prematurely stopped lopinavir/ritonavir therapy due to a moderate adverse drug reaction, mainly hepatic and gastrointestinal disorders. Conclusion: Patients with Covid-19 pneumonitis treated with lopinavir/ritonavir have plasma concentrations dramatically higher than expected. Owing to that high plasma concentration may be required for antiviral activity against SARS-CoV-2, it appears that lopinavir dosage should not be reduced in the absence of adverse effect. About 80% of the patients well tolerated lopinavir/ritonavir therapy under these plasma concentrations. However, cautious is necessary as drug repurposing can be associated with a new drug safety profile. [**note: wow, is this unexpected. More needs to be done to understand this! I wonder if there is a similar impact on other drug therapies.**] https://www.medrxiv.org/content/10.1101/2020.05.18.20105650v1

DIAGNOSTIC DEVELOPMENT

- Virus neutralization remains the gold standard for determining antibody efficacy. Therefore, a high-throughput assay to measure SARS-CoV-2 neutralizing antibodies is urgently needed for COVID-19 serodiagnosis, convalescent plasma therapy, and vaccine development. Here we report on a fluorescence-based SARS-CoV-2 neutralization assay that detects SARS-CoV-2 neutralizing antibodies in COVID-19 patient specimens and yields comparable results to plaque reduction neutralizing assay, the gold standard of serological testing. Our approach offers a rapid platform that can be scaled to screen people for antibody protection from COVID-19, a key parameter necessary to safely reopen local communities. [**note: another approach to rapid screening.**] https://www.biorxiv.org/content/10.1101/2020.05.21.109546v1
- Little is known about the quality of polyclonal antibody responses in COVID-19 patients, and how it correlates with disease severity or patients' prior exposure to other pathogens. The whole polyclonal antibody repertoire in a retrospective cohort of 538 individuals was mapped against SARS-CoV-2 spike (S) glycoprotein, the main target of antibody immune responses in SARS-CoV-2 infection. Bioinformatic predictions identified 15 major B cell epitopes for S of SARS-CoV-2. Several epitopes localised in RBD of S including those spanning the ACE2-binding site, the highly conserved cryptic epitope of the neutralizing antibody of SARS-CoV, and fusion/entry domains of HR1 and HR2 of S protein of SARS-CoV-2. Intriguingly, some of these epitopes have cross-reactivity to antigens of common pathogens, potentially affecting SARS-CoV-2 infection

outcome. High level of anti-Spike SARS-CoV-2 seroreactivity in populations with no history of exposure to SARS-CoV-2 is of clinical relevance and could underpin better understanding of COVID-19 pathophysiology in different populations and provide a blueprint for design of effective vaccines and developing better strategies for antibody testing. [**note: way cool, paper is partially funded by Protobios LLC, an Estonian biotech company!! We are all in this together, big countries and small. Little known fact: Estonia is well known for choral singing of all kinds. Some good composers are from there!!! Get this pandemic over so I can get back to rehearsals!**] https://www.medrxiv.org/content/10.1101/2020.05.18.20105189v1

- Real-time reverse transcription polymerase-chain reaction (RT-PCR) is the mainstay of Covid-19 diagnosis. False-negative RT-PCR results may hamper clinical management of patients and hinder the adoption of epidemiological measures to control the pandemic. The current study was aimed at assessing whether amplification of β-glucoronidase (GUSB) gene would help estimate the accuracy of SARS-CoV-2 RT-PCR negative results in upper respiratory tract (URT) specimens. URT specimens that tested negative by SARS-CoV-2 RT-PCR displayed higher GUSB RT-PCR cycle thresholds (CT) (P=0.070) than those testing positive (median, 30.7; range, 27.0-40.0, and median 29.7; range 25.5-36.8, respectively), this reflecting poorer cellularity. Receiver operating characteristic (roc) curve analysis indicated that a CT threshold of 31.2 discriminated best between positive and negative SARS CoV-2 RT-PCRs (area under a curve, 0.66; 95% CI, 0.50-0.81; P=0.08). This cut-off yielded a true negative ratio of 89% and accuracy of 70%. The data suggested that amplification of the GUSB gene by RT-PCR may help to appraise the accuracy of negative SARS-CoV-2 RT-PCR results in patients in whom Covid-19 is eventually diagnosed. [**note: this Spanish group is trying to solve the problem of poor reliability in upper respiratory tract specimens.**] https://www.medrxiv.org/content/10.1101/2020.05.20.20105312v1

