2020-06-29

Welcome to Week 15 of the newsletter!!

I could do several weeks on Motown and still not cover all the singers and songs that were part of my high school and college memories. Let's spend some time with the all-time faves. Summer is a time for taking it to the street and this can be done safely even in a time of pandemic distancing. Who else to get us off our easy chairs and moving but Martha and the Vandellas! The iconic 'Dancing in the Streets' is just the recipe: https://www.youtube.com/watch?v=CdvITn5cAVc and here is a great summer twofer with the same group doing 'Heat Wave': https://www.youtube.com/watch?v=dAvq_gimOto

Some bleak news from Texas and Florida who have seen large outbreaks. By most counts, a quarter to a third of all hospitalizations are for those under the age of 40. While this age group may experience low mortality, the bed occupancy in hospitals is near or at capacity in hard hit areas. COVID-19 delirium is no laughing matter.

From The New Yorker, the pandemic's wider health care crisis with a focus on patent care and hospital finances.

The Washington Post has a good article on the common mutation in the viral genome that apparently arose in Europe and moved to New York City. There is still debate about whether this caused an increase in infectivity. It's fascinating how a single amino acid substitution can have such an impact.

STAT on the establishment of a high level commission to look at what has gone wrong with the US response to COVID-19. I am ready to serve!! Honesty and fairness will be my mantra. Also from STAT, Gilead announces the price for remdesivir. I think this is about right given it's use pattern and probable lack of a long term market (one hopes!); and a new journal that will vet COVID-19 reprints (good news for this beleaguered newsletter curator).

As usual for Mondays, the reading is noticeably light today. I am still waiting for results from some of the large trials that are ongoing.

MODELING

- A pipeline involving data acquisition, curation, carefully chosen graphs and mathematical models, allows analysis of COVID-19 outbreaks at 3,546 locations world-wide (all countries plus smaller administrative divisions with data available). Comparison of locations with over 50 deaths shows all outbreaks have a common feature: H(t) defined as loge(X(t)/X(t 1)) decreases linearly on a log scale, where X(t) is the total number of Cases or Deaths on day, t (we use ln for loge). The downward slopes vary by about a factor of three with time constants (1/slope) of between 1 and 3 weeks; this suggests it may be possible to predict when an outbreak will end. Is it possible to go beyond this and perform early prediction of the outcome in terms of the eventual plateau number of total confirmed cases or deaths? We test this hypothesis by showing that the trajectory of cases or deaths in any outbreak can be converted into a straight line. Specifically , is a straight line for the correct plateau value N, which is determined by a new method, Best-Line Fitting (BLF). BLF involves a straight-line facilitation extrapolation needed for prediction; it is blindingly fast and amenable to optimization. We find that in some locations that

entire trajectory can be predicted early, whereas others take longer to follow this simple functional form. Fortunately, BLF distinguishes predictions that are likely to be correct in that they show a stable plateau of total cases or death (N value). We apply BLF to locations that seem close to a stable predicted N value and then forecast the outcome at some locations that are still growing wildly. Our accompanying web-site will be updated frequently and provide all graphs and data described here. [**note: these days I only look at models with catch titles! 'Predicting the Trajectory of any COVID-19 Epidemic From the Best Straight Line' meets this criterion. Do not be misled, the approach involves more than a pencil and a ruler and the Gompertz distribution plays a role here (good that I brushed up on this last week). Kudos to these researchers for making these tools available for use and scrutiny. I'm going to spend some more time with this model and continue to follow this group's work. It is too long to add, but do read the humble conclusions section on page 15.**]
https://www.medrxiv.org/content/10.1101/2020.06.26.20140814v1

NEWLY REGISTERED CLINICAL TRIALS

- Will Check Tomorrow

CLINICAL TRIAL RESULTS

- Nothing Posted on the usual sites.

DRUG DEVELOPMENT

- According to The Guardian, the CanSino adenovirus vector vaccine has been approved for use within the Chinese military. The company said in a securities filing on the Hong Kong exchange the vaccine is approved for one year and they cannot guarantee that it will be commercialized.

VIRUS BIOCHEMISTRY

- Nothing new

DIAGNOSTIC DEVELOPMENT

- Assays to monitor SARS-CoV-2 growth depend on time-consuming and costly RNA extraction steps, hampering progress in basic research and drug development efforts. Here we developed a facile Q-RT-PCR assay that bypasses viral RNA extraction steps and can monitor SARS-CoV-2 replication kinetics from a small amount of cell culture supernatants. Using this assay, we screened the activities of a number of entry, SARS-CoV-2- and HIV-1-specific inhibitors in a proof of concept study. In line with previous studies which has shown that processing of the viral Spike protein by cellular proteases and endosomal fusion are required for entry, we found that E64D and apilimod potently decreased the amount of SARS-CoV-2 RNA in cell culture supernatants with minimal cytotoxicity. Surprisingly, we found that macropinocytosis inhibitor EIPA similarly decreased viral RNA in supernatants suggesting that entry may additionally be mediated by an alternative pathway. HIV-1-specific inhibitors nevirapine (an NNRTI), amprenavir (a protease inhibitor), and ALLINI-2 (an allosteric integrase inhibitor) modestly inhibited SARS-CoV-2 replication, albeit the IC_{50} values were much higher than that required for HIV-1. Taken together, this facile assay will undoubtedly expedite basic SARS-CoV-2 research, be amenable to mid-throughput screens to identify chemical inhibitors of SARS-CoV-2, and be applicable to a

broad number of RNA and DNA viruses. [**note: this is more of a research tool for drug screening.**] https://www.biorxiv.org/content/10.1101/2020.06.26.174698v1

>>>

2020-06-30

Keeping with the Motown vibe, let's look at one of the big male groups that rocked during the 1960s. The Temptations were just a huge hit machine for Barry Gordy's record label. Even though the personnel changed over the years, they always had a crisp sound. My favorite recording is 'My Girl': https://www.youtube.com/watch?v=C_CSjcm-z1w

James Fallows weighs in on Three Weeks that Changed Everything. He approaches it from the perspective of an NTSB investigation following a plane crash (Fallows is an amateur pilot).

The Washington Post has an interesting story on the use of the Johns Hopkins COVID-19 Tracker.

The New York Times has a good story on why most people with SARS-CoV-2 won't spread it but a few infect many. Good read!

STAT offers a piece on how bureaucratic regulations get in the way of COVID-19 progress and how Taiwan's robust electronic health records system (EHR) helped that country minimize the impact of SARS-CoV-2 infection. Don't even get me started talking about the inefficiencies of the US EHR system.

Maybe we need to rethink mask fabrics! Here is a preprint that looks at silk for DIY masks and compares it to cotton. "As a protective barrier and face covering, silk is more effective at impeding the penetration and absorption of droplets due to its greater hydrophobicity relative to other tested fabrics. Silk face coverings repelled droplets as well as masks, but unlike masks they are hydrophobic and can be readily sterilized for immediate reuse. Conclusions Silk is an effective hydrophobic barrier to droplets, more breathable than other fabrics that trap humidity, and are readily re-useable via cleaning. Therefore, silk can serve as an effective material for protecting respirators under clinical conditions and as a material for face coverings."

Tuesdays are always tough with lots of preprints that get posted after the weekend. There is the first report on the use of baricitinib showing some positive data.

MODELING

- After the outbreak of Coronavirus disease in 2019 (COVID-19), stringent lockdown measures were imposed in Wuhan between January 23, 2020 and April 8, 2020. To provide evidence on the post-lockdown risk of COVID-19 epidemic in Wuhan, the city government conducted a citywide nucleic acid screening of SARS-CoV-2 infection between May 14 and June 1, 2020. Methods: All city residents aged ≥6 years were potentially eligible to participate the screening programme. The rate of detection of asymptomatic infected cases was calculated, and their demographic and geographic distributions were investigated. ArcGIS 10.0 was used to draw a

geographic distribution of asymptomatic infected persons. Results: The screening programme recruited a total of 9,899,828 persons (response rate, 92.9%). The screening found no newly confirmed patients with COVID-19, and identified 300 asymptomatic infected cases (detection rate 0.303/10,000). In addition, 107 of 34,424 previously recovered patients with a history of COVID-19 diagnosis were tested positive (relapse rate, 0.31%). Virus culture of SARS-CoV-2 was negative for all 300 asymptomatic cases and all 107 recovered COVID-19 patients. A total of 1,174 close contacts of asymptomatic cases were traced and all of them had a negative nucleic acid testing result. Conclusions: Prevalence of COVID-19 nucleic acid test positivity was very low in the Wuhan general population, in recovered cases and in contacts of asymptomatic cases, five to eight weeks after the end of lockdown. These findings help resolve concerns about the post-lockdown risk of COVID-19 epidemic, and promote the recovery of economy and normal social life in Wuhan. [**note: this is an incredible feat that is unlikely to be repeated in other countries.**] https://www.medrxiv.org/content/10.1101/2020.06.29.20142554v1

- By conducting a retrospective, cross-sectional analysis of SARS-CoV-2 seroprevalence in a sentinel group (enriched for SARS-CoV-2 infections) and a screening group (representative of the general population) using >5,000 plasma samples from patients at Mount Sinai Hospital in New York City (NYC), we identified seropositive samples as early as in the week ending February 23, 2020. A stark increase in seropositivity in the sentinel group started the week ending March 22 and in the screening group in the week ending March 29. By the week ending April 19, the seroprevalence in the screening group reached 19.3%, which is well below the estimated 67% needed to achieve community immunity to SARS-CoV-2. These data potentially suggest an earlier than previously documented introduction of SARS-CoV-2 into the NYC metropolitan area. [**note: from the excellent Mt. Sinai group. Seropositive samples from late February in New York City**] https://www.medrxiv.org/content/10.1101/2020.06.28.20142190v1
- During March 1-May 16, 2020, 191,392 laboratory-confirmed COVID-19 cases were diagnosed and reported and 20,141 confirmed and probable COVID-19 deaths occurred among New York City (NYC) residents. We applied a network model-inference system developed to support the City's pandemic response to estimate underlying SARS-CoV-2 infection rates. Based on these estimates, we further estimated the infection fatality risk (IFR) for 5 age groups (i.e. <25, 25-44, 45-64, 65-74, and 75+ years) and all ages overall, during March 1-May 16, 2020. We estimated an overall IFR of 1.45% (95% Credible Interval: 1.09-1.87%) in NYC. In particular, weekly IFR was estimated as high as 6.1% for 65-74 year-olds and 17.0% for 75+ year-olds. These results are based on more complete ascertainment of COVID-19-related deaths in NYC and thus likely more accurately reflect the true, higher burden of death due to COVID-19 than previously reported elsewhere. It is thus crucial that officials account for and closely monitor the infection rate and population health outcomes and enact prompt public health responses accordingly as the pandemic unfolds. [**note: this is useful as it comes from the major US epicenter with the highest mortality. It will be important to do similar analyses in other geographical areas.**] https://www.medrxiv.org/content/10.1101/2020.06.27.20141689v1
- The COVID-19 pandemic has created unprecedented challenges worldwide. Strained healthcare providers make difficult decisions on patient triage, treatment and care management on a daily basis. Policy makers have imposed social distancing measures to slow the disease, at a steep economic price. We design analytical tools to support these decisions and combat the pandemic. Specifically, we propose a comprehensive data-driven approach to understand the

clinical characteristics of COVID-19, predict its mortality, forecast its evolution, and ultimately alleviate its impact. By leveraging cohort-level clinical data, patient-level hospital data, and census-level epidemiological data, we develop an integrated four-step approach, combining descriptive, predictive and prescriptive analytics. First, we aggregate hundreds of clinical studies into the most comprehensive database on COVID-19 to paint a new macroscopic picture of the disease. Second, we build personalized calculators to predict the risk of infection and mortality as a function of demographics, symptoms, comorbidities, and lab values. Third, we develop a novel epidemiological model to project the pandemic's spread and inform social distancing policies. Fourth, we propose an optimization model to reallocate ventilators and alleviate shortages. Our results have been used at the clinical level by several hospitals to triage patients, guide care management, plan ICU capacity, and re-distribute ventilators. At the policy level, they are currently supporting safe back-to-work policies at a major institution and equitable vaccine distribution planning at a major pharmaceutical company, and have been integrated into the US Center for Disease Control's pandemic forecast. [**note: this model is from a large group of researchers at the Sloan School of Management at MIT and is a worthwhile read. It provides useful information about resource allocation as well as triage in hospital settings.**] https://www.medrxiv.org/content/10.1101/2020.06.26.20141127v1

NEWLY REGISTERED CLINICAL TRIALS

-

CLINICAL TRIAL RESULTS

- One of the more disturbing clinical manifestations of COVID-19 is the multisystem inflammatory syndrome in children and adolescents. It occurs in a small number of those infected, manifesting itself 2-4 weeks following infection. The New England Journal of Medicine has two original research papers, HERE and HERE along with an editorial.
- Children have a lower rate of COVID-19, potentially related to cross-protective immunity conferred by seasonal coronaviruses (HCoVs). We tested if prior infections with seasonal coronaviruses impacted SARS-CoV-2 infections and related Multisystem Inflammatory Syndrome (MIS). Methods: This cross-sectional observational study in Paris hospitals enrolled 739 pauci or asymptomatic children (HOS group) plus 36 children with suspected MIS (MIS group). Prevalence, antigen specificity and neutralizing capability of SARS-CoV-2 antibodies were tested. Antibody frequency and titres against Nucleocapsid (N) and Spike (S) of the four seasonal coronaviruses (NL63, HKU1, 229E, OC43) were measured in a subset of seropositive patients (54 SARS-CoV-2 (HOS-P subgroup) and 15 MIS (MIS-P subgroup)), and in 118 matched SARS-CoV-2 seronegative patients (CTL subgroup). Findings: SARS-CoV-2 mean prevalence rate in HOSP children was 11.7% from April 1 to June 1. Neutralizing antibodies were found in 55.6% of seropositive children, and their relative frequency increased with time (up to 100 % by mid-May). A majority of MIS children (25/36) were SARS-CoV-2 seropositive, of which all tested (n=15) had neutralizing antibodies. On average, seropositive MIS children had higher N and S1 SARS-CoV-2 titres as compared to HOS children. Patients from HOS-P, MIS-P, and CTL subgroups had a similar prevalence of antibodies against the four seasonal HCoVs (66.9 -100%). The level of anti-SARS-CoV-2 antibodies was not significantly different in children who had prior seasonal coronavirus infection. Interpretation: Prior infection with HCoVs does not prevent SARS-CoV-2

infection and related MIS in children. Children develop neutralizing antibodies after SARS-CoV-2 infection. [**note: here is some data on multi-inflammatory syndrome in youth from France. Past exposure to other coronaviruses does not prevent SARS-CoV-2 infection.**] https://www.medrxiv.org/content/10.1101/2020.06.29.20142596v1

- Because they transport oxygen, red blood cells (RBCs) may play a role in the severity of hypoxemia in COVID-19 patients. The present study combines state-of-the-art metabolomics, proteomics, and lipidomics approaches to investigate the impact of COVID-19 on RBCs from 23 healthy subjects and 29 molecularly-diagnosed COVID-19 patients. RBCs from COVID-19 patients had increased levels of glycolytic intermediates, accompanied by oxidation and fragmentation of ankyrin, spectrin beta, and the N-terminal cytosolic domain of band 3 (AE1). Significantly altered lipid metabolism was also observed, especially short and medium chain saturated fatty acids, acyl-carnitines, and sphingolipids. Nonetheless, there were no alterations of clinical hematological parameters, such as RBC count, hematocrit, and mean corpuscular hemoglobin concentration, with only minor increases in mean corpuscular volume. Taken together, these results suggest a significant impact of SARS-CoV-2 infection on RBC structural membrane homeostasis at the protein and lipid levels. Increases in RBC glycolytic metabolites are consistent with a theoretically improved capacity of hemoglobin to off-load oxygen as a function of allosteric modulation by high-energy phosphate compounds, perhaps to counteract COVID-19-induced hypoxia. Conversely, because the N-terminus of AE1 stabilizes deoxyhemoglobin and finely tunes oxygen off-loading, RBCs from COVID-19 patients may be incapable of responding to environmental variations in hemoglobin oxygen saturation when traveling from the lungs to peripheral capillaries and, as such, may have a compromised capacity to transport and deliver oxygen. [**note: another clinical manifestation of COVID-19: damage to red blood cells that might be key to lack of oxygen delivery.]** https://www.medrxiv.org/content/10.1101/2020.06.29.20142703v1
- SARS-CoV-2-specific memory T cells will likely prove critical for long-term immune protection against COVID-19. We systematically mapped the functional and phenotypic landscape of SARS-CoV-2-specific T cell responses in a large cohort of unexposed individuals as well as exposed family members and individuals with acute or convalescent COVID-19. Acute phase SARS-CoV-2-specific T cells displayed a highly activated cytotoxic phenotype that correlated with various clinical markers of disease severity, whereas convalescent phase SARS-CoV-2-specific T cells were polyfunctional and displayed a stem-like memory phenotype. Importantly, SARS-CoV-2-specific T cells were detectable in antibody-seronegative family members and individuals with a history of asymptomatic or mild COVID-19. Our collective dataset shows that SARS-CoV-2 elicits robust memory T cell responses akin to those observed in the context of successful vaccines, suggesting that natural exposure or infection may prevent recurrent episodes of severe COVID-19 also in seronegative individuals. [**note: I am reminded of the tagline from the long-running daytime show, 'Days of our Lives' that started with "Like the Sands of the Hourglass…" The same thing might be applied to the slowly unravelling of the immune system's response to SARS-CoV-2 infection. This Swedish paper adds to our knowledge and shows that a robust memory T cell response is important to long-term immune protection. BTW, I have never been a fan of daytime 'soap operas' though there was a momentary infatuation with 'Dark Shadows' in the late 1960s**] https://www.biorxiv.org/content/10.1101/2020.06.29.174888v1