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2020-05-24

For reflection Sunday, let's add a bit of optimism. What better and uplifting way to move our spirits than the Beethoven 9th Symphony. We turn to the Frankfurt Radio Symphony under the baton of Andrés Orozco-Estrada. I really like how he only modestly accepts the audience applause, preferring to acknowledge all the ensemble members and soloists: https://www.youtube.com/watch?v=gT91esZK90I Collectively, we are all brothers and sisters and we will get through this!

I got back to web site coding this week and am getting my old-style website spiffed up. The first thing to get a fresh coat of paint is the SARS-CoV-2 page. You can see the results HERE! I really like the font. It is 'Lato' from the Google Font collection.

One of my loyal readers is a partner at a multi-stage investment firm dedicated to evidence-based investing in public and private healthcare and life science companies. They have developed some nice COVID-19 resources. There is a good technical atlas on the site! Make sure you read the 'Strategic Overview & How to Use the Map' first.

From STAT an opinion piece on the benefits of remdesivir treatment and limitations; also who should get the COVID-19 vaccine first; and a proposal for a health policy holiday (*I am more interested in a pre-print holiday so I can take a day off from the newsletter*). STAT also reported late on Friday that CDER

director, Janet Woodcock has been moved to focus on the COVID-19 vaccine project. This one is behind the paywall and I have not seen an FDA press statement on this.

There was no rest for the weary this morning with a lot of interesting preprints to review and post. I can't wait to see how the Egyptian baking soda trial sorts out. We do need a low-cost therapeutic intervention!!

Enjoy the Sunday where every you may live.

MODELING

- Background: As the coronavirus (COVID-19) epidemic passes the peak infection rate in some states and counties a phased re-opening with changes of stay-at-home restrictions and social distancing recommendations may lead to an increase of non-essential work, social activities and gathering, especially among younger persons. Methods: A longitudinal cohort analysis of Washington State Department of Health COVID-19 confirmed case age distribution March 1-April 19 2020 for proportional change over time using chi square tests for significance (N = 13,934). Results: From March 1st to April 19, 2020 age distribution shifted with a 10% decline in cases age 60 years and older and a 20% increase in age 0-19/20-39 years (chi-square = 223.10, p <.001). Number of cases over the eight-week analysis period were 0-19 years n = 515, 20-39 years n = 4078, 40-59 years n =4788, 60-79 years n = 3221, 80+ years n = 1332. New cases increased steadily among 0-19 and 20-39-year olds. After the peak (March 22, 2020), there was no decline among age 0-19 and a lesser decline among age 20-39 than older groups. As incidence declined in older age groups, the combined percentage of cases age 0-19 and 20-39 increased from 20% to 40% of total cases. Conclusions: Increased COVID-19 infection among children and young adults is not without serious morbidity and mortality risk to them and others they may come in contact with, indicating a targeted approach for awareness and safety measures is advisable to reduce incidence among the supposedly less vulnerable but more mobile young population age 0-19 and 20-39 years. [**note: good data from Washington showing the age related disease progression from older persons to younger. Though symptoms in the young are usually benign there is still a disturbing number of problems in this population.**] https://www.medrxiv.org/content/10.1101/2020.05.21.20109389v1
- Wastewater surveillance of SARS-CoV-2 has become an attractive tool for combating the spread of COVID-19 by assessing the presence or levels of the virus shed in a population. However, the methods to quantify viral RNA and to link those quantities to the level of infection within the community vary. In this study, we sought to identify and optimize scalable methods for recovery of viral nucleic acids from wastewater and attempted to use a constitutive member of the gut virome, human-specific crAssphage, to help account for unknown levels of SARS-CoV-2 decay and dilution in the wastewater infrastructure. Results suggest that ultracentrifugation of a small volume of wastewater through a 50% sucrose cushion followed by total nucleic acid extraction yielded quantifiable virus in an area with a modest number of COVID-19 cases. Further, the ratio of log10(SARS-CoV-2):log10(crAssphage) appears to be associated with the cumulative incidence of COVID-19 in the Syracuse, NY area. In areas where ultracentrifuges are available, these

methods may be used to link SARS-CoV-2 quantities in wastewater to levels of transmission within communities with sewer service. [**note: some good work from these Syracuse researchers on an improved way to monitor wastewater. This can help target more aggressive person screening by identifying hot areas.**] https://www.medrxiv.org/content/10.1101/2020.05.21.20109181v1

- Objectives: To investigate COVID-19 risk by occupational group. Design: Prospective study of linked population-based and administrative data. Setting: UK Biobank data linked to SARS-CoV-2 test results from Public Health England from 16 March to 3 May 2020. Participants: 120,621 UK Biobank participants who were employed or self-employed at baseline (2006-2010) and were 65 years or younger in March 2020. Overall, 29% (n=37,890) were employed in essential occupational groups, which included healthcare workers, social and education workers, and other essential workers comprising of police and protective service, food, and transport workers. Poisson regression models, adjusted for baseline sociodemographic, work-related, health, and lifestyle-related risk factors were used to assess risk ratios (RRs) of testing positive in hospital by occupational group as reported at baseline relative to non-essential workers. Main outcome measures: Positive SARS-CoV-2 test within a hospital setting (i.e. as an inpatient or in an Emergency Department). Results: 817 participants were tested for SARS-CoV-2 and of these, 206 (0.2%) individuals had a positive test in a hospital setting. Relative to non-essential workers, healthcare workers (RR 7.59, 95% CI: 5.43 to 10.62) and social and education workers (RR 2.17, 95% CI: 1.37 to 3.46) had a higher risk of testing positive for SARS-CoV-2 in hospital. Using more detailed groupings, medical support staff (RR 8.57, 95% CI: 4.35 to 16.87) and social care workers (RR 2.99, 95% CI: 1.71 to 5.24) had highest risk within the healthcare worker and social and education worker categories, respectively. In general, adjustment for covariates did not substantially change the pattern of occupational differences in risk. Conclusions: Essential workers in health and social care have a higher risk of severe SARS-CoV-2 infection. These findings underscore the need for national and organisational policies and practices that protect and support workers with elevated risk of SARS-CoV-2 infection. [**note: one of the good things about the UK NHS is the ability to do large longitudinal studies such as this one. It's not surprising that medical staff are more at risk but good to get confirmation and figure out how to manage the risk.**] https://www.medrxiv.org/content/10.1101/2020.05.22.20109892v1
- Objectives To explore the impact of COVID-19 lockdown on premature birth rates in Denmark Design Nationwide register-based prevalence proportion study. Participants 31,180 live singleton infants born in Denmark between March 12, and April 14, from 2015 to 2020 Main outcome measures The Main outcome measure was the odds ratio of premature birth, per preterm category, during the lockdown period compared with the calendar match period in the five previous years. Results A total of 31 180 newborns were included in the study period, of these 58 were born extremely premature (gestational age below 28 weeks). The distribution of gestational ages was significantly different (p = 0.004) during the lockdown period compared to the previous five years. *The extremely premature birth rate during the lockdown was significantly lower than the corresponding mean rate for the same dates in the previous years (odds ratio 0.09 [95 % CI 0.01 - 0.04], p < 0.001).* No significant difference between the lockdown and previous years was found for other gestational age categories. Conclusions The birth rate of extremely premature infants decreased significantly (~90 % reduction) during the Danish nationwide lockdown from a stable rate in the preceding five years. The reasons for this

decrease are unclear. Identification of possible causal mechanisms might stimulate changes in clinical practice. Ideally, some cases of extreme prematurity are preventable which may decrease infant morbidity and mortality. [**note: here is another good use of a national health system database, this time from Denmark. The drop in extremely premature births is fascinating. One might think that this is counter-intuitive given stress put on citizens by the lockdown.**] https://www.medrxiv.org/content/10.1101/2020.05.22.20109793v1