- We assessed the expression of the cell adhesion molecule Sialoadhesin (CD169), a type I interferon-inducible receptor, on monocytes (mCD169) in 53 adult patients admitted to the hospital during the COVID-19 outbreak for a suspicion of SARS-CoV-2 infection. mCD169 was strongly overexpressed in 30 out of 32 (93.7%) confirmed COVID-19 cases, compared to three out of 21 (14.3%) patients for whom the diagnosis of COVID-19 was finally ruled out. mCD169 was associated with the plasma interferon alpha level and thrombocytopenia. mCD169 testing may be helpful for the rapid triage of suspected COVID-19 patients during an outbreak. [**note: another potential marker for COVID-19 progression.**] https://www.medrxiv.org/content/10.1101/2020.06.28.20141556v1
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the ongoing pandemic coronavirus disease 2019 (COVID-19). The majority of patients with COVID-19 have a good prognosis, but variable percentages in different countries develop pneumonia associated with lymphocytopenia and severe inflammatory response due to uncontrolled release of cytokines. These immune mediators are transcriptionally regulated by JAK-STAT molecular pathways, which can be disabled by small molecules. Here, we provide evidences on the efficacy of baricitinib, a JAK1/JAK2 inhibitor, in correcting the immune abnormalities observed in patients hospitalized with COVID-19. Indeed, we demonstrate a significant reduction in serum levels of interleukin (IL)-6, IL-1β and tumor necrosis factor (TNF)α, a rapid recovery in circulating T and B cell frequencies and an increased antibody production against SARS-CoV-2 spike protein in baricitinib-treated patients. Moreover, treated patients underwent a rapid reduction in oxygen flow need and progressive increase in the P/F. Our work provides the basis on developing effective treatments against COVID-19 pathogenesis using on-target therapy. [**note: FINALLY some clinical data on a therapeutic! The study is from Italy and baricitinib is in trials at fifteen locations both in the US and abroad. This study is a little difficult to unravel as patients were treated with HCQ and in some case lopinavir/ritonavir as they were SOC drugs during this period of time. Cortico-steroids were avoided. 88 patients with COVID-19 pneumonia were followed and 20 were given baricitinib. There was only one death compared to 25 dead in the non-baricitinib arm. TIWWDCT! We will need confirmation of this obviously.**] https://www.medrxiv.org/content/10.1101/2020.06.26.20135319v1
- Hydroxychloroquine sulphate (HCQ) is being scrutinized for repositioning in the treatment and prevention of SARS-Cov-2 infection. This antimalarial drug is also chronically used to treat patients with autoimmune diseases. Methods: By analyzing the Portuguese anonymized data on private and public based medical prescriptions we have identified all cases chronically receiving HCQ for the management of diseases such as systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune diseases. Additionally, we have detected all laboratory confirmed cases of SARS-CoV-2 infection and all laboratory confirmed negative cases in the Portuguese population (mandatorily registered in a centrally managed database). Cross linking the two sets of data has allowed us to compare the proportion of HCQ chronic treatment (at least 2 grams per month) in laboratory confirmed cases of SARS-CoV-2 infection with laboratory confirmed negative cases. Results: Out of 26,815 SARS-CoV-2 positive patients, 77 (0.29%) were chronically treated with HCQ, while 1,215 (0.36%) out of 333,489 negative patients were receiving it chronically (P=0.04). After adjustment for age, sex, and chronic treatment with corticosteroids and/or immunosuppressants, the odds ratio of SARS-CoV-2 infection for chronic treatment with HCQ has been 0.51 (0.37-0.70). Conclusions: Our data suggest that chronic

treatment with HCQ confer protection against SARS-CoV-2 infection. [**note: HCQ is turning into a 'Zombie' drug, refusing to go away. Here is an observational study from Portugal on the possible protection in patients who are being treated for other medical conditions. I know the OHDSI group has this as one of their drugs to study but have not seen any information from them. I guess we need to wait for the big Duke study to come in, if it is ever fully enrolled.**] https://www.medrxiv.org/content/10.1101/2020.06.26.20056507v1

- PTX3 is an essential component of humoral innate immunity, involved in resistance to selected pathogens and in the regulation of inflammation. PTX3 plasma levels are associated with poor outcome in systemic inflammatory conditions and vascular pathology. The present study was designed to assess expression and significance of PTX3 in COVID-19. By bioinformatics analysis of public databases PTX3 expression was detected in lung respiratory cell lines exposed to SARS-CoV-2. By analysis at single cell level of COVID-19 circulating mononuclear cells, we found that PTX3 was selectively expressed by monocytes among circulating leukocytes. Moreover, in lung bronchoalveolar lavage fluid, single cell analysis revealed selective expression of PTX3 in neutrophils and macrophages, which play a major role in the pathogenesis of the disease. By immunohistochemistry, PTX3 was expressed by lung myelomocytic cells, type 2 pneumocytes and vascular endothelial cells. PTX3 plasma levels were determined by ELISA in 96 consecutive patients with a laboratory-confirmed diagnosis of COVID-19. Higher PTX3 plasma levels were observed in 52 (54.2%) patients admitted in ICU (median 21.0ng/mL, IQT 15.5-46.3 vs 12.4ng/mL IQT 6.1-20.2 in ward patients; p=0.0017) and in 22 (23%) patients died by 28 days (39.8ng/mL, IQT 20.2-75.7 vs 15.7ng/mL, IQT 8.2-21.6 in survivors; p=0.0001). After determining an optimal PTX3 cut-off for the primary outcome, the Kaplan-Meier curve showed an increased mortality in patients with PTX3>22.25ng/mL (Log-rank tests p<0.0001). In Cox regression model, PTX3>22.25ng/mL showed an adjusted Hazard Ratio (aHR) of 7.6 (95%CI2.45-23.76) in predicting mortality. Performing a multivariate logistic regression including all inflammatory markers (PTX3, ferritin, D-Dimer, IL-6, and CRP), PTX3 was the only marker significantly associated with death (aHR 1.13;95%CI1.02-1.24; p=0.021). The results reported here suggest that circulating and lung myelomonocytic cells are a major source of PTX3 and that PTX3 plasma levels can serve as a strong prognostic indicator of short-term mortality in COVID-19. [**note: add another biomarker to the increasing list of things to look for in disease progression.**] https://www.medrxiv.org/content/10.1101/2020.06.26.20139923v1
- Background In the absence of a standard of treatment for COVID-19, the combined use of anti-inflammatory (corticosteroids and Enoxaparin) and antiviral drugs may be more effective than using either modality alone in the treatment of COVID-19. Methods Patients hospitalized between April 10th, 2020, through May 10th, 2020, who had confirmed COVID-19 infection with clinical or radiographic evidence of pneumonia, in which 65 patients have moderate COVID-19 pneumonia, and 63 patients have severe COVID-19 pneumonia. All patients received early combination therapy of anti-inflammatory (corticosteroids and Enoxaparin) and antiviral drugs. They assessed for type and duration of treatment, and days need to wean from oxygen therapy, length of stay, virus clearance time, and complication or adverse events. All patients had more than 28 days follow up after discharge from the hospital. Results Moderate COVID-19 pneumonia group were 65 patients who received Enoxaparin, antiviral drugs, empirical antibiotics for pneumonia, and standard treatment for comorbidity. Male patients were 50 (76.9 %) and female patients were 15 (23.1 %). 34 (52.3 %) patients have comorbidity, 25 (38.5%)

patients have Diabetes Mellitus and 2 (3.1 %) pregnant ladies. 19 (29.2 %) patients were on low flow oxygen therapy, 3L oxygen or less to maintain oxygen saturation more than 92%. All patients discharged home with no major or minor bleeding complications or significant complications. Severe COVID-19 pneumonia group were 63 patients who received methylprednisolone, enoxaparin, antiviral drugs, empirical antibiotics for pneumonia, and standard treatment for comorbidity. Male patients were 55 (87.3 %) and female patients were 8 (12.7 %). 37 (58.7 %) patients have comorbidity, and 24 (38.1%) patients have Diabetes Mellitus. 32 (50.8 %) patients were on low flow oxygen therapy, 4-9L oxygen, and 31 (49.2 %) patients were on low flow oxygen therapy, 10L oxygen or more, including 12 patients on a non-rebreathing mask. Patients received methylprednisolone were 37 (58.7 %) for 3 days, 16 (25.4 %) for 5 days and 10 (15.9 %) for more than 5 days. Sixty-two patients discharged home with one patient had a long stay, and the other two transferred to ICU. One long-stay patient transferred to ICU on low flow oxygen therapy. Conclusion Early use of a combined anti-inflammatory (corticosteroids and Enoxaparin) and antiviral drugs treatment in patients with moderate to severe COVID-19 pneumonia prevent complications of the disease and improve clinical outcomes. [**note: from Saudi Arabia, use of a combination steroid, anti-coagulant, anti-viral therapy to treat COVID-19 pneumonia.**]
https://www.medrxiv.org/content/10.1101/2020.06.22.20134957v1

DRUG DEVELOPMENT

- Cytokine storm and multi-organ failure are the main causes of SARS-CoV-2-related death. However, the origin of the virus' excessively damaging abilities remains unknown. Here we show that the SARS-CoV-2 envelope (2-E) protein alone is sufficient to cause acute respiratory distress syndrome (ARDS)-like damage in vitro and in vivo. Overexpression of 2-E protein induced rapid pyroptosis-like cell death in various susceptible cells and robust secretion of cytokines and chemokines in macrophages. Intravenous administration of purified 2-E protein into mice caused ARDS-like pathological damage in lung and spleen. Overexpressed 2-E protein formed cation channels in host cell membranes, eventually leading to membrane rupture. Newly identified channel inhibitors exhibited potent anti-SARS-CoV-2 activity and excellent protective effects against the 2-E-induced damage both in vitro and in vivo. Importantly, their channel inhibition, cell protection and antiviral activities were positively correlated with each other, supporting 2-E is a promising drug target against SARS-CoV-2.
https://www.biorxiv.org/content/10.1101/2020.06.27.174953v1
- Since the emergence of CoVID-19 pandemic in China in late 2019, scientists are striving hard to explore non-toxic, viable anti-SARS-CoV-2 compounds or medicines. We determined In Vitro anti-SARS-CoV-2 activity of oral formulations (syrup and capsule) of an Iodine-complex (Renessance). A monolayer of vero cells were exposed to SARS-CoV-2 in the presence and absence of different concentrations (equivalent to 50, 05 and 0.5 ug/ml of I2) of Renessans. Anti-SARS-CoV-2 activity of each of the formulation was assessed in the form of cell survival, SARS-CoV-2-specific cytopathic effect (CPE) and genome quantization. With varying concentrations of syrup and capsule, a varying rate of inhibition of CPE, cells survival and virus replication was observed. Compared to 0.5 ug/ml concentration of Renessans syrup, 5 and 50 ug/ml showed comparable results where there was a 100% cell survival, no CPEs and a negligible viral replication (change in CT= 0.11 and 0.13, respectively). This study indicates that

RENESSANS, containing iodine, may have potential activity against SARS-CoV-2 which needs to be further investigated in human clinical trials. [**note: while interesting, there are already some trials going on with iodine nasal lavage and gargles}**
https://www.biorxiv.org/content/10.1101/2020.06.29.171173v1

- We developed primary human lung epithelial infection models to understand responses of proximal and distal lung epithelium to SARS-CoV-2 infection. Differentiated air-liquid interface cultures of proximal airway epithelium and 3D organoid cultures of alveolar epithelium were readily infected by SARS-CoV-2 leading to an epithelial cell-autonomous proinflammatory response. We validated the efficacy of selected candidate COVID-19 drugs confirming that Remdesivir strongly suppressed viral infection/replication. We provide a relevant platform for studying COVID-19 pathobiology and for rapid drug screening against SARS-CoV-2 and future emergent respiratory pathogens. [**note: another cell system for drug discovery**]
https://www.biorxiv.org/content/10.1101/2020.06.29.174623v1

VIRUS BIOCHEMISTRY

- There is a well-known and established link between high lipopolysaccharide (LPS) levels in blood and the metabolic syndrome (MS). MS is a risk factor for developing severe COVID-19 and acute respiratory distress syndrome (ARDS). Here we define an interaction between SARS-CoV-2 Spike (S) protein and LPS and its link to aggravated inflammation in vitro and in vivo. Electrophoresis under native conditions demonstrated that SARS-CoV-2 S protein binds to *Escherichia coli* LPS, forming high molecular weight aggregates. Microscale thermophoresis analysis further defined the interaction, having a K_D of ~47 nM, similar to the observed affinity between LPS and the human receptor CD14. Moreover, S protein, when combined with low levels of LPS, boosted nuclear factor-kappa B (NF-κB) and cytokine responses in monocytic THP-1 cells and human blood, respectively. In an experimental model of localized inflammation, employing NF-κB reporter mice and in vivo bioimaging, S protein in conjunction with LPS significantly increased the inflammatory response when compared with S protein and LPS alone. Apart from providing information on LPS as a ligand for S protein, our results are of relevance for studies on comorbidities involving bacterial endotoxins, such as the MS, or co-existing acute and chronic infections in COVID-19 patients. [**note: an interesting biochemical finding that may have clinical consequences.**] https://www.biorxiv.org/content/10.1101/2020.06.29.175844v1
- Owing to poor understanding of pathogenicity, the virus is eluding treatment and complicating recovery. Regulatory roles of long non-coding RNAs (lncRNAs) during viral infection and associated antagonism of host antiviral immune responses has become more evident in last decade. To elucidate possible functions of lncRNAs in the COVID-19 pathobiology, we have utilized RNA-seq dataset of SARS-CoV-2 infected lung epithelial cells. Results: Our analyses uncover 21 differentially expressed lncRNAs whose functions are broadly involved in cell survival and regulation of gene expression. By network enrichment analysis we find that these lncRNAs can directly interact with differentially expressed protein-coding genes ADAR, EDN1, KYNU, MALL, TLR2 and YWHAG; and also AKAP8L, EXOSC5, GDF15, HECTD1, LARP4B, LARP7, MIPOL1, UPF1, MOV10 and PRKAR2A, host genes that interact with SARS-CoV-2 proteins. These genes are involved in cellular signaling, metabolism, immune response and RNA homeostasis. Since lncRNAs have been known to sponge microRNAs and protect expression of upregulated genes, we also identified 9 microRNAs that are induced in viral infections; however, some lncRNAs are

able to block their usual suppressive effect on overexpressed genes and consequently contribute to host defense and cell survival. Conclusions: Our investigation determines that deregulated lncRNAs in SARS-CoV-2 infection are involved in viral proliferation, cellular survival, and immune response, ultimately determining disease outcome and this information could drive the search for novel RNA therapeutics as a treatment option. [**note: kudos to these Bangladeshi researchers for adding to our knowledge. I believe this is the first paper from that country to make the newsletter. We are all in this together!**]
https://www.biorxiv.org/content/10.1101/2020.06.29.177204v1

- Unprecedented quantities of sequence data have been generated from the newly emergent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causative agent of COVID-19. We document here the presence of s2m, a highly conserved, mobile genetic element with unknown function, in both the SARS-CoV-2 genome and a large number of insect genomes. Although s2m is not universally present among coronaviruses and appears to undergo horizontal transfer, the high sequence conservation and universal presence of s2m among isolates of SARS-CoV-2 indicate that, when present, the element is essential for viral function. [**note: another unusual genetic finding about the SARS-CoV-2 virus.**]
https://www.biorxiv.org/content/10.1101/2020.06.29.177030v1

DIAGNOSTIC DEVELOPMENT

- Rapid point-of-care (PoC) diagnostics to detect the causative virus, SARS-CoV-2, are urgently needed to identify and isolate patients, contain its spread and guide clinical management. In this work, we report the development of a rapid PoC diagnostic test (< 20 min) based on reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) and semiconductor technology for the detection of SARS-CoV-2 from extracted RNA samples. The developed LAMP assay was tested on a real-time benchtop instrument (RT-qLAMP) showing a lower limit of detection of 10 RNA copies per reaction. It was validated against 183 clinical samples including 127 positive samples (screened by the CDC RT-qPCR assay). Results showed 90.55% sensitivity and 100% specificity when compared to RT-qPCR and average positive detection times of 15.45 ± 4.43 min. For validating the incorporation of the RT-LAMP assay onto our PoC platform (RT-eLAMP), a subset of samples was tested (n=40), showing average detection times of 12.89 ± 2.59 min for positive samples (n=34), *demonstrating a comparable performance to a benchtop commercial instrument. Paired with a smartphone for results visualization and geo-localization, this portable diagnostic platform with secure cloud connectivity will enable real-time case identification and epidemiological surveillance.* [**note: another quick PCR test platform**]
https://www.medrxiv.org/content/10.1101/2020.06.29.20142349v1
- As the United States prepares to return to work and open up the economy in the midst of the COVID-19 pandemic without an available vaccine or effective therapy, testing and contact tracing are essential to contain and limit the spread of the COVID-19 virus. In response to the urgent public health need for accurate, effective, low-cost, and scalable COVID-19 testing technology, we evaluated and identified diagnostic solutions with potential for use as an at-home product. We conducted a deep horizon scan for antigen and serology-based diagnostics and down-selected to the most promising technologies. A total of 303 candidate products (138 antibody and 44 antigen tests) were identified. Product evaluations were based entirely on company-provided data. 73 serology-based antibody tests passing an initial scoring algorithm

based on specificity and sensitivity data were then further evaluated using a second scoring algorithm. This second algorithm included a review of additional technical specifications of the devices, an analysis of supply chain, manufacturing, and distribution capacity of each vendor. 24 potential antibody products met the selection criteria for further direct laboratory evaluation. The performance metrics for selection of these 24 products are currently being evaluated in a Mass General Brigham laboratory. Testing alone might not be sufficient to prevent the spread of a highly contagious disease like COVID-19. Manual contact tracing could complement testing, but it is likely to fail in identifying many individuals who were in contact with a given COVID patient. The proliferation of smartphones in the population has enabled the development of solutions that can provide public health officials with valuable information for rapid and accurate contact tracing. Besides, electronic-based contact tracing solutions can be augmented by symptom self-reports gathered using electronic patient reported outcome (ePRO) platforms and by physiological data collected using wearable sensors. We performed a detailed assessment of 12 ePRO solutions, 27 wearable sensors, and 44 electronic-based contact tracing solutions. These technologies were evaluated using criteria developed to assess their suitability to address the COVID-19 pandemic. We identified a number of solutions that could augment if not provide a more effective alternative to manual contact tracing. Finally, we propose a theoretical framework in which ePRO platforms, wearable sensors, and electronic-based contact tracing solutions would be utilized in combination with molecular and serological tests to identify and isolate COVID-19 cases rapidly. [**note: this is a useful compendium of information to scan. It gives some idea of what types of technologies can be employed to augment track and trace. Of course most of this should have already been done by now and put into the field.**] https://www.medrxiv.org/content/10.1101/2020.06.25.20140236v1