NEWLY REGISTERED CLINICAL TRIALS

- Here is one that might lead to a very inexpensive course of treatment. These Egyptian researchers have some preliminary evidence that inhaled sodium bicarbonate might be useful. "in our practice we faced with 3 cases with clinical and ct pictures as well as laboratory findings suggestive of COVID-19 pneumonia. We treated them with antibiotics, oseltamivir as well as inhalation of sodium bicarbonate. They showed marked clinical, laboratory and radiological improvements." They will be expanding this into a small trial, [**note: I'll have to check the baking supply shelf at the market to see if they still have the trusty Arm and Hammer baking soda; maybe it's all be diverted into DIY cures for COVID-19!**] NCT04374591
- Amyndas Pharmaceuticals S.A. is conducting a study with one of its compounds: AMY-101, a potent C3 inhibitor, for the management of patients with ARDS caused by SARS-CoV-2 infection. NCT04395456
- Here is a French trial with a liposomal formulation containing trans crocetin. [**note: I'm starting to see some weird compounds going into trials. We are approaching the throw everything at the wall and see what sticks period.**] NCT04378920
- Yet another Egyptian trial this time a Combination With Alvelestat and Isotretinoin May Enhances Neutralizing Antibodies in COVID -19 Infected Patients [**note: this one had one of the longest "brief" summaries of any CT I've seen.**] NCT04396067
- Here is a trial from Veru, Inc. To demonstrate the efficacy of VERU-111 (an anti-tubulin compound that is being looked at for prostate cancer) in the treatment of SARS-Cov-2 Infection by assessing its effect on the proportion of subjects that are alive without respiratory failure at Day 22. Respiratory failure is defined as non-invasive ventilation or high-flow oxygen, intubation and mechanical ventilation, or ventilation with additional organ support (e.g., pressors, RRT, ECMO). NCT04388826
- This Israeli study appeals to me!!! The current study examines an adapted guided self-help stress reduction program, focusing on reducing stress in the time of COVID-19. Two studies are proposed: 1) an international study in English in which individuals proficient in English throughout the world will participate and 2) a study in Hebrew. The first study will publicize via social media and participants will be randomized to either immediate participation or a 6 week waitlist period. Assessments will be conducted pre, (again after waitlist for the control group), posttx (after completion/cessation of the program), 6 month and 1 year followup. Two forms of assessment will be conducted: pre-post-follow-up (with the CRISIS measure) and weekly during the program (via the DASS-21). For the full program see: https://www.iterapi.se/sites/coronastress/ NCT04394403