>>

2020-07-01

Let's continue with Motown. Smokey Robinson and the Miracles were another one of the staples in the Motown hit parade. One of their great songs was 'Tracks of My Tears.' While Smokey was great with the song, I'm choosing the phenomenal cover by the phenomenal (why have I not had her in the newsletter?) Linda Rondstadt: https://www.youtube.com/watch?v=OYLSvXYp_5U Rondstadt wowed a lot of us back in the mid-1960 with The Stone Poneys where she was the featured vocalist on 'Different Drum': https://www.youtube.com/watch?v=w9qsDgA1q8Y (I had an upstairs neighbor in college who played this so many times that the grooves on the record wore out) and there is this bizarre live version with a pick up band that seems to be playing something totally different: https://www.youtube.com/watch?v=TGZznJXY1Xc it's from a NYC night club as per the comment by Alan White. Rondstadt was a great rock and roller as well with this version of Back in the USA accompanied by Chuck Berry, Keith Richard and George Harrison!!!: https://www.youtube.com/watch?v=u7MwyZnMdYY Showing her versatility, Rondstadt did a run in 'The Pirates of Penzance' first in Central Park and then on Broadway: https://www.youtube.com/watch?v=RfjAJn4SC-w I'll have to stop here before this departs from COVID-19 and becomes a Linda Ronstadt fan blog!

Here is a vaccine update from Derek Lowe.

Goldman Sachs weighs in on masks! A national face mask mandate could potentially substitute for lockdowns that would otherwise subtract nearly 5% from GDP. The Guardian also has a good article on masks. I do believe I look better in a mask than Tony Fauci. Perhaps I can be the poster boy/man for the mask industry!

The large global HCQ prevention trial run by Oxford is resuming after a review by British regulators. The lead investigator noted that HCQ might still prevent infections and this needs to be determined by a randomized trial.

The Lancet has a nice reflection piece on the experiences of social distancing. This STAT piece asks the question whether there is a middle ground for containing COVID-19.

FDA takes action to help COVID-19 vaccine development. I need to finish my vaccine registry paper and get it out!! I think there are opportunities here that need to be taken to look at vaccination, safety, efficacy, and antibody response. This makes a good case for a BIG DATA project.

This preprint goes above the fold (no pun intended as you will see). "The SARS-CoV-2/COVID-19 pandemic continues to threaten global health and socioeconomic stability. Experiments have revealed snapshots of many of the viral components but remain blind to moving parts of these molecular machines. To capture these essential processes, over a million citizen scientists have banded together through the Folding@home distributed computing project to create the worlds first Exascale computer and simulate protein dynamics. An unprecedented 0.1 seconds of simulation of the viral proteome reveal how the spike complex uses conformational masking to evade an immune response, conformational changes implicated in the function of other viral proteins, and "cryptic" pockets that are absent in experimental snapshots. These structures and mechanistic insights present new targets for the design of therapeutics. This living document will be updated as we perform further analysis and make the data publicly accessible." https://www.biorxiv.org/content/10.1101/2020.06.27.175430v1 **Note: see https://foldingathome.org/ for more information. I donated computer time to a folding project several years ago and will look at this one to see how I can help! Remember, we are all in this together and even the humblest of citizens can help out here even if it's just a small monetary donation. Citizen science in action.**

Now that all of you have finished listening to your Linda Ronstadt play lists on YouTube you can begin reading! There is a lot of stuff to digest today!!!! Do go all the way down and read the cool DIY paper in the diagnostics section.

MODELING

- To date, no study has examined the effectiveness of social distancing, while controlling for social mobility and social distancing restrictions in the United States. We utilize the quasi-experimental setting created by the nationwide protests precipitated by George Floyd's tragic death on May 25, 2020, to assess the causal impact of social distancing on the spread of SARS-CoV-2. Methods: Our sample period spans from January 22, 2020, to June 20, 2020, and consists of 474,422 county-days representing 3,142 counties from all 50 states and the District of Columbia. To assess the change in COVID-19 case counts following the protests, we employ a differences in differences estimation strategy in a multivariate setting, in which we control for social distancing

restrictions and social mobility across counties. We also control for covariates that may influence COVID-19 transmission, and implement placebo tests using a Monte Carlo simulation. Findings: We document a country wide increase of over 3.06 cases per day, per 100,000 population, following the onset of the protests (95%CI: 2.47-3.65), and a further increase of 1.73 cases per day, per 100,000 population, in the counties in which the protests took place (95%CI: 0.59- 2.87). Relative to the week preceding the onset of the protests, this represents a 61.2% country wide increase in COVID-19 cases, and a further 34.6% increase in the protest counties. Interpretation: Our study documents a significant increase in COVID-19 case counts in counties that experienced a protest, and we conclude that social distancing practices causally impact the spread of SARS-CoV-2. The observed effect cannot be explained by changes in social distancing restrictions and social mobility, and placebo tests rule out the possibility that this finding is attributable to chance. [**note: it is with some trepidation that I post this preprint. There has been controversy regarding the recent US protests and whether there was an impact on increased SARS-CoV-2 infections. This is the first study I have seen that tries to tease this out.**] https://www.medrxiv.org/content/10.1101/2020.06.29.20143131v1

- As a pandemic of coronavirus spreads across the globe, people debate policies to mitigate its severity. Many complex, highly detailed models have been developed to help policy setters make better decisions. However, the basis of these models is unlikely to be understood by non-experts. We describe the advantages of simple models for covid-19. We say a model is simple if its only parameter is the rate of contact between people in the population. This contact rate can vary over time, depending on choices by policy setters. Such models can be understood by a broad audience, and thus can be helpful in explaining the policy decisions to the public. They can be used to evaluate the outcomes of different policy strategies. However, simple models have a disadvantage when dealing with inhomogeneous populations. To augment the power of a simple model to evaluate complicated situations, we add what we call satellite equations that do not change the original model. For example, with the help of a satellite equation, one could know what his/her chance is of remaining uninfected through the end of epidemic. Satellite equations can model the effect of the epidemic on high-risk individuals, or death rates, or on nursing homes, and other isolated populations. To compare simple models with complex models, we introduce our slightly complex Model J. We find the conclusions of simple and complex models can be quite similar. But, for each added complexity, a modeler may have to choose additional parameter values describing who will infect whom under what conditions, choices for which there is often little rationale but that can have a big impact on predictions. Our simulations suggest that the added complexity offers little predictive advantage. [**note: as I have often noted, catch modeling titles warrant highlighting and "The advantages of the simplest pandemic models" certainly fits this to a 'T'. I am always fond of concepts fitting Occam's Razor.**] https://www.medrxiv.org/content/10.1101/2020.06.23.20132522v1

NEWLY REGISTERED CLINICAL TRIALS

- This multi-center, open, randomized study will evaluate the efficacy and safety of BDB-001 injection in severe COVID-19 with severe pneumonia, or acute lung injury/acute respiratory distress syndrome. Patients will be randomized to two treatment arms (Arm A: Conventional treatment + BDB-001; Arm B: Conventional treatment alone). [**note: I have no idea what this**

drug is as there is little information on the NIH trial site. The company is Stadson Biopharmaceuticals, a Chinese company.] NCT04449588

- The study is a prospective, randomized, placebo-controlled, single-blind phase 2 clinical study of the efficacy and safety of CERC-002, a potent inhibitor of LIGHT, for the treatment of patients with COVID-19 pneumonia who have mild to moderate ARDS. LIGHT is a cytokine in the TNF super family (TNFSF14) which drives inflammation and induces many other cytokines including IL-1, IL-6 and GM-CSF. LIGHT levels have been shown to be elevated in COVID-19 infected patients and inhibiting LIGHT is hypothesized to ameliorate the cytokine storm which has shown to be a major factor in progression of ARDS. [**note: trial sponsored by Cerecor.]** NCT04412057
- The purpose of this study is to evaluate the safety and effectiveness of NasoVAX in preventing worsening of symptoms and hospitalization in patients with early COVID-19. [**note: this is a nasal flu vaccine developed by Altimmune.**] NCT04442230
- A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study Assessing the Efficacy and Safety of Anti-Spike SARS-CoV-2 Monoclonal Antibodies in Preventing SARS-CoV-2 Infection in Household Contacts of Individuals Infected With SARS-CoV-2 [**note: this is another Regeneron mAb study, this time looking to see if it can be used for protection. I conjectured this might be an application several months ago.**] NCT04452318

- Since the 1960s, studies have shown that oral polio vaccine (OPV) may have beneficial non-specific effects, reducing morbidity and mortality from other infections than polio. Such beneficial non-specific effect have been observed for other live vaccines, including measles, smallpox and BCG vaccine. For BCG, the vaccine for which the mechanism has been studied the most, the effects appear to be mediated through the innate immune system. The COVID-19 pandemic caused by the novel coronavirus SARS-CoV-2 has now caused over 7.1 million cases and >400,000 deaths worldwide. As everywhere else, it is anticipated that in Africa the older part of the population will be at risk of severe COVID-19. OPV is widely used in Africa, but for children. Both polio and coronavirus are positive-strand RNA viruses, therefore it is likely that they may induce and be affected by common innate immune mechanisms. In a randomised trial at the Bandim Health Project in Guinea-Bissau, we will assess the effect of providing OPV vs no vaccine to 3400 persons above 50 years of age. The trial will have the power to test the hypothesis that OPV reduces the combined risk of morbidity admission or death (composite outcome) by at least 28% over the subsequent 6 months. [**note: there was discussion last week about using the Sabin oral polio vaccine as an immune booster. Here is an African trial that will assess this.**] NCT04445428

CLINICAL TRIAL RESULTS

- More data are in from the large UK RECOVERY trial. The anti-viral combination of lopinavir/ritonavir showed no clinical benefit in hospitalized patients. 1596 patients were randomized on treatment compared with 3376 that received SOC. Earlier data from China also showed no efficacy.
- To the best of our knowledge, our study is the first to provide biochemical evidence that endotheliopathy is an important feature in the coagulopathy of COVID-19. Although other studies have shown elevated von Willebrand factor (VWF) in critically ill patients with COVID-19, this is the first to measure VWF in both critically ill and non-critically ill patients, along with

several other previously unreported endothelial markers, including soluble P-selectin and soluble thrombomodulin. We show that endotheliopathy is widespread among hospitalised patients with COVID-19 and is more extensive in critically ill patients than in non-critically ill patients. We describe, for the first time, that soluble thrombomodulin concentrations might predict mortality and other clinical outcomes in patients with COVID-19. This finding requires further validation. Our study provides convincing biochemical evidence for endothelial involvement in COVID-19-associated coagulopathy and critical illness. Our preliminary findings identify a potential prognostic role for measurement of endothelial markers in patients with COVID-19 and suggest a need for future investigations of therapeutic strategies aimed at preserving endothelial function in COVID-19 and other related infectious processes. [**note: this is from Yale researchers and shows the relationship between biochemical markers and coagulation problems of severe COVID-19. Commentary on the study is HERE.**] https://www.thelancet.com/journals/lanhae/article/PIIS2352-3026(20)30216-7/fulltext

- We analyzed data from 92,664 clinically and molecularly confirmed Covid-19 cases in Brazil to understand the potential associations between influenza vaccination and Covid-19 outcomes. Controlling for health facility of treatment, comorbidities as well as an extensive range of sociodemographic factors, we show that patients who received a recent influenza vaccine experienced on average 8% lower odds of needing intensive care treatment (95% CIs [0.86, 0.99]), 18% lower odds of requiring invasive respiratory support (0.74, 0.88) and 17% lower odds of death (0.75, 0.89). Large scale promotion of influenza vaccines seems advisable, especially in populations at high risk of severe SARS-CoV-2 infection. [**note: from hard hit Brazil, more observational data on the protective nature of influenza vaccine. I would like to see data on other vaccines as well. It would also be good to see if there is a time dependency of vaccination here. Perhaps this effect is only observed in recently vaccinated individuals where residual antibody response is still fresh.**] https://www.medrxiv.org/content/10.1101/2020.06.29.20142505v1
- We administered tocilizumab into 13 severe-to-critically ill patients with coronavirus disease 2019 (COVID-19) for compassionate use in combination with potential anti-viral agents in those who required an oxygen supply and showed increased laboratory inflammatory markers such as C-reactive protein (CRP) and ferritin. One injection of tocilizumab led to rapid improvements in clinical features, inflammatory findings, and oxygen supply in seven patients with severe COVID-19 and substantial amelioration in two patients who were critically ill, whereas four patients, who exhibited rapidly worsened respiratory function, required artificial ventilatory support even after tocilizumab treatment. Three of these four patients ultimately recovered from deterioration after methylprednisolone treatment. Administration of tocilizumab did not affect viral elimination nor IgG production specific for the virus. Compared with well-responding patients, rapidly-worsened patients showed a significantly higher ratio of ferritin vs. CRP. These findings suggest that tocilizumab has beneficial effects in severe-to-critically ill patients with COVID-19; however, in some cases, addition of methylprednisolone is required for disease rescue. [**note: very small number of patients. I remain curious why a DSMB has not reported out preliminary results from some of the ongoing clinical trials on tocilizumab. Is the data not robust enough?**] https://www.medrxiv.org/content/10.1101/2020.06.24.20134288v1

DRUG DEVELOPMENT

- There is some data that Vitamin D may have protective effect, so authors decided to analyze European country-wide data to determine if Vitamin D levels are associated with COVID-19 population death rate. Methods: To retrieve the Vitamin D levels data, authors analyzed the Vitamin D European population data compiled by 2019 ECTS Statement on Vitamin D Status published in the European Journal of Endocrinology. For the data set to used for analysis, only recently published data, that included general adult population of both genders ages 40-65 or wider, and must have included the prevalence of Vitamin D deficiency. Results: There were 10 countries data sets that fit the criteria and were analyzed. Severe Vitamin D deficiency was defined as 25(OH)D less than 25 nmol/L (10 ng/dL). Pearson correlation analysis between death rate per million from COVID-19 and prevalence of severe Vitamin D deficiency shows a strong correlation with r = 0.76, p = 0.01, indicating significant correlation. Correlation remained significant, even after adjusting for age structure of the population. Additionally, over time, correlation strengthened, and r coefficient asymptoticaly increased. Conclusions: Authors recommend universal screening for Vitamin D deficiency, and further investigation of Vitamin D supplementation in randomized control studies, which may lead to possible treatment or prevention of COVID-19. [**note: I never know which category is right for preprints such as this one. This provides evidence about severe Vitamin D deficiency and COVID-19 severity.**] https://www.medrxiv.org/content/10.1101/2020.06.24.20138644v1
- COVID-19 is undoubtedly the most impactful viral disease of the current century, afflicting millions worldwide. As yet, there is not an approved vaccine, as well as limited options from existing drugs for treating this disease. We hypothesized that combining drugs with independent mechanisms of action could result in synergy against SARS-CoV-2. Using in silico approaches, we prioritized 73 combinations of 32 drugs with potential activity against SARS-CoV-2 and then tested them in vitro. Overall, we identified 16 synergistic and 8 antagonistic combinations, 4 of which were both synergistic and antagonistic in a dose-dependent manner. Among the 16 synergistic cases, combinations of nitazoxanide with three other compounds (remdesivir, amodiaquine and umifenovir) were the most notable, all exhibiting significant synergy against SARS-CoV-2. The combination of nitazoxanide, an FDA-approved drug, and remdesivir, FDA emergency use authorization for the treatment of COVID-19, demonstrate a strong synergistic interaction. Notably, the combination of remdesivir and hydroxychloroquine demonstrated strong antagonism. Overall, our results emphasize the importance of both drug repurposing and preclinical testing of drug combinations for potential therapeutic use against SARS-CoV-2 infections. [**note: interesting paper on drug development and the information on HCQ and remdesivir may be important. It is interesting that umifenovir (arbidol) shows up as synergistic. There are some trials, largely outside the US going on with this drug.**] https://www.biorxiv.org/content/10.1101/2020.06.29.178889v1
- The coronavirus SARS-CoV-2 causing the COVID-19 pandemic uses −1 programmed ribosomal frameshifting (−1 PRF) to control the expression levels of key viral proteins. Because modulating −1 PRF can attenuate viral propagation, ligands binding to the viral RNA pseudoknot that stimulates −1 PRF may prove useful as therapeutics. Mutations in the pseudoknot have been observed over the course of the pandemic, but how they affect −1 PRF and the activity of inhibitors is unknown. Cataloguing natural mutations in all parts of the SARS-CoV-2 pseudoknot, we studied a panel of 6 mutations in key structural regions. Most mutations left the −1 PRF efficiency unchanged, even when base-pairing was disrupted, but one led to a remarkable three-

fold decrease, suggesting that SARS-CoV-2 propagation may be less sensitive to modulation of −1 PRF efficiency than some other viruses. Examining the effects of one of the few small-molecule ligands known to suppress −1 PRF significantly in SARS-CoV, we found that it did so by similar amounts in all SARS-CoV-2 mutants tested, regardless of the basal −1 PRF efficiency, indicating that the activity of anti-frameshifting ligands can be resistant to natural pseudoknot mutations. These results have important implications for therapeutic strategies targeting SARS-CoV-2 through modulation of −1 PRF. [**note: this is an interesting drug target but it may require a lot of work to figure out a good drug that works at realistic concentrations.**] https://www.biorxiv.org/content/10.1101/2020.06.29.178707v1

- Drug repurposing is the only method capable of delivering treatments on the shortened time-scale required for patients afflicted with lung disease arising from SARS-CoV-2 infection. Mucin-1 (MUC1), a membrane-bound molecule expressed on the apical surfaces of most mucosal epithelial cells, is a biochemical marker whose elevated levels predict the development of acute lung injury (ALI) and respiratory distress syndrome (ARDS), and correlate with poor clinical outcomes. In response to the pandemic spread of SARS-CoV-2, we took advantage of a high content screen of 3,713 compounds at different stages of clinical development to identify FDA-approved compounds that reduce MUC1 protein abundance. Our screen identified Fostamatinib (R788), an inhibitor of spleen tyrosine kinase (SYK) approved for the treatment of chronic immune thrombocytopenia, as a repurposing candidate for the treatment of ALI. In vivo, Fostamatinib reduced MUC1 abundance in lung epithelial cells in a mouse model of ALI. In vitro, SYK inhibition by Fostamatinib promoted MUC1 removal from the cell surface. Our work reveals Fostamatinib as a repurposing drug candidate for ALI and provides the rationale for rapidly standing up clinical trials to test Fostamatinib efficacy in patients with COVID-19 lung injury. [**note: another drug candidate for clinical trials.**] https://www.biorxiv.org/content/10.1101/2020.06.30.180380v1
- The COVID-19 pandemic continues to spread throughout the world with an urgent need for a safe and protective vaccine to effectuate herd immunity to control the spread of SARS-CoV-2. Here, we report the development of a SARS-CoV-2 subunit vaccine (NVX-CoV2373) produced from the full-length spike (S) protein, stabilized in the prefusion conformation. Purified NVX-CoV2373 S form 27.2nm nanoparticles that are thermostable and bind with high affinity to the human angiotensin-converting enzyme 2 (hACE2) receptor. In mice and baboons, low-dose NVX-CoV2373 with saponin-based Matrix-M adjuvant elicits high titer anti-S IgG that is associated with blockade of hACE2 receptor binding, virus neutralization, and protection against SARS-CoV-2 challenge in mice with no evidence of vaccine-associated enhanced respiratory disease (VAERD). NVX-CoV2373 vaccine also elicits multifunctional CD4 and CD8 T cells, CD4 T follicular helper T cells (Tfh), and the generation of antigen-specific germinal center (GC) B cells in the spleen. These results support the ongoing phase 1/2 clinical evaluation of the safety and immunogenicity of NVX-CoV2327 with Matrix-M (NCT04368988). [**note: this is the Novavax vaccine that is in trials.**] https://www.biorxiv.org/content/10.1101/2020.06.29.178509v1
- The COVID-19 pandemic caused by SARS-CoV-2 has escalated into a global crisis. The spike (S) protein that mediates cell entry and membrane fusion is the current focus of vaccine and therapeutic antibody development efforts. The S protein, like many other viral fusion proteins such as HIV-1 envelope (Env) and influenza hemagglutinin, is glycosylated with both complex and high mannose glycans. Here we demonstrate binding to the SARS-CoV-2 S protein by a

category of Fab-dimerized glycan-reactive (FDG) HIV-1-induced broadly neutralizing antibodies (bnAbs). A 3.1 Å resolution cryo-EM structure of the S protein ectodomain bound to glycan-dependent HIV-1 bnAb 2G12 revealed a quaternary glycan epitope on the spike S2 domain involving multiple protomers. These data reveal a new epitope on the SARS-CoV-2 spike that can be targeted for vaccine design. [**note: there appears to be no shortage of epitopes for vaccine development and here is another one.**]
https://www.biorxiv.org/content/10.1101/2020.06.30.178897v1

- Vaccination efficacy is enhanced by targeting the antigen-presenting cell compartment. Here, we show that S1-Fc antigen delivery targeting the FcgammaR+ antigen-presenting cell compartment elicits anti-SARS-CoV-2 S1-antigen specific IgG production in vivo exerting biologically functional and protective activity against live virus infection, assessed in a stringent experimental virus challenge assay in vitro. The S1-domain of the SARS-CoV-2 spike protein was genetically fused to a human immunoglobulin Fc moiety, which contributes to mediate S1-Fc cellular internalization by FcgammaR+ antigen-presenting cells. Immediately upon administration intramuscularly, our novel vaccine candidate recombinant rS1-Fc homes to lymph nodes in vivo where FcgammaR+ antigen-presenting cells reside. Seroconversion is achieved as early as day 7, mounting considerably increased levels of anti-S1 IgGs in vivo. Interestingly, immunization at elevated doses with non-expiring S1-Fc encoding dsDNA favors the education of a desired antigen-specific adaptive T cell response. However, low-dose immunization, safeguarding patient safety, using recombinant rS1-Fc, elicits a considerably elevated protection amplitude against live SARS-CoV-2 infection. Our promising findings on rS1-Fc protein immunization prompted us to further develop an affordable and safe product for delivery to our communities in need for COVID-19 vaccinations. [**note: another vaccine approach!!!**] https://www.biorxiv.org/content/10.1101/2020.06.29.178616v1

VIRUS BIOCHEMISTRY

- The trimeric spike (S) glycoprotein interacts with its receptor human ACE2 to mediate viral entry into host-cells. Here we present cryo-EM structures of an uncharacterized tightly closed SARS-CoV-2 S-trimer and the ACE2-bound-S-trimer at 2.7-angstrom and 3.8-angstrom-resolution, respectively. The tightly closed S-trimer with inactivated fusion peptide may represent the ground prefusion state. ACE2 binding to the up receptor-binding domain (RBD) within S-trimer triggers continuous swing-motions of ACE2-RBD, resulting in conformational dynamics of S1 subunits. Noteworthy, SARS-CoV-2 S-trimer appears much more sensitive to ACE2-receptor than SARS-CoV S-trimer in terms of receptor-triggered transformation from the closed prefusion state to the fusion-prone open state, potentially contributing to the superior infectivity of SARS-CoV-2. We defined the RBD T470-T478 loop and residue Y505 as viral determinants for specific recognition of SARS-CoV-2 RBD by ACE2, and provided structural basis of the spike D614G-mutation induced enhanced infectivity. Our findings offer a thorough picture on the mechanism of ACE2-induced conformational transitions of S-trimer from ground prefusion state towards postfusion state, thereby providing important information for development of vaccines and therapeutics aimed to block receptor binding. [**note: more information on the Spike protein.**]
https://www.biorxiv.org/content/10.1101/2020.06.30.177097v1
- The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein mediates infection of cells expressing angiotensin-converting enzyme 2 (ACE2). ACE2 is also the viral

receptor of SARS-CoV (SARS-CoV-1), a related coronavirus that emerged in 2002-2003. Horseshoe bats (genus Rhinolophus) are presumed to be the original reservoir of both viruses, and a SARS-like coronavirus, RaTG13, closely related SARS-CoV-2, has been isolated from one horseshoe-bat species. Here we characterize the ability of S-protein receptor-binding domains (RBDs) of SARS-CoV-1, SARS-CoV-2, and RaTG13 to bind a range of ACE2 orthologs. We observed that the SARS-CoV-2 RBD bound human, pangolin, and horseshoe bat (R. macrotis) ACE2 more efficiently than the SARS-CoV-1 or RaTG13 RBD. Only the RaTG13 RBD bound rodent ACE2 orthologs efficiently. Five mutations drawn from ACE2 orthologs of nine Rhinolophus species enhanced human ACE2 binding to the SARS-CoV-2 RBD and neutralization of SARS-CoV-2 by an immunoadhesin form of human ACE2 (ACE2-Fc). Two of these mutations impaired neutralization of SARS-CoV-1. An ACE2-Fc variant bearing all five mutations neutralized SARS-CoV-2 five-fold more efficiently than human ACE2-Fc*. These data narrow the potential SARS-CoV-2 reservoir, suggest that SARS-CoV-1 and -2 originate from distinct bat species, and identify a more potently neutralizing form of ACE2-Fc*. [**note: mutations that may point to the origin of SARS-CoV-2 and perhaps an alternative therapy.**]
https://www.biorxiv.org/content/10.1101/2020.06.29.178459v1 and if you are really interested, here is a transcriptional atlas of the Chinese horseshoe bat that is the presumptive reservoir for this class of viruses: https://www.biorxiv.org/content/10.1101/2020.06.30.175778v1

- Research efforts of the ongoing SARS-CoV-2 pandemic have focused on viral genome sequence analysis to understand how the virus spread across the globe. Here, we assess three recently identified SARS-CoV-2 genomes in Beijing from June 2020 and attempt to determine the origin of these genomes, made available in the GISAID database. The database contains fully or partially sequenced SARS-CoV-2 samples from laboratories around the world. Including the three new samples and excluding samples with missing annotations, we analyzed 7,643 SARS-CoV-2 genomes. Using principal component analysis computed on a similarity matrix that compares all pairs of the SARS-CoV-2 nucleotide sequences at all loci simultaneously, using the Jaccard index, we find that the newly discovered virus genomes from Beijing are in a genetic cluster that consists mostly of cases from Europe and South(east) Asia. The sequences of the new cases are most related to virus genomes from a small number of cases from China (March 2020), cases from Europe (February to early May 2020), and cases from South(east) Asia (May to June 2020). *These findings could suggest that the original cases of this genetic cluster originated from China in March 2020 and were re-introduced to China by transmissions from samples from South(east) Asia between April and June 2020*. [**note: Louis Pasteur and Robert Koch would have a field day with all this new technology were they alive today. This is interesting genetic stuff on the recent Beijing infection cluster in China.**]
https://www.biorxiv.org/content/10.1101/2020.06.22.165936v1

DIAGNOSTIC DEVELOPMENT

- The loop-mediated isothermal amplification (LAMP) assay has proven to be a reliable and simple protocol that can detect small amounts of viral RNA in patient samples (<10 genomes per µL) (Nagamine, Hase, and Notomi 2002) Recently, Rabe and Cepko at Harvard published a sensitive and simple protocol for COVID-19 RNA detection in saliva using an optimized LAMP assay (Rabe and Cepko, 2020). This LAMP protocol has the benefits of being simple, requiring no specialized equipment; rapid, requiring less than an hour from sample collection to readout; and

cheap, costing around $1 per reaction using commercial reagents. The pH based colorimetric readout also leaves little ambiguity and is intuitive. However, a shortfall in many nucleic acid-based methods for detection in saliva samples has been the variability in output due to the presence of inhibitory substances in saliva. Centrifugation to separate the reaction inhibitors from inactivated sample was shown to be an effective way to ensure reliable LAMP amplification. However, a centrifuge capable of safely achieving the necessary speeds of 2000 RPM for several minutes often costs hundreds of dollars and requires a power supply. We present here an open hardware solution- Handyfuge - that can be assembled with readily available components for the cost of <5 dollars a unit and could be used together with the LAMP assay for point of care detection of COVID-19 RNA from saliva. The device is then validated using the LAMP protocol from Rabe and Cepko. With the use of insulated coolers for reagent supply chain and delivery, the assay presented can be completed without the need for electricity or any laboratory scale infrastructure. [**note: THIS IS WAY COOL DIY TECHNOLOGY AND CHEAP. If you read one paper this week, let it be this one if just for the diagrams. Props to these Stanford engineers for total out of the box thinking!!!! They deserve a prize!**]
https://www.medrxiv.org/content/10.1101/2020.06.30.20143255v1

- a surge in diagnostic testing quickly resulted in worldwide competition for the same sample preparation and real-time RT-PCR diagnostic reagents (rRT-PCR). Consequently, Hampshire Hospitals NHS Foundation Trust, UK sought to diversify their diagnostic portfolio by exploring alternative amplification chemistries including those that permit direct testing without RNA extraction. This study describes the validation of a SARS-CoV-2 RT-LAMP assay, which is an isothermal, autocycling, strand displacement nucleic acid amplification technique which can be performed on extracted RNA, (RNA RT-LAMP) or directly from swab (Direct RT-LAMP). Analytical specificity (ASp) of this new RT-LAMP assay was 100% and analytical sensitivity (ASe) was between 1x101 and 1x102 copies when using a synthetic DNA target. The overall diagnostic sensitivity (DSe) and specificity (DSp) of RNA RT LAMP was 97% and 99% respectively, relative to the standard of care (SoC) rRT-PCR. When a CT cut-off of 33 was employed, above which increasingly, evidence suggests there is a very low risk of patients shedding infectious virus, the diagnostic sensitivity was 100%. The DSe and DSp of Direct-RT LAMP was 67% and 97%, respectively. When setting CT cut-offs of <33 and <25, the DSe increased to 75% and 100%, respectively. Time from swab-to-result for a strong positive sample (CT < 25) was < 15 minutes. We propose that RNA RT-LAMP could replace rRT-PCR where there is a need for increase in throughput, whereas Direct RT-LAMP could be used as a screening tool for triaging patients into appropriate hospitals wards, at GP surgeries and in care homes, or for population screening to identify highly contagious individuals (super shedders). Direct RT-LAMP could also be used during times of high prevalence to save critical extraction and rRT-PCR reagents by screening out those strong positives from diagnostic pipelines. [**note: from the UK a proposal for using high throughput RT-LAMP for triaging.**]
https://www.medrxiv.org/content/10.1101/2020.06.30.20142935v1
- The ongoing pandemic of SARS-CoV-2 calls for rapid and cost-effective methods to accurately identify infected individuals. The vast majority of patient samples is assessed for viral RNA presence by RT-qPCR. Our biomedical research institute, in collaboration between partner hospitals and an accredited clinical diagnostic laboratory, established a diagnostic testing pipeline that has reported on more than 40,000 RT-qPCR results since its commencement at the

beginning of April 2020. However, due to ongoing demand and competition for critical resources, alternative testing strategies were sought. In this work, we present a clinically-validated standard operating procedure (SOP) for *high-throughput SARS- CoV-2 detection by RT-LAMP in 25 minutes that is robust, reliable, repeatable, sensitive, specific, and inexpensive*. [**note: good validation study for high throughput use of RT-LAMP**] https://www.medrxiv.org/content/10.1101/2020.06.29.20142430v1

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

2020-07-02

The Band were one of the iconic groups of the 1965-75 era. They produced a lot of influential work and their tours with Bob Dylan were legendary. I was fortunate enough to see one of the performances on the final tour with Dylan when I was in grad school at IU. The Band's final concert was recorded by the great American filmmaker, Martin Scorsese and released as 'The Last Waltz.' It is still a staple on cable television. Here is one of their iconic songs, 'The Weight': https://www.youtube.com/watch?v=q-w9OclUnns it is from the Scorsese film and backed up by the Staple Singers. Let's give you a Thursday twofer with this all-star version sung in memory of Levon Helm's passing: https://www.youtube.com/watch?v=BlgDC6mOU0o

The Washington Post has a good article on how autopsies are helping to decipher COVID-19. Here is a classic cautionary tale of best intentions gone awry.

The New York Times offers a story on whether human challenge trials of a COVID-19 vaccine are warranted. Pooled sample COVID-19 testing seems to be a good way to go for moderate sized groups. There is an interesting Upshot column in the Times on how natural experiments can assist physicians as they wait for randomized trials to be completed.

The **biggest news** from yesterday is the Pfizer announcement of preliminary results from their COVID-19 vaccine trials. Robust antibody generation was observed in vaccinated individuals and the company is preparing for a larger trial. The major side effect was fever, chills and other symptoms following the second shot of an intermediate dose. This is similar to effects from the GSK shingles vaccine as many of have experienced. Here is the preprint of the interim report on the vaccine.

Medscape has a story on dermatological signs accompanying COVID-19.

JAMA has a paper that estimates the excess deaths associated with the COVID-19 pandemic in the US. An associated commentary is HERE. There is also a thoughtful piece on reopening schools based on other country's experiences. Finally, in the category of 'number of angels dancing on the head of a pin' category, a commentary on whether "cytokine storm" is relevant to COVID-19 (maybe it is worthwhile to debate the usefulness of the term but since it is so widely used in the COVID-19 literature, it is now the lingua franca for symptom categorization).