CLINICAL TRIAL RESULTS

- Temporal inference from laboratory testing results and their triangulation with clinical outcomes as described in the associated unstructured text from the provider notes in the Electronic Health Record (EHR) is integral to advancing precision medicine. Here, we studied 181 COVIDpos and 7,775 COVIDneg patients subjected to 1.3 million laboratory tests across 194 assays during a two-month observation period centered around their SARS-CoV-2 PCR testing dates. We found that compared to COVIDneg at the time of clinical presentation and diagnostic testing, COVIDpos patients tended to have higher plasma fibrinogen levels and similarly low platelet counts, with approximately 25% of patients in both cohorts showing outright thrombocytopenia. However, these measures show opposite longitudinal trends as the infection evolves, with declining fibrinogen and increasing platelet counts to levels that are lower and higher compared to the COVIDneg cohort, respectively. Our EHR augmented curation efforts *suggest a minority of patients develop thromboembolic events after the PCR testing date, including rare cases with disseminated intravascular coagulopathy (DIC), with most patients lacking the platelet reductions typically observed in consumptive coagulopathies. These temporal trends present, for the first time, fine-grained resolution of COVID-19 associated coagulopathy (CAC), via a digital framework that synthesizes longitudinal lab measurements with structured medication data and neural network-powered extraction of outcomes from the unstructured EHR*. This study demonstrates how a precision medicine platform can help contextualize each patients specific coagulation profile over time, towards the goal of informing better personalization of thromboprophylaxis regimen. [**note: good use of EHRs to better understand the thromboembolic events in some patients.**]
  https://www.medrxiv.org/content/10.1101/2020.05.21.20109439v1
- Importance Treatment options for Severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2) are limited with no clarity on the efficacy and safety profiles. Objective To assess if the effect estimate of any intervention improves the outcomes and safety profile. Data sources PubMed, Embase, Cochrane Central were searched from December 1, 2019 to May 11, 2020. Study selection Any prospective/retrospective clinical study on SARS-CoV-2 patients above 18 years of age with report on therapeutic interventions. Data synthesis and extraction Data was screened and extracted by two independent investigators. Main outcomes and measures The primary outcome was all-cause in-hospital mortality. The secondary outcomes were rates of mechanical ventilation, viral clearance, adverse events, discharge, progression to severe disease, median time for clinical recovery and anti-viral clearance. Pooled rates and odds ratios (OR) were calculated. Results A total of 29 studies with 5207 participants were included in the analysis. The pooled all-cause in-hospital mortality rate was 12.8% (95%CI: 8.1%-17.4%) in intervention arm. There was no significant difference in mortality between both arms overall (OR: 1.36, 95% CI: 0.97-1.89). The mortality was significantly higher in the Hydroxychloroquine (HCQ) group compared to control: (1.86, 95% CI: 1.38-2.50). The need for mechanical ventilation in patients with mild-moderate disease was 13.5% vs 9.8% in intervention and control groups, with no significant difference (OR: 1.58, 95% CI: 0.60-4.15).The median duration for viral clearance in the intervention arm was 6.1 (IQR: 4.3-8.8) days and control arm was 9 (IQR: 4.5-14) days, with no significant difference between the groups (p = 0.37). There was no significant difference between pooled adverse event rates in intervention and control groups: 34% vs 29.5% (OR: 1.44, 95% CI: 0.70-2.94), respectively. However, incidence of adverse events was significantly higher in HCQ sub-group (OR: 3.88, 95% CI: 1.60-9.45, I2 = 0%). There was no

significant difference in other secondary outcomes. Conclusion and relevance *The use of hydroxychloroquine was associated with increased mortality and adverse event rates. No other therapeutic intervention including Lopinavir/Ritonavir, Remdesivir or Tocilizumab seem to alter the natural course of the disease.* There is a further need for well-designed randomized clinical trials. [**note: another broad literature survey of therapeutic results. I think this will be the last one I post as it's clear that the clinical trial results will be the most meaningful to clinicians. As I often say, TIWWDCT!** https://www.medrxiv.org/content/10.1101/2020.05.20.20108365v1