STAT has an interesting article on whether smell tests might help for routine screening for SARS-CoV-2 infection. I can see immediate riches coming my way as TSA will need high volume amounts of my COVID-19 Scent Strip™. I may have to put the newsletter on hiatus to gear up manufacturing!!! There is a second article on whether asthma increases COVID-19 risk. The answer appears to be no and I've linked to some past preprints on this topic.

MODELING

- We analyzed the daily incidence of newly reported COVID-19 cases among adults aged 20-39 years, 40-59 years, and 60 or more years in the sixteen most populous counties of the state of Florida from March 1 through June 27, 2020. In all 16 counties, an increase in reported COVID-19 case incidence was observed in all three age groups soon after the governor-ordered Full Phase 1 reopening went into effect. Trends in testing, hospitalization and mortality do not support the hypothesis that the observed increase in case incidence was merely the result of liberalization of testing criteria. Parameter estimates from a parsimonious two-group heterogeneous SIR model strongly support the hypothesis that younger persons, having first acquired their infections through increasing social contact with their peers, then transmitted their infections to older, less socially mobile individuals. [**note: an obvious finding but someone had to do this type of study.**]
  https://www.medrxiv.org/content/10.1101/2020.06.30.20143842v1
- Background: Nations are imposing unprecedented measures at large-scale to contain the spread of COVID-19 pandemic. Recent studies indicate that measures such as lockdowns may have slowed down the growth of COVID-19. However, in addition to substantial economic and social costs, these measures also limit the exposure to Ultraviolet-B radiation (UVB). Emerging observational evidence indicate the protective role of UVB and vitamin D in reducing the severity and mortality of COVID-19 deaths. In this observational study, we empirically outline the independent protective roles of lockdown and UVB exposure as measured by ultraviolet index (UVI), whilst also examining whether the severity of lockdown is associated with a reduction in the protective role. Methods. We apply a log-linear fixed-effects model to a panel dataset of 162 countries over a period of 108 days (n=6049). We use the cumulative number of COVID-19 deaths as the dependent variable and isolate the mitigating influence of lockdown severity on the association between UVI and growth-rates of COVID-19 deaths from time-constant country-specific and time-varying country-specific potentially confounding factors. Findings: After controlling for time-constant and time-varying factors, we find that a unit increase in UVI and lockdown severity are independently associated with 17% [-1.8 percentage points] and 77% [-7.9 percentage points] decline in COVID-19 deaths growth rate, indicating their respective protective roles. However, the widely utilized and least severe lockdown (recommendation to not leave the house) already fully mitigates the protective role of UVI by 95% [1.8 percentage points] indicating its downside. Interpretation: We find that lockdown severity and UVI are independently associated with a slowdown in the daily growth rates of cumulative COVID-19 deaths. However, we find consistent evidence that increase in lockdown severity is associated with a significant reduction in the protective role of UVI in reducing COVID-19 deaths. Our results suggest that lockdowns in conjunction with adequate exposure to UVB radiation might have provided even more substantial health benefits, than lockdowns alone. For example, we estimate that there would be 21% fewer deaths on average with sufficient UVB exposure while people were recommended not to leave their house. Therefore, our study outlines the importance of considering UVB exposure, especially while implementing lockdowns and may support policy decision making in countries imposing such measures. [**note: it is good to get out of the house, even if for a short walk in the sunlight. Do put on sunscreen or wear a hat to protect your face. Remember to get that yearly (or as in my case twice) dermatology check up.**] https://www.medrxiv.org/content/10.1101/2020.06.30.20143586v1

- After the first case of COVID-19 in Japan on 15 January 2020, multiple nationwide COVID-19 clusters were identified by the end of February. The Japanese government focused on mitigating emerging COVID-19 clusters by conducting active nationwide epidemiological surveillance. However, an increasing number of cases appeared until early April, many with unclear infection routes exhibiting no recent history of travel outside Japan. We aimed to evaluate the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genome sequences from COVID-19 cases until early April and characterise the genealogical networks to demonstrate possible routes of spread in Japan. Methods: Nasopharyngeal specimens were collected from patients and a quantitative reverse transcription polymerase chain reaction testing for SARS-CoV-2 was performed. Positive RNA samples were subjected whole genome sequencing and a haplotype network analysis was performed. Findings: Some of the primary clusters identified during January and February in Japan directly descended from Wuhan-Hu-1-related isolates in China and other distinct clusters. Clusters were almost contained until mid-March; the haplotype network analysis demonstrated that COVID-19 cases from late March through early April may have caused an additional large cluster related to the outbreak in Europe, leading to additional spread within Japan. National self-restraint during February was effective in mitigating the COVID-19 spread, but late action on stopping immigration and declaring national emergency in Japan might be involved in the later increase in cases. Interpretation: Genome surveillance suggested that at least two distinct SARS-CoV-2 introductions from China and other countries occurred. [**note: genomic analyses of SARS-CoV-2 outbreaks in Japan.]**
  https://www.medrxiv.org/content/10.1101/2020.07.01.20143958v1
- there remains little knowledge regarding droplet dissemination during airway management procedures in real life settings. Methods: 12 different airway management procedures were investigated during routine clinical care. A high-speed video camera (1000 frames/second) was for imaging. Quantitative droplet characteristics as size, distance traveled, and velocity were computed. Results: Droplets were detected in 8/12 procedures. The droplet trajectories could be divided into two distinctive patterns (type 1/2). Type 1 represented a ballistic trajectory with higher speed droplets whereas type 2 represented a random trajectory of slower particles that persisted longer in air. Speaking and coughing lead to a larger amount of droplets than non-invasive ventilation therapy. The use of tracheal cannula filters reduced the amount of droplets. Conclusions: Respiratory droplet patterns generated during airway management procedures follow two distinctive trajectories based on the influence of aerodynamic forces. Speaking and coughing produce more droplets than non-invasive ventilation therapy confirming these behaviors as exposure risks. Even large droplets may exhibit patterns resembling the fluid dynamics smaller airborne aerosols that follow the airflow convectively and may place the healthcare provider at risk. [**note: some very good diagrams on droplet trajectory in this paper. It is worth taking a look at the data as well.**]
  https://www.medrxiv.org/content/10.1101/2020.07.01.20144386v1
- The rising COVID-19 pandemic caused many governments to impose policies restricting social interactions. These policies have slowed down the spread of the SARS-CoV-2 virus to the extent that restric- tions can be gradually lifted. Models can be useful to assess the consequences of deconfinement strategies with respect to business, school and leisure activities. Methods. We adapted the individual-based model "STRIDE" to simulate interactions between the 11 million inhabitants of Belgium at the levels of households, workplaces, schools and communities. We

calibrated our model to observed hospital incidence and seroprevalence data. STRIDE can explore contact tracing options and account for repetitive leisure contacts in extended household settings (so called "household bubbles") with varying levels of connectivity. Findings. Household bubbles have the potential to reduce the number of COVID-19 hospital admissions by up to 90%. The effectiveness of contact tracing depends on its timing, as it becomes futile more than 4 days after the index case developed symptoms. Assuming that children have a lower level of susceptibility and lower probability to experience symptomatic SARS-CoV-2 infection, (partial) school closure options have relatively little impact on COVID-19 burden. Interpretation. Not only the absolute number and intensity of physical contacts drive the transmission dynamics and COVID-19 burden, also their repetitiveness is influential. Contact tracing seems essential for a controlled and persistent release of lockdown measures, but requires timely compliance to testing, reporting and self-isolation. Rapid tracing and testing, and communication ensuring continued involvement of the population are therefore essential. [**note: from a Belgian group, a nice model on how contact tracing can work. This is good reading for all of the public health professionals who get my newsletter. One thing is clear, we really need good data on infection/transmission rates for children as decisions on school openings are being made**]
https://www.medrxiv.org/content/10.1101/2020.07.01.20144444v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow

CLINICAL TRIAL RESULTS

- Importance: The COVID-19 antibody response is a critical indicator for evaluating immunity and also serves as the knowledge base for vaccine development. The picture is still not clear because of many limitations including testing tools, time of sampling, and the unclear impact of varying clinical status. In addition to these problems, antibody levels may not be equivalent to protective capacity. Objective: To define the key factor for the different patterns of COVID-19 antibody response. Design: We elucidated the antibody response with time-series throat and serum samples for viral loads and antibody levels, then used a neutralization test to evaluate protectiveness. Setting: A medical center that typically cares for patients with moderate to severe diseases. Because of the low prevalence of COVID-19 in Taiwan and local government policy, however, we also admit COVID-19 patients with mild disease or even those without symptoms for inpatient care. Participants: RT-PCR-confirmed COVID-19 patients. Results: We found that only patients with relative persistence of virus at pharynx displayed strong antibody responses that were proportional to the pharyngeal viral load. They also had proportional neutralization titers per unit of serum. Although antibody levels decreased around 2 weeks after symptom onset, the neutralization efficacy per unit antibody remained steady and even continued to increase over time. The antibody response in patients with rapid virus clearance was weak, but the neutralization efficacy per unit antibody in these patients was comparable to those with persistent presence of virus. The deceased were with higher viral load, higher level of antibody, and higher neutralization titers in the serum, but the neutralization capacity per unit antibody is relatively low. Conclusions and Relevance: Strong antibody response depends on the relative persistence of the virus, instead of the absolute virus amount. The antibody response is

still weak if large amount of virus is cleared quickly. The neutralization efficacy per unit antibody is comparable between high and low antibody patterns. Strong antibody response contains more inefficient and maybe even harmful antibodies. Low antibody response is also equipped with a capable B cell pool of efficient antibodies, which may expand with next virus encounter and confer protection. [**note: from Taiwan, more on the antibody response and these researcher also look at viral persistence**]
https://www.medrxiv.org/content/10.1101/2020.07.01.20143917v1

- Abnormal coagulation and an increased risk of thrombosis are features of severe COVID-19, with parallels proposed with hemophagocytic lymphohistiocytosis (HLH), a life-threating condition associated with hyperinflammation. The presence of HLH was described in severely ill patients during the H1N1 influenza epidemic, presenting with pulmonary vascular thrombosis. We tested the hypothesis that genes causing primary HLH regulate pathways linking pulmonary thromboembolism to the presence of SARS-CoV-2 using novel network-informed computational algorithms. This approach led to the identification of Neutrophils Extracellular Traps (NETs) as plausible mediators of vascular thrombosis in severe COVID-19 in children and adults. Taken together, the network-informed analysis led us to propose the following model: the release of NETs in response to inflammatory signals acting in concert with SARS-CoV-2 damage the endothelium and direct platelet-activation promoting abnormal coagulation leading to serious complications of COVID-19. The underlying hypothesis is that genetic and/or environmental conditions that favor the release of NETs may predispose individuals to thrombotic complications of COVID-19 due to an increase risk of abnormal coagulation. This would be a common pathogenic mechanism in conditions including autoimmune/infectious diseases, hematologic and metabolic disorders. [**note: this McGill University study proposes neutrophil extracellular traps as a mediator of thrombosis.**]
https://www.medrxiv.org/content/10.1101/2020.07.01.20144121v1
- The angiotensin-converting enzyme 2 (ACE2) on the cell surface is crucial for SARS-COV-2 entering into the cells. The expression of ACE2 in skin suggested that skin might be a way of transmitting SARS-CoV-2. We found the elevated ACE2 level of patients with psoriasis but downregulated after IL-17 antibody treatment. Further results showed that ACE2 expression increased either in psoriasis or in atopic dermatitis, which were typical inflammatory skin disorders with barrier dysfunction. And elevated ACE2 level was also detected in mouse models of dermatitis induced by imiquimod, calcipotriol, repeated tape-stripping or 1-Fluoro-2,4-dinitrobenzene (DNFB) respectively. Moreover, alleviation of cutaneous inflammation with skin recovery moisture also lowered expression of ACE2 in mouse models with barrier deteriorated inflammatory skin disorders. Furthermore, inflammatory skin disorders with barrier dysfunction increased the penetration of topical FITC conjugated spike protein into the skin. Conversely, improvement in skin permeability barrier could prevent this penetration. Thus, indicating the special link between inflammatory skin disorders with skin barrier dysfunction and increasing risk of COVID-19. [**note: oh no, another thing to worry about! While it is interesting to consider skins transmission of SARS-CoV-2, I am putting this way down on the COVID concern list.**] https://www.biorxiv.org/content/10.1101/2020.06.30.181297v1
- The current pandemic is caused by the SARS-CoV-2 virus and large progress in understanding the pathology of the virus has been made since its emergence in late 2019. Several reports indicate short lasting immunity against endemic coronaviruses, which contrasts repeated

reports that biobanked venous blood contains SARS-CoV-2 reactive T cells even before the outbreak in Wuhan. This suggests there exists a preformed T cell memory in individuals not exposed to the pandemic virus. Given the similarity of SARS-CoV-2 to other members of the Coronaviridae family , the endemic coronaviruses appear likely candidates to generate this T cell memory. However, given the apparent poor immunological memory created by the endemic coronaviruses, other immunity against other common pathogens might offer an alternative explanation. Here, we utilize a combination of epitope prediction and similarity to common human pathogens to identify potential sources of the SARS-CoV-2 T cell memory. We find that no common human virus, other than beta-coronaviruses, can explain the pre-existing SARS-CoV-2 reactive T cells in uninfected individuals. *Our study suggests OC43 and HKU1 are the most likely pathogens giving rise to SARS-CoV-2 preformed immunity*. [**note: this is an intriguing paper regarding the presence of pre-existing SARS-CoV-2 T-cells. The two strains mentioned in the abstract's final sentence are two endemic coronaviruses. There is so much interesting immunology that is being uncovered these days!**]
https://www.biorxiv.org/content/10.1101/2020.07.01.182741v1

- Favipiravir is a broad−spectrum oral antiviral agent that shows in vitro activity against SARS−CoV−2. Presently, data on the effectiveness and optimal dosage of favipiravir for treating COVID−19 is limited. Methods. We conducted a retrospective observational study of hospitalized adult patients with COVID-19 at five tertiary care hospitals in Thailand. We reviewed patient charts to obtain all necessary data. Results. Among 247 COVID-19 patients, 63 (23.0%) received ≥1 dose of favipiravir. Of these, 27.0% required an O2−nasal cannula, 9.5% required non−invasive ventilation and/or high−flow O2−therapy, and 6.4% required invasive mechanical ventilation and/or ECMO. The median baseline NEWS2 score was 5(0−16). The Day−7 clinical improvement rate [95%CI] was 66.7%[53.7−78.0%] in all patients, 92.5%[75.7%−99.1%] in patients who did not require O2−supplementation, and 47.2%[0.4%−64.5%] in patients who required O2−supplementation. No life-threatening adverse events were identified. The 28-day mortality rate was 4.8%. Multivariate analysis revealed three poor prognostic factors for Day−7 clinical improvement [odds ratio (95%CI); p−value]: older age [0.94 (0.89 to 0.99); p=0.04], higher baseline NEWS2 score [0.64 (0.47 to 0.88); p=0.006], and lower favipiravir loading dose (≤45 mg/kg/day) [0.04 (0.005 to 0.4); p=0.006]. Conclusions. Our study reports the promising effectiveness of favipiravir for treating COVID−19 patients. In addition to older age and a high baseline NEWS2 score, a low loading dose of favipiravir (≥45 mg/kg/day) was also identified as a poor prognostic factor for early clinical improvement. Further studies to explore the optimal dose and the optimal timing of drug initiation for favipiravir should be performed. [**note: There have been a couple of preprints on favipiravir that were inconclusive about the drug's utility. This is an observational study from Thailand and of course it goes without saying TIWWDCT. There are 28 studies registered including one at Stanford that is looking at the use in mild COVID-19 cases**. **We still need an oral drug that can be used to treat mild COVID-19**]
https://www.medrxiv.org/content/10.1101/2020.06.24.20133249v2

DRUG DEVELOPMENT

- Strategies to develop therapeutics for SARS-CoV-2 infection may be informed by experimental identification of viral-host protein interactions in cellular assays and measurement of host

response proteins in COVID-19 patients. Identification of genetic variants that influence the level or activity of these proteins in the host could enable rapid "in silico" assessment in human genetic studies of their causal relevance as molecular targets for new or repurposed drugs to treat COVID-19. We integrated large-scale genomic and aptamer-based plasma proteomic data from 10,708 individuals to characterize the genetic architecture of 179 host proteins reported to interact with SARS-CoV-2 proteins or to participate in the host response to COVID-19. We identified 220 host DNA sequence variants acting in cis (MAF 0.01-49.9%) and explaining 0.3-70.9% of the variance of 97 of these proteins, including 45 with no previously known protein quantitative trait loci (pQTL) and 38 encoding current drug targets. Systematic characterization of pQTLs across the phenome identified protein-drug-disease links, evidence that putative viral interaction partners such as MARK3 affect immune response, and establish the first link between a recently reported variant for respiratory failure of COVID-19 patients at the ABO locus and hypercoagulation, i.e. maladaptive host response. Our results accelerate the evaluation and prioritization of new drug development programmes and repurposing of trials to prevent, treat or reduce adverse outcomes. Rapid sharing and dynamic and detailed interrogation of results is facilitated through an interactive webserver (https://omicscience.org/apps/covidpgwas/). [**note: this is similar to the work that the large UCSF group has done.**] https://www.biorxiv.org/content/10.1101/2020.07.01.182709v1

VIRUS BIOCHEMISTRY

- A novel coronavirus (SARS-CoV-2) has emerged to a global pandemic and caused significant damages to public health. Human angiotensin-converting enzyme 2(ACE2) was identified as the entry receptor for SARS-CoV-2. As a carboxypeptidase, ACE2 cleaves many biological substrates besides Ang II to control vasodilatation and permeability. Given the nanomolar high affinity between ACE2 and SARS-CoV-2 spike protein, we wonder how this interaction would affect the enzymatic activity of ACE2. Surprisingly, SARS-CoV-2 trimeric spike protein increased ACE2 proteolytic activity ~3-10 fold when fluorogenic caspase-1 substrate and Bradykinin-analog peptides were used to characterize ACE2 activity. In addition, the enhancement was mediated by ACE2 binding of RBD domain of SARS-CoV-2 spike. These results highlighted the altered activity of ACE2 during SARS-CoV-2 infection and would shed new lights on the pathogenesis of COVID-19 and its complications for better treatments. [**note: interesting finding about the enhanced activity of ACE2 upon biding of the Spike protein]**
https://www.biorxiv.org/content/10.1101/2020.07.01.182659v1

DIAGNOSTIC DEVELOPMENT

- Population-wide serological testing is an essential component in understanding the COVID-19 pandemic. The logistical challenges of undertaking widespread serological testing could be eased through use of a reliable dried blood spot (DBS) sampling method. Objective: To validate the use of dried blood spot sampling for the detection of SARS-CoV-2-specific antibodies. Design, setting and participants: Eighty-seven matched DBS and serum samples were obtained from eighty individuals, including thirty-one who were previously PCR-positive for SARS-CoV-2. DBS eluates and sera were used in an ELISA to detect antibodies to the viral spike protein. Results: Specific anti-SARS-Cov-2 spike glycoprotein antibodies were detectable in both serum and DBS eluate and there was a significant correlation between the antibody levels detected in

matched samples (r = 0.96, p<0.0001). Using serum as the gold standard in the assay, matched DBS samples achieved a Cohens kappa coefficient of 0.975 (near-perfect agreement), a sensitivity of 98.1% and specificity of 100%, for detecting anti-spike glycoprotein antibodies. Conclusions and relevance: Eluates from DBS samples are a reliable and reproducible source of antibodies to be used for the detection of SARS-CoV-2-specific antibodies. The use of DBS sampling could complement the use of venepuncture in the immunosurveillance of COVID-19 in both low and high income settings. [**note: this is a useful finding that dried blood spots can be used for serology testing.**]
https://www.medrxiv.org/content/10.1101/2020.07.01.20144295v1

- We established a translational approach combining home blood sampling by finger-pricking with multiplexed serology to assess the exposure to the SARS-CoV-2 virus in a general population. The developed procedure determines the immune response in multiplexed assays against several spike (S, here denoted SPK), receptor binding domain (RBD) and nucleocapsid (NCP) proteins in eluates from dried capillary blood. The seroprevalence was then determined in two study sets by mailing 1000 blood sampling kits to random households in urban Stockholm during early and late April 2020, respectively. After receiving 55% (1097/2000) of the cards back within three weeks, 80% (878/1097) were suitable for the analyses of IgG and IgM titers. The data revealed diverse pattern of immune response, thus seroprevalence was dependent on the antigen, immunoglobulin class, stringency to include different antigens, as well as the required analytical performance. Applying unsupervised dimensionality reduction to the combined IgG and IgM data, 4.4% (19/435; 95% CI: 2.4%-6.3%) and 6.3% (28/443; 95% CI: 4.1%-8.6%) of the samples clustered with convalescent controls. Using overlapping scores from at least two SPK antigens, prevalence rates reached 10.1% (44/435; 95% CI: 7.3%-12.9%) in study set 1 and 10.8% (48/443; 95% CI: 7.9%-13.7%). Measuring the immune response against several SARS-CoV-2 proteins in a multiplexed workflow can provide valuable insights about the serological diversity and improve the certainty of the classification. Combining such assays with home-sampling of blood presents a viable strategy for individual-level diagnostics and towards an unbiased assessment of the seroprevalence in a population and may serve to improve our understanding about the diversity of COVID-19 etiology. [**note: Sweden has taken a different approach to dealing with the SARS-CoV-2 pandemic. Here is an interesting approach to collecting and analyzing serology samples. I would like to see something like this done at the community level here in the US to see how viable it might be to a larger rollout. Somebody step up and do this!**] https://www.medrxiv.org/content/10.1101/2020.07.01.20143966v1

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

2020-07-03

Martha Argerich is a very young 79 years of age and like some of her male predecessors still playing phenomenally well. She has played pieces from virtually all the top composers of the piano rep since her debut recital at the age of 8. There are a number of fine YouTube videos but let's just look and revel in the most recent from pre-pandemic December 2019. It is the Beethoven Piano Concerto #1 and a couple of nice encores: https://www.youtube.com/watch?v=BF7pmimzjBs Let this be an inspiration to all of us over the age of 65!

Let's get to the important stuff first! Do your glasses fog up when you are wearing a mask? Here are some helpful hints to avoid this problem.

One of my loyal readers sent me this story from Kaiser Health News on the sad state of funding for our Public Health System. The government manages to throw money into a vaccine acceleration project, a disjointed clinical trial system, and some other things without thinking about what really can make a difference. There has been little imagination since the beginning of the pandemic and this is just another example.

Derek Lowe on the Pfizer vaccine results and human challenge testing.

There has been a lot of discussion about colleges opening up for in class instruction this fall. The big question is the willingness of professors to teach as this New York Times story notes. Maybe small schools in rural areas can control SARS-CoV-2 but large commuter schools in urban areas will find this difficult. Remote learning may work for lecture classes but it is not going to substitute for laboratory instruction. The Times has a nice story on a contract tracer supervisor. In one New York county, public health authorities had to use subpoenas to do contact tracing. This is just too weird for me.

There has been a lot written about the observed D614G mutation in the European SARS-CoV-2 variant, most recently by the New York Times. I have linked to some preprints on this and now here is a paper in Cell from Los Alamos researchers. There is a lot we still don't understand about the virus and what exactly this mutation means. Viral mutations are common and we probably don't even know how frequently this happens as there may be mutations that reduce infectivity leading to that version disappearing. Thus far I haven't seen anything to suggest that current vaccine and mAb efforts are impacted by this variant.

While we had good news from Pfizer yesterday, STAT reports that the Moderna vaccine 30K Phase 3 trial is delayed. I hope this goes smoothly otherwise it will be a black eye for NIH who have been the principal funder of the trial (Pfizer has not taken any government funding for their work).

MODELING

- After yesterday's deluge of models, there is nothing novel to report today.

NEWLY REGISTERED CLINICAL TRIALS (I am seeing a lot of trials from small companies these days. I wonder if these are just being registered to prop up stock prices as I cannot see the clinical rationale in some of them. It may be they never enroll patients.)

- The rationale of the use of tramadol for COVID-19 patients is attributed to its anti-inflammatory, hypocagulatory, antioxidant, cardio-protective, analgesic, antitussive, bactericidal and antidepressant effect. [**note: this is an Egyptian study**[NCT04454307
- This is a randomised, double-blind, placebo-controlled phase 2 trial investigating the safety and efficacy of C21 in subjects who are hospitalised with COVID-19 infection, but not in need of mechanical invasive or non-invasive ventilation. [**note: I don't know much about the drug. Sponsor is a Swedish company, Vicore Pharma AB and more info is at the link**] NCT04452435
- This is a Phase 1 randomized study to evaluate the safety, tolerability and efficacy of IV Ampion in improving the clinical course and outcomes of patients hospitalized with COVID-19 infection who require supplemental oxygen. [**note: another drug I know little about. The primary**

constituent ingredient of Ampion is aspartyl-alanyl diketopiperazine, or DA-DKP, an endogenous immunomodulatory molecule derived from the N-terminus of HSA. Sponsor is Ampio Pharmaceuticals, a Colorado-based company] NCT04456452

- This randomized, open-label, prospective, parallel-group controlled clinical study that aims to explore the natural history of COVID-19 illness and the safety of KB109, a novel glycan, plus SSC versus SSC alone and measures of health in outpatients with mild-to-moderate COVID-19. [**note sponsor is Kaleido Biosciences**] NCT04414124
- The clinical Phase 2/3 evaluates the safety and efficacy of NA-831 alone, and a combination therapy comprises NA-831 with an anti-viral drug Atazanavir, NA-831 with an anti-inflammatory drug, Dexamethasone and a potential synergy between Atazanavir and Dexamethasone. NA-831 is also known as Traneurocin is a neuroprotective drug that is in clinical study for the treatment of Alzheimer's Disease. Participants will receive NA-831 or Atazanavir with or without Dexamethasone. Investigators are primarily interested in the time to recovery. [**note: sponsor is NeuroActiva.**] NCT04452565

CLINICAL TRIAL RESULTS

- Between Dec 1, 2005, and May 20, 2020, we included 230 patients with Kawasaki disease. The median number of Kawasaki disease hospitalisations estimated by the quasi-Poisson model was 1·2 per month (IQR 1·1–1·3). In April, 2020, we identified a rapid increase of Kawasaki disease that was related to SARS-CoV-2 (six cases per month; 497% increase [95% CI 72–1082]; p=0·0011), starting 2 weeks after the peak of the COVID-19 epidemic. SARS-CoV-2 was the only virus circulating intensely during this period, and was found in eight (80%) of ten patients with Kawasaki disease since April 15 (SARS-CoV-2-positive PCR or serology). A second peak of hospital admissions due to Kawasaki disease was observed in December, 2009 (six cases per month; 365% increase ([31–719]; p=0.0053), concomitant with the influenza A H1N1 pandemic. Our study further suggests that viral respiratory infections, including SAR-CoV-2, could be triggers for Kawasaki disease and indicates the potential timing of an increase in incidence of the disease in COVID-19 epidemics. Health-care providers should be prepared to manage an influx of patients with severe Kawasaki disease, particularly in countries where the peak of COVID-19 has recently been reached. [**note: it appears that Kawasaki disease is increased as a result of SARS-CoV-2 infection as noted in the French study. The intriguing question is whether the Euro variant as described in the intro to this newsletter is responsible for this. As the commentary notes, few if any cases have been described in China or other Asian areas where SARS-CoV-2 outbreaks have taken place.**] https://www.thelancet.com/journals/lanchi/article/PIIS2352-4642(20)30175-9/fulltext and here is a good commentary on this issue https://www.thelancet.com/journals/lanchi/article/PIIS2352-4642(20)30207-8/fulltext
- A number of in vitro as well as in vivo model animal studies have shown that widely used compound hydroxychloroquine (HCQ) is able to cause anti-viral effect on SARS-CoV-2. While there is no enough clinical data to support the use of HCQ, several countries including Russia have already approved HCQ as treatment and prophylactic option. In the current study we analyzed the dynamics of the SARS-CoV-2 RNA quantity change in nasopharynx swabs of infected patients in mild condition and compared that of patients receiving HCQ and receiving no antiviral pharmacological therapy. We found that most of the patients demonstrated gradual decrease in the number of SARS-CoV-2 RNA copies in the swab regardless of the HCQ receiving.

Noteworthy that patients with RNA load higher than 106 copies were hospitalized due to condition deteriorating significantly more frequently compared to those with RNA load below 106 copies even with HCQ administration. In addition, the results of the current study indicate that recovering patients may produce viruses at least during 18 days from the onset of symptoms and HCQ therapy does not block or reduce it. [**note: this HCQ study is from Russia. HCQ shows no effect on nasopharynx viral load in mild COVID-19 patients. More interesting is the finding that patients may produce virus 18 days from the onset of symptoms. I wonder if this is a common phenomenon with other respiratory viruses.**]
https://www.medrxiv.org/content/10.1101/2020.06.30.20143289v1

- Patients with severe asthma may have a greater risk of dying from COVID-19 disease caused by SARS-CoV-2 virus. Angiotensin converting enzyme 2 (ACE2) receptor and enzyme proteases, transmembrane protease, serine 2 (TMPRSS2) and furin are needed for the attachment and invasion of the virus into host cells. We determined whether their expression in the airways of severe asthma patients is increased. Method. We examined the microarray mRNA expression of ACE2, TMPRSS2 and furin in the sputum, bronchial brush and bronchial biopsies of participants in the European U-BIOPRED cohort. Results. ACE2 and furin sputum gene expression was significantly increased in severe non-smoking asthma compared to mild-moderate asthma and healthy volunteers. By contrast, TMPRSS2 expression in bronchial biopsy and bronchial brushings was increased in severe smoking and ex-smoking asthmatics, and so was furin expression in bronchial brushings. Several clinical parameters including male gender, oral steroid use and nasal polyps were positively associated with ACE2, TMPRSS2 and furin expression levels. There was a higher expression of ACE2 and furin in the sputum neutrophilic molecular phenotype with inflammasome activation compared to the eosinophilic Type2-high or paucigranulocytic phenotypes. The enrichment score of the IL-13-Type2 gene signature was positively correlated with ACE2, TMPRSS2 and furin levels. Conclusion. These key determinants of virus entry into the lungs may contribute to the poorer outcomes from COVID-19 disease in patients with severe asthma. [**note: a multi-national team looks at various factors that might contribute to poor clinical outcomes in patients with severe asthma.**]
https://www.medrxiv.org/content/10.1101/2020.06.29.20142091v1
- Background. It can be a diagnostic challenge to identify COVID-19 patients without bacterial co-infection in whom antibiotics can be safely stopped. We sought to evaluate the validity of a guideline that recommends withholding antibiotics in patients with a low serum procalcitonin (PCT). Methods. We retrospectively collected 28-day outcome data on patients admitted to Sheffield Teaching Hospitals NHS Foundation Trust, UK, between 5 March and 15 April 2020, with a positive SARS-CoV-2 polymerase chain reaction (PCR) and PCT within 48 hours of diagnosis. PCT was considered negative if ≤0.25ng/ml and positive if >0.25ng/ml. Primary outcomes included antibiotic consumption, mortality, intensive care admission and length of hospital stay. Results. 368 patients met the inclusion criteria; 218 (59%) had a negative PCT and 150 (41%) positive. At 48 hours post-diagnosis, 73 (33%) of those with a negative PCT were receiving antimicrobials compared to 126 (84%) with a positive PCT ($p<0.001$), with a corresponding reduction in antimicrobial usage over 28 days (median DDD of 3.0 vs 6.8 ($p<0.001$); median DOT 2 vs 5 days ($p<0.001$) between the negative and positive PCT groups.) In the negative PCT group, there were fewer deaths (62 (28%) vs. 54 (36%), ($p=0.021$)) and critical care admissions (19 (9%) vs. 28 (19%), ($p=0.007$)) than in the positive PCT group. Median length

of hospital stay was 8.7 and 9 days in the negative and positive PCT groups respectively. Conclusions. Procalcitonin is a valuable tool in the assessment of patients with SARS-CoV-2 infection, safely reducing the potential burden of unnecessary antibiotic usage. [**note: this is really useful in that it can cut down on routine use of antibiotics in hospitalized COVID-19 patients.**] https://www.medrxiv.org/content/10.1101/2020.06.29.20136572v1

DRUG DEVELOPMENT

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). To identify factors of the respiratory tract that suppress SARS-CoV-2, we screened a peptide/protein library derived from bronchoalveolar lavage, and identified α1-antitrypsin (α1-AT) as specific inhibitor of SARS-CoV-2. α1-AT targets the viral spike protein and blocks SARS-CoV-2 infection of human airway epithelium at physiological concentrations. Our findings show that endogenous α1-AT restricts SARS-CoV-2 and repurposes α1-AT-based drugs for COVID-19 therapy. [**note: some good *in vitro* work on α1-antitrypsin as an inhibitor of SARS-CoV-2**] https://www.biorxiv.org/content/10.1101/2020.07.02.183764v1
- Modified Vaccinia Ankara (MVA) is a highly attenuated poxvirus vector that is widely used to develop vaccines for infectious diseases and cancer. We developed a novel vaccine platform based on a unique three-plasmid system to efficiently generate recombinant MVA vectors from chemically synthesized DNA. In response to the ongoing global pandemic caused by SARS coronavirus-2 (SARS-CoV-2), we used this novel vaccine platform to rapidly produce fully synthetic MVA (sMVA) vectors co-expressing SARS-CoV-2 spike and nucleocapsid antigens, two immunodominant antigens implicated in protective immunity. Mice immunized with these sMVA vectors developed robust SARS-CoV-2 antigen-specific humoral and cellular immune responses, including potent neutralizing antibodies. These results demonstrate the potential of a novel vaccine platform based on synthetic DNA to efficiently generate recombinant MVA vectors and to rapidly develop a multi-antigenic poxvirus-based SARS-CoV-2 vaccine candidate. [**note: another new vaccine candidate from the City of Hope researchers! They also provide some animal data**] https://www.biorxiv.org/content/10.1101/2020.07.01.183236v1
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a worldwide health threat. Here, we report that low plasma interleukin-3 (IL-3) levels were associated with increased severity and mortality during SARS-CoV-2 infections. IL-3 promoted the recruitment of antiviral circulating plasmacytoid dendritic cells (pDCs) into the airways by stimulating CXCL12 secretion from pulmonary CD123+ epithelial cells. This study identifies IL-3 as a predictive disease marker and potential therapeutic target for SARS-CoV-2 infections. [**note: yet another immune system modulator this time, IL-3, that might be both a marker for disease progression and a therapeutic.**] https://www.biorxiv.org/content/10.1101/2020.07.02.184093v1
- Coronavirus papain-like protease (PLpro) is an attractive drug target as it is essential for viral polyprotein cleavage and for deconjugation of ISG15, an antiviral ubiquitin-like protein. We show here that 6-Thioguanine (6-TG) inhibits SARS-CoV-2 PLpro-catalyzed viral polyprotein cleavage and ISG15 deconjugation in cells and inhibits replication of SARS-CoV-2 in Vero-E6 cells and Calu3 cells at submicromolar levels. As a well-characterized FDA-approved orally delivered drug, 6-TG represents a promising therapeutic for COVID-19 and other emerging coronaviruses. [**note: this is a new drug target that I have not seen before. The inhibitory concentration from**

***in vitro* studies looks good but is there anyone who will put this In trial?**]
https://www.biorxiv.org/content/10.1101/2020.07.01.183020v1

VIRUS BIOCHEMISTRY

- Nothing today.