- Purpose. To report a comparison between fatalities and recovery of the severe Covid-19 infected patients based on demographic and clinical characteristics. Methods. Between 5 March and 12 May 2020, of 4,000 patients, 1,278 were laboratory confirmed to be Covid-19 infection in Mashhad, Iran. Finally, 334 deceased and 733 recovered cases were assessed in terms of demography, exposure history, health outcomes and clinical symptoms. Results. Mean (SD) age for confirmed patients, for deceased cases and for recovered cases. Mean (SD) age for all confirmed-patients was 56.9 (18.74), for the fatalities 67.26 (15.77) and for those recovered 52.82 (17.91) years. The rise of the mortality rate in relation to seniority was statistically significant. Despite a high frequency of Covid-19 infections accrued in the age groups of 30-39 and 40-49 years, most of these cases (88.2 and 85.8%, respectively) recovered. The median (IQR) duration of hospitalization was 9.0 (9) days. The most prevalent co-morbidities were cardiovascular disorders (21%) and diabetes (16.3%). Fever (63.8%), cough (68.1%), and dyspnoea (72.7%) were the most frequent clinical symptoms. 5.2% of infected-cases were healthcare workers that two (3%) of them died. Most patients (48%) received both antiviral and antibiotic therapy. The mortality rate of Kaletra combination prescribed for severe cases was 46.7%. Conclusions. The characteristics of Covid-19 varied from died to survived infected patients. *There were a higher number of fatalities in younger patients than in international studies.* Diabetes and cardiovascular disorders were most prevalent co-morbidities. The study could not address the case-fatality rate of Covid-19 infection that remains for future studies. [**note: outcome demographics from one medical center in hard hit Iran.}** https://www.medrxiv.org/content/10.1101/2020.05.20.20108068v1
- The emergence of the SARS-CoV-2 novel coronavirus has led to a global pandemic (COVID-19), with more than 5 million cases as of May 2020. Available data suggest that severe illness and death from COVID-19 are rare in the pediatric population. Integrating single-cell RNA sequencing of the developing mouse lung with temporally-resolved RNA-in-situ hybridization (ISH) in mouse and human lung tissue, we found expression of SARS-CoV-2 Spike protein primer TMPRSS2 was highest in ciliated cells and type I alveolar epithelial cells (AT1) and increased with aging in mice and humans. SARS-CoV-2 RNA colocalized with TMPRSS2 mRNA in lung cells from a patient who died of SARS-CoV-2. Together, these data suggest developmental regulation of TMPRSS2 may underlie the relative protection of infants and children from severe respiratory illness. [**note: a possible explanation for the lower rate of severe symptoms in young patients.**] https://www.biorxiv.org/content/10.1101/2020.05.22.111187v1
- Background: Infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes an acute illness termed coronavirus disease 2019 (COVID-19). Humoral immune responses likely play an important role in containing SARS-CoV-2, however, the determinants of SARS-CoV-2-specific antibody responses are unclear. Methods: Using immunoassays specific for the SARS-CoV-2 spike protein, we determined SARS-CoV-2-specific immunoglobulin A (IgA) and

immunoglobulin G (IgG) in sera and mucosal fluids of two cohorts, including patients with quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR)-confirmed SARS-CoV-2 infection (n = 56; median age 61 years) with mild versus severe COVID-19, and SARS-CoV-2-exposed healthcare workers (n = 109; median age 36 years) with or without symptoms and tested negative or positive by RT-qPCR. Findings: On average, SARS-CoV-2-specific serum IgA titers in mild COVID-19 cases became positive eight days after symptom onset and were often transient, whereas serum IgG levels remained negative or reached positive values 9-10 days after symptom onset. Conversely, patients with severe COVID-19 showed a highly significant increase of SARS-CoV-2-specific serum IgA and IgG titers as a function of duration since symptom onset, independent of patient age and comorbidities. Very high levels of SARS-CoV-2-specific serum IgA correlated with severe acute respiratory distress syndrome (ARDS). Interestingly, some of the SARS-CoV-2-exposed healthcare workers with negative SARS-CoV-2-specific IgA and IgG serum titers had detectable SARS-CoV-2-specific IgA antibodies in their nasal fluids and tears. Moreover, SARS-CoV-2-specific IgA levels in nasal fluids of these healthcare workers were inversely correlated with patient age. Interpretation: These data show that systemic IgA and IgG production against SARS-CoV-2 develops mainly in severe COVID-19, with very high IgA levels seen in patients with severe ARDS, whereas mild disease may be associated with transient serum titers of SARS-CoV-2-specific antibodies but stimulate mucosal SARS-CoV-2-specific IgA secretion. The findings suggest four grades of antibody responses dependent on COVID-19 severity. [**note: more on the antibody response.**]
https://www.biorxiv.org/content/10.1101/2020.05.21.108308v1