DIAGNOSTIC DEVELOPMENT

- Commercially available SARS-CoV-2 serological assays based on different viral antigens have been approved for the qualitative determination of anti-SARS-CoV-2 antibodies. However, there is limited published data associating the results from commercial assays with neutralizing antibodies. Methods: 67 specimens from 48 patients with PCR-confirmed COVID-19 and a positive result by the Roche Elecsys SARS-CoV-2, Abbott SARS-CoV-2 IgG, or EUROIMMUN SARS-CoV-2 IgG assays and 5 control specimens were analyzed for the presence of neutralizing antibodies to SARS-CoV-2. Correlation, concordance, positive percent agreement (PPA), and negative percent agreement (NPA) were calculated at several cutoffs. Results were compared in patients categorized by clinical outcomes. Results: The correlation between SARS-CoV-2 neutralizing titer (EC50) and the Roche, Abbott, and EUROIMMUN assays was 0.29, 0.47, and 0.46 respectively. At an EC50 of 1:32, the concordance kappa with Roche was 0.49 (95% CI; 0.23-0.75), with Abbott was 0.52 (0.28-0.77), and with EUROIMMUN was 0.61 (0.4-0.82). At the same neutralizing titer, the PPA and NPA for the Roche was 100% (94-100) & 56% (30-80); Abbott was 96% (88-99) & 69% (44-86); and EUROIMMUN was 91% (80-96) & 81% (57-93) for distinguishing neutralizing antibodies. Patients who died, were intubated, or had a cardiac injury from COVID-19 infection had significantly higher neutralizing titers relative to those with mild symptoms. Conclusion: COVID-19 patients generate an antibody response to multiple viral proteins such that the calibrator ratios on the Roche, Abbott, and EUROIMMUN assays are all associated with SARS-CoV-2 neutralization. Nevertheless, commercial serological assays have poor NPA for SARS-CoV-2 neutralization, making them imperfect proxies for neutralization. [**note: not all SARS-CoV-2 antibodies are create equal as some may not be effective in neutralizing ability. Mayo Clinic has announced a neutralizing antibody test that addresses this issue (there may be other labs that have done this as well). I think this type of work is very important as it could serve as a clinical proxy for vaccine potency and accelerate vaccine development in a meaningful manner.**] https://www.biorxiv.org/content/10.1101/2020.07.01.182220v1

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

2020-07-04

Happy Independence Day to all my American Readers!

The Eurovision Song Contest has been around for a long time. The best known winners were ABBA (1974) and Celine Dion (1988) though there have been some others who have had moderate careers such as Lulu (1969). Now we have a movie, Eurovision Song Contest: The Story of Fire Saga, with Will Farrell and Rachel McAdams that is a clever parody of the annual event. Here we have a wonderful song

sung by McAdams from the movie: https://www.youtube.com/watch?v=AvzotsxlJLI the movie is streaming on Netflix

Here are the experts on how to deal with issues in everyday lives. This one is mandatory reading!! The Washington Post also has a good story on the use of ventilators in the treatment of severe COVID-19. Mortality rates have come down considerable relative to what was observed in the early days of the pandemic.

How about easy to use home test strips? Of course they won't have the accuracy of a validated lab test but they will have a role as this opinion piece notes. I've been critical of the lack of imagination in confronting the pandemic. Let's use this July 4th to make a sweeping turn towards getting things done!

The New York Times has a story on the reduced mortality currently observed in the new outbreak of SARS-CoV-2 infections. As I have noted, we are likely not going to know the CRF until we have robust numbers for infections. The paper also has a nice story on the possible end of the 5-day office work week. One of my other activities (yes, I don't spend all my time writing this newsletter!) is tracking some of the economic indicators impacted by the COVID-19 pandemic. There are a number of thoughtful analysts who think office real estate is going to take a hit because employers understand that remote work can effectively substitute for full time in office work. There are a lot of offices in our metro area that are just beginning to reopen and it is unclear whether they will go back to 100% staffing. This is an indicator to watch.

The Washington Post offers this story on the German anti-vaxxer movement. The paper also has the cautionary tale of COVID-19 which only recently has hit Joplin Missouri with a vengeance. The City Council defeated a mask ordinance by one vote. Too sad.

Following up on yesterday's link to the Kaiser Health News article on public health funding, STAT has an opinion piece on the topic worth reading.

I am putting this Lancet 'personal view' piece above the fold as it is a good summary of SARS-CoV-2 relative to some other pandemics. Recommended reading! Also worth reading is this Lancet piece on viral presence on surfaces. Money quote, "In my opinion, the chance of transmission through inanimate surfaces is very small, and only in instances where an infected person coughs or sneezes on the surface, and someone else touches that surface soon after the cough or sneeze (within 1–2 h)." If you want a current scientific paper this one just came out from the USAMRIID up at Fr. Dietrick. They observe culturable virus on skin (pig), currency, and clothing for a significant period of time. Whether this reflects a 'real world' risk is not known. I haven't used any paper currency in the past four months.

Lots of stuff today and of course more confounding information on the 'Zombie' drug HCQ.

MODELING

- Mortality in care homes has had a prominent focus during the COVID-19 outbreak. Multiple and interconnected challenges face the care home sector in the prevention and management of outbreaks of COVID-19, including adequate supply of personal protective equipment, staff shortages, and insufficient or lack of timely COVID-19 testing. Care homes are particularly vulnerable to infectious diseases. Aim: To analyse the mortality of older care home residents in

Wales during COVID-19 lockdown and compare this across the population of Wales and the previous 4-years. Study Design and Setting: We used anonymised Electronic Health Records (EHRs) and administrative data from the Secure Anonymised Information Linkage (SAIL) Databank to create a cross-sectional cohort study. We anonymously linked data for Welsh residents to mortality data up to the 14th June 2020. Methods: We calculated survival curves and adjusted Cox proportional hazards models to estimate hazard ratios (HRs) for the risk of mortality. We adjusted hazard ratios for age, gender, social economic status and prior health conditions. Results: Survival curves show an increased proportion of deaths between 23rd March and 14th June 2020 in care homes for older people, with an adjusted HR of 1.72 (1.55, 1.90) compared to 2016. Compared to the general population in 2016-2019, adjusted care home mortality HRs for older adults rose from 2.15 (2.11,2.20) in 2016-2019 to 2.94 (2.81,3.08) in 2020. Conclusions: *The survival curves and increased HRs show a significantly increased risk of death in the 2020 study periods*. [**note: this is a nice study nursing homes in Wales with a somewhat obvious outcome but someone had to do it.**]
https://www.medrxiv.org/content/10.1101/2020.07.03.20145839v1

- A recent genetic association study (Ellinghaus et al. 2020) identified a gene cluster on chromosome 3 as a risk locus for respiratory failure in SARS-CoV-2. Recent data comprising 3,199 hospitalized COVID-19 patients and controls reproduce this and find that it is the major genetic risk factor for severe SARS-CoV-2 infection and hospitalization (COVID-19 Host Genetics Initiative). Here, we show that the risk is conferred by a genomic segment of ~50 kb that is inherited from Neandertals and occurs at a frequency of ~30% in south Asia and ~8% in Europe. [**note: this is an interesting phylogenetic paper on the source of the risk locus for respiratory failure in SARS-CoV-2. I need to spend a bit more time reading it.**]
https://www.biorxiv.org/content/10.1101/2020.07.03.186296v1
- Background: Identification of healthy people at high risk for severe COVID-19 is a global health priority. We investigated whether blood biomarkers measured by high-throughput metabolomics could be predictive of severe pneumonia and COVID-19 hospitalisation years after the blood sampling. Methods: Nuclear magnetic resonance metabolomics was used to quantify a comprehensive biomarker profile in 105,146 plasma samples collected in the UK Biobank during 2007-2010 (age range 39-70). The biomarkers were tested for association with severe pneumonia (2507 cases, defined as diagnosis in hospital or death record occurring during a median of 8.1-year follow-up) and with severe COVID-19 (195 cases, defined as diagnosis in hospital between mid-March to mid-June 2020). A multi-biomarker score was derived for prediction of severe pneumonia based on half of the study population and validated in the other half. We explored how this biomarker score relates to the risk of severe COVID-19. Findings: The biomarker associations with risk of severe COVID-19 followed an overall pattern similar to associations with risk of severe pneumonia (correlation 0.83). The multi-biomarker score, comprised of 25 blood biomarkers including inflammatory proteins, fatty acids, amino acids and advanced lipid measures, was strongly associated with risk of severe pneumonia (odds ratio 1.67 per standard deviation [95% confidence interval 1.59-1.76]; 3.8-fold risk increase for individuals in upper vs lower quintile). The multi-biomarker score was also associated with risk of severe COVID-19 (odds ratio 1.33 [1.17-1.53]; 2.5-fold risk for upper vs lower quintile) and remained significant when adjusting for body mass index, smoking, and existing respiratory and cardiometabolic diseases. Mimicking the decade lag from blood sampling to COVID-19, severe

pneumonia events occurring after 7-11 years associated with the multi-biomarker score to a similar magnitude (odds ratio 1.43 [1.29-1.59]; 2.6-fold risk for upper vs lower quintile) as for severe COVID-19. However, the short-term risk of severe pneumonia events associated to the multi-biomarker score at even 3 times higher magnitude (odds ratio 2.21 [1.95-2.50]; 8.0-fold risk for upper vs lower quintile in analysis of the first 2 years after blood sampling). Interpretation: In decade-old blood samples from the UK Biobank, a biomarker score measured by high-throughput metabolomics is indicative of the risk for severe COVID-19. The molecular signature of biomarker changes reflective of risk for severe COVID-19 is similar to that for severe pneumonia, in particular when accounting for the time lag to the COVID-19 pandemic. The even stronger association of the biomarker score with 2-year risk for severe pneumonia lends support to promising screening possibilities for identifying people at high risk for severe COVID-19. [**note: another study on biomarkers using the UK Biobank. They were able to go back a whole decade to look at data. Does anyone think a comparable study could be done here In the US??**] https://www.medrxiv.org/content/10.1101/2020.07.02.20143685v1

- Disease transmission is notoriously heterogeneous, and SARS-CoV-2 is no exception. A skewed distribution where few individuals or events are responsible for the majority of transmission can result in explosive, superspreading events, which produce rapid and volatile epidemic dynamics, especially early or late in epidemics. Anticipating and preventing superspreading events can produce large reductions in overall transmission rates. Here, we present a compartmental (SEIR) epidemiological model framework for estimating transmission parameters from multiple imperfectly observed data streams, including reported cases, deaths, and mobile phone-based mobility that incorporates individual-level heterogeneity in transmission using previous estimates for SARS-CoV-1 and SARS-CoV-2. We parameterize the model for COVID-19 epidemic dynamics by estimating a time-varying transmission rate that incorporates the impact of non-pharmaceutical intervention strategies that change over time, in five epidemiologically distinct settings---Los Angeles and Santa Clara Counties, California; Seattle (King County), Washington; Atlanta (Dekalb and Fulton Counties), Georgia; and Miami (Miami-Dade County), Florida. We find the effective reproduction number R_E dropped below 1 rapidly following social distancing orders in mid-March, 2020 and remained there into June in Santa Clara County and Seattle, but climbed above 1 in late May in Los Angeles, Miami, and Atlanta, and has trended upward in all locations since April. With the fitted model, we ask: how does truncating the tail of the individual-level transmission rate distribution affect epidemic dynamics and control? We find interventions that truncate the transmission rate distribution while partially relaxing social distancing are broadly effective, with impacts on epidemic growth on par with the strongest population-wide social distancing observed in April, 2020. Given that social distancing interventions will be needed to maintain epidemic control until a vaccine becomes widely available, "chopping off the tail" to reduce the probability of superspreading events presents a promising option to alleviate the need for extreme general social distancing. [**note: if you want a theoretical paper on the value of social distancing, this paper from Stanford may just be your cup of tea.**] https://www.medrxiv.org/content/10.1101/2020.06.30.20143115v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- Of 2,541 patients, with a median total hospitalization time of 6 days (IQR: 4-10 days), median age was 64 years (IQR:53-76 years), 51% male, 56% African American, with median time to follow-up of 28.5 days (IQR:3-53). Overall in-hospital mortality was 18.1% (95% CI:16.6%-19.7%); by treatment: hydroxychloroquine + azithromycin, 157/783 (20.1% [95% CI: 17.3%-23.0%]), hydroxychloroquine alone, 162/1202 (13.5% [95% CI: 11.6%-15.5%]), azithromycin alone, 33/147 (22.4% [95% CI: 16.0%-30.1%]), and neither drug, 108/409 (26.4% [95% CI: 22.2%-31.0%]). Primary cause of mortality was respiratory failure (88%); no patient had documented torsades de pointes. From Cox regression modeling, predictors of mortality were age>65 years (HR:2.6 [95% CI:1.9-3.3]), white race (HR:1.7 [95% CI:1.4-2.1]), CKD (HR:1.7 [95%CI:1.4-2.1]), reduced O2 saturation level on admission (HR:1.5 [95%CI:1.1-2.1]), and ventilator use during admission (HR: 2.2 [95%CI:1.4-3.3]). Hydroxychloroquine provided a 66% hazard ratio reduction, and hydroxychloroquine + azithromycin 71% compared to neither treatment ($p < 0.001$). In this multi-hospital assessment, when controlling for COVID-19 risk factors, treatment with hydroxychloroquine alone and in combination with azithromycin was associated with reduction in COVID-19 associated mortality. Prospective trials are needed to examine this impact. **[note: yet more confounding information on HCQ from this observational trial at the Henry Ford Health System. Thanks to a loyal reader for flagging this one. We do have one controlled trial, the UK RECOVERY whose results were pretty convincing against HCQ. I don't have a full tally of all the observational trials I've noted but I think the negative trials are greater than the positive ones. I don't know what to make of these results. OHDSI does have these drugs on the list for their large observational study.**] https://www.ijidonline.com/article/S1201-9712(20)30534-8/fulltext
- Between March 20 and March 30, 2020, 6228 patients with autoimmune rheumatic diseases were included in the study. The overall rate of COVID-19 in patients with an autoimmune rheumatic disease in our study population was 0·43% (27 of 6228 patients). We identified 42 families in which COVID-19 was diagnosed between Dec 20, 2019, and March 20, 2020, in either patients with a rheumatic disease or in a family member residing at the same physical address during the outbreak. Within these 42 families, COVID-19 was diagnosed in 27 (63%) of 43 patients with a rheumatic disease and in 28 (34%) of 83 of their family members with no rheumatic disease (adjusted odds ratio [OR] 2·68 [95% CI 1·14–6·27]; p=0·023). Patients with rheumatic disease who were taking hydroxychloroquine had a lower risk of COVID-19 infection than patients taking other disease-modifying anti-rheumatic drugs (OR 0·09 [95% CI 0·01–0·94]; p=0·044). Additionally, the risk of COVID-19 was increased with age (adjusted OR 1·04 [95%CI 1·01–1·06]; p=0·0081). [**note: the HCQ numbers are too small to be meaningful. At the beginning of the outbreak there was interest in whether those on HCQ therapy for rheumatoid arthritis might be protected from SARS-CoV-2 infection. We still do not know the answer to this question.**] https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(20)30227-7/fulltext
- To evaluate associations of plasma 25(OH)D status with the likelihood of coronavirus disease (COVID-19) infection and hospitalization. Methods: The study population included the 14,000 members of Leumit Health Services who were tested for COVID-19 infection from February 1st to April 30th, 2020, and who had at least one previous blood test for plasma 25(OH)D level. "Suboptimal" or "low" plasma 25(OH)D level was defined as plasma 25-hydroxyvitamin D, or 25(OH)D, concentration below 30 ng/mL. Results: Of 7,807 individuals, 782 (10.1%) were COVID-

19-positive, and 7,025 (89.9%) COVID-19-negative. The mean plasma vitamin D level was significantly lower among those who tested positive than negative for COVID-19 [19.00 ng/mL (95% confidence interval [CI] 18.41-19.59) vs. 20.55 (95% CI 20.32-20.78)]. Univariate analysis demonstrated an association between low plasma 25(OH)D level and increased likelihood of COVID-19 infection [crude odds ratio (OR) of 1.58 (95% CI 1.24-2.01, p<0.001)], and of hospitalization due to the SARS-CoV-2 virus [crude OR of 2.09 (95% CI 1.01-4.30, p<0.05)]. In multivariate analyses that controlled for demographic variables and psychiatric and somatic disorders, the adjusted OR of COVID-19 infection [1.45 (95% CI 1.08-1.95, p<0.001)], and of hospitalization due to the SARS-CoV-2 virus [1.95 (95% CI 0.98-4.845, p=0.061)] were preserved. In the multivariate analyses, age over 50 years, male gender and low-medium socioeconomic status were also positively associated with the risk of COVID-19 infection; age over 50 years was positively associated with the likelihood of hospitalization due to COVID-19. [**note: make sure you known what your plasma vitamin D3 levels are and take a supplement if it is low! This is a large Israeli population study.**]
https://www.medrxiv.org/content/10.1101/2020.07.01.20144329v1