DRUG DEVELOPMENT

- Background: Coronavirus Disease 2019 (COVID-19) has no known specific treatments. However, there might be in vitro and early clinical data as well as evidence from Severe Acute Respiratory Syndrome and Middle Eastern Respiratory Syndrome that could inform clinicians and researchers. This systematic review aims to create priorities for future research of drugs repurposed for COVID-19. Methods: This systematic review will include in vitro, animal, and clinical studies evaluating the efficacy of a list of 34 specific compounds and four groups of drugs identified in a previous scoping review. Studies will be identified both from traditional literature databases and pre-print servers. Outcomes assessed will include time to clinical improvement, time to viral clearance, mortality, length of hospital stay, and proportions transferred to the intensive care unit and intubated, respectively. We will use the GRADE methodology to assess the quality of the evidence. Discussion: The challenge posed by COVID-19 requires not just a rapid review of drugs that can be repurposed but also a sustained effort to integrate new evidence into a living systematic review. Systematic review registration: PROSPERO 2020 CRD42020175648 [**note: this is an obvious effort that should have been started earlier (perhaps the planning did). They have set up a website: https://www.coviddrugs.org/ that will be populated with results.**]
https://www.medrxiv.org/content/10.1101/2020.05.21.20109074v1
- Effective therapies for COVID-19 are urgently needed. Presently there are more than 800 COVID-19 clinical trials globally, many with drug combinations, resulting in an empirical process with an enormous number of possible combinations. To identify the most promising potential therapies, we developed a biophysical model for the SARS-CoV-2 viral cycle and performed a

sensitivity analysis for individual model parameters and all possible pairwise parameter changes (16^2 = 256 possibilities). We found that model-predicted virion production is fairly insensitive to changes in viral entry, assembly, and release parameters, but highly sensitive to some viral transcription and translation parameters. Furthermore, we found a cooperative benefit to pairwise targeting of transcription and translation, predicting that combined targeting of these processes will be especially effective in inhibiting viral production. [**note: not clear how useful this will be in the current pandemic but it highlights a key point I've been noting regarding combination therapies.**] https://www.biorxiv.org/content/10.1101/2020.05.22.111237v1

- Immune dysregulation and cytokine release syndrome have emerged as pathological hallmarks of severe Coronavirus Disease 2019 (COVID-19), leading to the evaluation of cytokine antagonists as therapeutic agents. A number of immune-directed therapies being considered for COVID-19 patients are already in clinical use in chronic inflammatory conditions like inflammatory bowel disease (IBD). These considerations led us to systematically examine the intersections between COVID-19 and the GI tract during health and intestinal inflammation. We have observed that IBD medications, both biologic and non-biologic, do not significantly impact ACE2 and TMPRSS2 expression in the uninflamed intestines. Additionally, by comparing SARS CoV2-induced epithelial gene signatures with IBD-associated genes, we have identified a shared molecular subnetwork between COVID-19 and IBD. These data generate a novel appreciation of the confluence of COVID-19- and IBD-associated inflammation and provide mechanistic insights supporting further investigation of specific IBD drugs in the treatment of COVID-19. [**note: good work from Mt. Sinai, Cornel, and Janssen Pharma researchers on the possible linkage between infection and IBD.**] https://www.biorxiv.org/content/10.1101/2020.05.21.109124v1
- Guided by a computational docking analysis, about 30 FDA/EMA-approved small molecule medicines were characterized on their inhibition of the SARS-CoV-2 main protease (MPro). Of these tested small molecule medicines, six displayed an IC50 value in inhibiting MPro below 100 micromolar. Three medicines pimozide, ebastine, and bepridil are basic small molecules that are expected to exert a similar effect as hydroxychloroquine in raising endosomal pH for slowing down the SARS-CoV-2 entry into human cell hosts. Bepridil has been previously explored in a high dose as 100 mg/kg for treating diseases. Its high dose use will likely achieve dual functions in treating COVID-19 by both raising the endosomal pH to slow viral entry and inhibiting MPro in infected cells. Therefore, the current study urges serious considerations of using bepridil in COVID-19 clinical tests. [**note: this is a very interesting paper to read. The Texas A&M researchers use docking analysis and then direct binding studies. Even though the abstract refers to HCQ, they go beyond that to look for direct inhibition as opposed to just change in lysosomal pH. The marked difference in therapeutic targets of the three drugs is interesting. I remember studying pimozide years ago in a pharmacology class as it's similar to chlorpromazine. Bepridil is no longer used for angina.**]
  https://www.biorxiv.org/content/10.1101/2020.05.23.112235v1
- COVID-19 has become a global pandemic. Immune dysregulation has been implicated, but immune responses remain poorly understood. We analyzed 71 COVID-19 patients compared to recovered and healthy subjects using high dimensional cytometry. Integrated analysis of ~200 immune and >30 clinical features revealed activation of T cell and B cell subsets, but only in some patients. A subgroup of patients had T cell activation characteristic of acute viral infection and plasmablast responses could reach >30% of circulating B cells. However, another subgroup

had lymphocyte activation comparable to uninfected subjects. Stable versus dynamic immunological signatures were identified and linked to trajectories of disease severity change. These analyses identified three immunotypes associated with poor clinical trajectories versus improving health. These immunotypes may have implications for therapeutics and vaccines. [**note: from UPenn, some interesting stuff about the immune response. At some point we will understand the differential clinical progression to severity.**]
https://www.biorxiv.org/content/10.1101/2020.05.20.106401v1

DIAGNOSTIC DEVELOPMENT

- Rapid generation of diagnostics is paramount to understand epidemiology and to control the spread of emerging infectious diseases such as COVID-19. Computational methods to predict serodiagnostic epitopes that are specific for the pathogen could help accelerate the development of new diagnostics. A systematic survey of 27 SARS-CoV-2 proteins was conducted to assess whether existing B-cell epitope prediction methods, combined with comprehensive mining of sequence databases and structural data, could predict whether a particular protein would be suitable for serodiagnosis. Nine of the predictions were validated with recombinant SARS-CoV-2 proteins in the ELISA format using plasma and sera from patients with SARS-CoV-2 infection, and a further 11 predictions were compared to the recent literature. Results appeared to be in agreement with 12 of the predictions, in disagreement with 3, while a further 5 were deemed inconclusive. We showed that two of our top five candidates, the N-terminal fragment of the nucleoprotein and the receptor-binding domain of the spike protein, have the highest sensitivity and specificity and signal-to-noise ratio for detecting COVID-19 sera/plasma by ELISA. Mixing the two antigens together for coating ELISA plates led to a sensitivity of 94% (N=80 samples from persons with RT-PCR confirmed SARS-CoV2 infection), and a specificity of 97.2% (N=106 control samples). [**note: this may be a useful approach to improving serology tests.**]
https://www.biorxiv.org/content/10.1101/2020.05.22.111526v1
- To detect the presence of antibodies in blood against SARS-CoV-2 in a highly sensitive and specific manner, here we describe a robust, inexpensive ($200), 3D-printable portable imaging platform (TinyArray imager) that can be deployed immediately in areas with minimal infrastructure to read coronavirus antigen microarrays (CoVAMs) that contain a panel of antigens from SARS-CoV-2, SARS-1, MERS, and other respiratory viruses. Application includes basic laboratories and makeshift field clinics where a few drops of blood from a finger prick could be rapidly tested in parallel for the presence of antibodies to SARS-CoV-2 with a test turnaround time of only 2-4 h. To evaluate our imaging device, we probed and imaged coronavirus microarrays with COVID-19-positive and negative sera and achieved a performance on par with a commercial microarray reader 100x more expensive than our imaging device. This work will enable large scale serosurveillance, which can play an important role in the months and years to come to implement efficient containment and mitigation measures, as well as help develop therapeutics and vaccines to treat and prevent the spread of COVID-19. [**note: great DIY work!! Cheap and quick will win the day.**]
https://www.biorxiv.org/content/10.1101/2020.05.22.111518v1