- Background After recovery from COVID-19, most patients have anti-SARS-CoV-2 neutralizing antibodies. Their convalescent plasma could be an inexpensive and widely available treatment for COVID-19. Methods The Convalescent-plasma-for-COVID (ConCOVID) study was a randomized trial comparing convalescent plasma with standard of care therapy in patients hospitalized for COVID-19 in the Netherlands. Patients were randomized 1:1 and received 300ml of plasma with anti-SARS-CoV-2 neutralizing antibody titers of at least 1:80. The primary endpoint was day-60 mortality and key secondary endpoints were hospital stay and WHO 8-point disease severity scale improvement on day 15. Results The trial was halted prematurely after 86 patients were enrolled. Although symptomatic for only 10 days (IQR 6-15) at the time of inclusion, 53 of 66 patients tested had anti-SARS-CoV-2 antibodies at baseline. A SARS-CoV-2 plaque reduction neutralization test showed neutralizing antibodies in 44 of the 56 (79%) patients tested with median titers comparable to the 115 donors (1:160 vs 1:160, p=0.40). These observations caused concerns about the potential benefit of convalescent plasma in the study population and after discussion with the data safety monitoring board, the study was discontinued. No difference in mortality (p=0.95), hospital stay (p=0.68) or day-15 disease severity (p=0.58) was observed between plasma treated patients and patients on standard of care. Conclusion Most COVID-19 patients already have high neutralizing antibody titers at hospital admission. Screening for antibodies and prioritizing convalescent plasma to risk groups with recent symptom onset will be key to identify patients that may benefit from convalescent plasma. [**note: WOW, I sure didn't expect this result. This is the first report on using convalescent serum from a Dutch multi-hospital group. They saw no difference between the treatment and SOC group and the DSMB halted the study. Since COVID-19 patients already have high antibody titers it may be only a subset will be helped. Pharmaceutical intervention to quell cytokine storm may be more important. So much we still do not know!**]
https://www.medrxiv.org/content/10.1101/2020.07.01.20139857v1
- Intensive Care Unit (ICU) admissions and mortality in severe COVID-19 patients are driven by cytokine storms and acute respiratory distress syndrome (ARDS). Interim clinical trial results suggest that the corticosteroid dexamethasone displays superior 28-day survival in severe COVID-19 patients requiring ventilation or oxygen. Among 16 patients with plasma IL-6

measurement post-corticosteroid administration, a higher proportion of patients with an IL-6 value over 10 pg/mL have worse outcomes (i.e. ICU Length of Stay > 15 days or death) when compared to 41 patients treated with non-corticosteroid drugs including antivirals, tocilizumab, azithromycin, and hydroxychloroquine (p-value = 0.0024). Given this unexpected clinical association between post-corticosteroid IL-6 levels and COVID-19 severity, we hypothesized that the Glucocorticoid Receptor (GR or NR3C1) may be coupled to IL-6 expression in specific cell types that govern cytokine release syndrome (CRS). Examining single cell RNA-seq data from bronchoalveolar lavage fluid of severe COVID-19 patients and nearly 2 million human cells from a pan-tissue scan shows that alveolar macrophages, smooth muscle cells, and endothelial cells co-express both NR3C1 and IL-6. The mechanism of Glucocorticoid Receptor (GR) agonists mitigating pulmonary and multi-organ inflammation in some COVID-19 patients with respiratory failure, may be in part due to their successful antagonism of IL-6 production within lung macrophages and vasculature. [**note: here is some good linkage between corticosteroid administration and IL-6 levels. It may be a viable biomarker**]
https://www.medrxiv.org/content/10.1101/2020.07.02.20144733v1

DRUG DEVELOPMENT

- The severity of the COVID-19 pandemic, caused by the SARS-CoV-2 coronavirus, calls for the urgent development of a vaccine. The primary immunological target is the SARS-CoV-2 spike (S) protein. S is exposed on the viral surface to mediate viral entry into the host cell. To identify possible antibody binding sites not shielded by glycans, we performed multi-microsecond molecular dynamics simulations of a 4.1 million atom system containing a patch of viral membrane with four full-length, fully glycosylated and palmitoylated S proteins. By mapping steric accessibility, structural rigidity, sequence conservation and generic antibody binding signatures, we recover known epitopes on S and reveal promising epitope candidates for vaccine development. We find that the extensive and inherently flexible glycan coat shields a surface area larger than expected from static structures, highlighting the importance of structural dynamics in epitope mapping. [**note: more an epitope mapping for vaccinologists to consider.**] https://www.biorxiv.org/content/10.1101/2020.07.03.186825v1

VIRUS BIOCHEMISTRY

- I open a new category and there is nothing new!!

DIAGNOSTIC DEVELOPMENT

- While health agencies around the world are exploring various options to contain the spread of this fatal viral infection, multiple strategies and guidelines are being issued to boost the fight against the disease. Identifying and isolating infected individuals at an early phase of the disease has been a very successful approach to stop the chain of transmission. But this approach faces a practical challenge of limited resources. Sample pooling solves this enigma by significantly improving the testing capacity and result turn around time while using no extra resources. However, the general sample pooling method also has the scope of significant improvements. This article describes a process to further optimize the resources with optimal sample pooling. This is a user-friendly technique, scalable on a national or international scale. A mathematical model has been built and validated for its performance using clinical data. [**note: it's always**

nice when the research paper is brief and to the point. This one is three pages and offers a good model for sample pooling.]
https://www.medrxiv.org/content/10.1101/2020.07.03.20145953v1

- Extensive testing is essential to break the transmission of the new coronavirus SARS-CoV-2, which causes the ongoing COVID-19 pandemic. Recently, CRISPR-based diagnostics have emerged as attractive alternatives to quantitative real-time PCR due to their faster turnaround time and their potential to be used in point-of-care testing scenarios. However, existing CRISPR-based assays for COVID-19 have not considered viral genome mutations and RNA editing in human cells. Here, we present the VaNGuard (Variant Nucleotide Guard) test that is not only specific and sensitive for SARS-CoV-2, but can also detect the virus when its genome or transcriptome has evolved or has been edited by deaminases in infected human cells. We show that an engineered AsCas12a enzyme is more tolerant of mismatches than wildtype LbCas12a and that multiplexed Cas12a targeting can overcome the presence of single nucleotide variations. *Our assay can be completed in 30 minutes with a dipstick for a rapid point-of-care test*. [**note: a new approach to CRISPR testing that addresses genome alterations and it's quick to do**] https://www.biorxiv.org/content/10.1101/2020.07.03.185850v1
- There is an urgent need for screening patients of having a communicable viral disease to cut infection chains. We could recently demonstrate that MCC-IMS of breath is able to identify Influenza-A infected patients. With decreasing Influenza epidemic and upcoming SARS-CoV-2 infections we extended our study to the analysis of patients with suspected SARS-CoV-2 infections. 51 patients, 23m, 28f, aged 64 +/- 16 years, were included in this study. Besides RT-PCR analysis of nasopharyngeal swabs all patients underwent MCC-IMS analysis of breath. 16 patients, 7m, 9f, were positive for SARS-CoV-2 by RT-PCR. There was no difference in gender or age according to the groups. Stepwise canonical discriminant analysis could correctly classify the infected and non- infected subjects in 98% by cross-validation. Afterwards we combined the Influenza-A sub study and the SARS-CoV-2- sub study for a total of 75 patients, 34m, 41f, aged 64.8 +/- 1.8 years, 14 positive for Influenza-A, 16 positive for SARS-CoV-2, the remaining 44 patients were used as controls. In one patient RT-PCR was highly suspicious of SARS-CoV-2 but inconclusive. There was no imbalance between the groups for age or gender. 97.3% of the patients could be correctly classified to the respective group by discriminant analysis. Even the inconclusive patient could be mapped to the SARS-CoV-2 group applying the discrimination function. Conclusion: MCC-IMS is able to detect SARS-CoV-2 infection and Influenza-A infection in breath. As this method provides exact, fast non-invasive diagnosis it should be further developed for screening of communicable viral diseases. [**note: this is a new diagnostic approach but I don't know how costly the instrumentation is. From a cursory reading of the paper, the sampling looks pretty complex**]
https://www.medrxiv.org/content/10.1101/2020.06.30.20143347v1

>>

2020-07-05

Let us turn to love for this reflection Sunday. What better way to explore love's passion than the second act love duet from Richard Wanger's Tristan and Isolde. It's a draining opera for both singers and audience and perhaps the best place to view it is the summer festival at Bayreuth where director are given freedom to experiment and the acoustics are exemplary. Here are Waltraud Meier and Siegfried

Jerusalem under the baton of Daniel Barenboim in a 1995 production by Heiner Müller: https://www.youtube.com/watch?v=lQNcTYVlcEg I would be remiss if I didn't provide my loyal readers with Isolde's Liebestod that concludes the opera: https://www.youtube.com/watch?v=OAEkTK6aKUM While we are on the subject of redemptive love, let's not forget the efforts that Maestro Barenboim has taken for peace in the mid-East with his West-Eastern Divan Orchestra. Here is a concert performance of the Liebestod with Meier and that orchestra (it looks to be from the 2008 Ravello Festival on the Amalfi Coast): https://www.youtube.com/watch?v=jtKxaUeAD5I Enjoy and think about better times that will come following the pandemic!!!

I've already mentioned Tim Harford's 'Cautionary Tales' podcast. This week he talks about the heroic efforts that the people of Eyam took in 1665 to prevent a spread of the plague in England. There is a lot to learn from the experience and things are not always as they seem. This runs about 25 minutes and is fascinating as usual.

The latest debate about SARS-CoV-2 is how concerned should we be about airborne transmission? Clearly, large droplets loaded with virus are something we all want to avoid. What about small particles, able to linger or travel in the air for some distance? A fight is brewing between a group of scientists and the WHO on this latter point. My bottom line is the ratio that yields personnel density; how many people are inside an enclosed space. The higher the density the greater the risk. I have provided some preprint and articles on building ventilation systems but those are not very useful if you are going a store or office where you don't know what type of HVAC system they employ or what the air exchange rate is. Mask wearing can help reduce the risk. IMO, it is advisable to go to stores during hours when there is low population density inside.

The Washington Post has a good story on contact tracing and what some states are doing. One thing (among many) that I find troubling is contact tracing and quarantine can be readily accomplished in small and moderate size population areas but there is no appetite to do so. Some of this is resource constraints and some political. The paper also has a nice analytical piece on the call for regulations by some hard hit industries. If we want an economic recovery that is not going to look like a yo-yo, this has to be taken seriously.

There are a fair number of clinical papers today along with a very good study on the conformational changes in the virus Spike protein.

MODELING

- The aim of this study is to assess exposure to airborne SARS-CoV-2 particles from breathing, speaking, coughing and sneezing in an indoor environment. Methods An exposure assessment model was developed to estimate numbers of SARS-CoV-2 particles in aerosol droplets, expelled during breathing, speaking, coughing and sneezing by an infected person in an unventilated indoor environment, and subsequent inhalation by one or more persons. Scenarios encompass a range of virus concentrations, room sizes and exposure times. Results The calculated total volume of expelled aerosol droplets was highest for a sneeze, followed by a cough and speaking for 20 minutes, and lastly breathing for 20 minutes. A few to as much as tens of millions of virus particles were expelled. Exposure probability strongly depends on the viral concentration in

mucus, as well as on the scenario. Exposure probabilities were generally below 1% at a virus concentration in mucus below 10^5 per mL for all scenarios, increasing steeply at different higher concentrations. According to nose / throat swab data collected from patients, 75%, 50% and 5% of infected individuals carry an estimated number of SARS-CoV-2 per mL mucus of at least 10^5, 10^6 and 10^8, respectively. Discussion Exposure to SARS-CoV-2 via aerosols generated during breathing, speaking, coughing and sneezing in an unventilated indoor environment is possible. This study forms a basis to estimate probabilities of exposure to SARS-Cov-2 by airborne transmission in indoor spaces. As long as it is uncertain what fraction of the airborne virus particles is infectious and as long as a dose response relation is lacking, it is recommended to be precautious. [**note: surprise!! A paper on aerosol generation right after I wrote this up in the general section of the newsletter. This is from Dutch researchers.**] https://www.medrxiv.org/content/10.1101/2020.07.02.20144832v1

- The COVID-19 pandemic has spread all over the globe. In the absence of a vaccine, a small number of countries have managed to control the diffusion of viruses by early detection and early quarantine. South Korea, one of the countries which have kept the epidemics well-controlled, has opened the infected patients' trajectory to the public. Such a reaction has been regarded as an effective method, however, serious privacy breach cases have been issued in South Korea. Furthermore, some suspected contacts have refused to take infection tests because they are afraid of being exposed. To solve this problem, we propose a privacy-preserving contact tracing method based on spatio-temporal trajectory which can be practically used in many quarantine systems. In addition, we develop a system to visualize the contact tracing workflow. [**note: a useful Korean paper on privacy protection and contact tracing**] https://www.medrxiv.org/content/10.1101/2020.06.29.20143180v1

NEWLY REGISTERED CLINICAL TRIALS

- Things are slowing down so I will only check every third day.

CLINICAL TRIAL RESULTS

- Summary Background Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a global pandemic of Coronavirus disease 2019 (COVID-19). However, microbial composition of the respiratory tract and other infected tissues, as well as their possible pathogenic contributions to varying degrees of disease severity in COVID-19 patients remain unclear. Method Between January 27 and February 26, 2020, serial clinical specimens (sputum, nasal and throat swab, anal swab and feces) were collected from a cohort of hospitalized COVID-19 patients, including 8 mildly and 15 severely ill patients (requiring ICU admission and mechanical ventilation), in the Guangdong province, China. Total RNA was extracted and ultra-deep metatranscriptomic sequencing was performed in combination with laboratory diagnostic assays. Co-infection rates, the prevalence and abundance of microbial communities in these COVID-19 patients were determined. Findings Notably, respiratory microbial co-infections were exclusively found in 84.6% of severely ill patients (11/13), among which viral and bacterial co-infections were detected by sequencing in 30.8% (4/13) and 69.2% (9/13) of the patients, respectively. In addition, for 23.1% (3/13) of the patients, bacterial co-infections with Burkholderia cepacia complex (BCC) and Staphylococcus epidermidis were also confirmed by bacterial culture. Further, a time-dependent, secondary infection of B. cenocepacia with

expressions of multiple virulence genes in one severely ill patient was demonstrated, which might be the primary cause of his disease deterioration and death one month after ICU admission. Interpretation Our findings identified distinct patterns of co-infections with SARS-CoV-2 and various respiratory pathogenic microbes in hospitalized COVID-19 patients in relation to disease severity. Detection and tracking of BCC-associated nosocomial infections are recommended to improve the pre-emptive treatment regimen and reduce fatal outcomes of hospitalized patients infected with SARS-CoV-2. [**note: this is data from China from early in the pandemic on nosocomial bacterial infections that might accompany SARS-CoV-2. There have been reports from other hospitals on this topic.**]
https://www.medrxiv.org/content/10.1101/2020.07.02.20143032v1

- We present here genetic risk factors for survivability from infection by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) responsible for coronavirus disease 19 (COVID-19). At the time of writing it is too early to determine comprehensively and without doubt all risk factors, but there is an urgency due to the global pandemic crisis that merits this early analysis. We have nonetheless discovered 5 novel risk variants in 4 genes, discovered by examining 193 deaths from 1,412 confirmed infections in a group of 5,871 UK Biobank participants tested for the virus. We also examine the distribution of these genetic variants across broad ethnic groups and compare it to data from the UK Office of National Statistics for increased risk of death from SARS-CoV-2. We confidently identify the gene ERAP2 with a high-risk variant, as well as three other genes of potential interest. Although mostly rare, a common theme of genetic risk factors affecting survival might be the inability to launch or modulate an effective immune and stress response to infection from the SARS-CoV-2 virus. [**Note: more good stuff from the UK Biobank! The paper is straight forward reading and provides a nice perspective. Recommended!**]
https://www.medrxiv.org/content/10.1101/2020.07.01.20144592v1
- Successful host defence against a pathogen can involve resistance or tolerance, with implications for prioritising either antimicrobial or immunomodulatory therapeutic approaches. Hyper-inflammation occurs in Covid-19 and is associated with worse outcomes. The efficacy of dexamethasone in preventing mortality in critical Covid-19 suggests that inflammation has a causal role in death. Whether this deleterious inflammation is primarily a direct response to the presence of SARS-CoV-2 requiring enhanced resistance, or an independent immunopathologic process necessitating enhanced tolerance, is unknown. Here we report an aberrant immune response in fatal Covid-19, principally involving the lung and reticuloendothelial system, that is not clearly topologically associated with the virus, indicating tissue-specific tolerance of SARS-CoV-2. We found that inflammation and organ dysfunction in fatal Covid-19 did not map to the widespread tissue and cellular distribution of SARS-CoV-2 RNA and protein, both between and within tissues. A monocyte/myeloid-rich vasculitis was identified in the lung, along with an influx of macrophages/monocytes into the parenchyma. In addition, stereotyped abnormal reticulo-endothelial responses (reactive plasmacytosis and iron-laden macrophages) were present and dissociated from the presence of virus in lymphoid tissues. *Our results support virus-independent immunopathology being one of the primary mechanisms underlying fatal Covid-19. This supports prioritising pathogen tolerance as a therapeutic strategy in Covid-19, by better understanding non-injurious organ-specific viral tolerance mechanisms and targeting aberrant macrophage and plasma cell responses*. [**note: from the UK and a very thorough pathological**

examination of tissue distribution of SARS-CoV-2 and damage.]
https://www.medrxiv.org/content/10.1101/2020.07.02.20145003v1

- The purpose of this study was to evaluate the effect of corticosteroids in non-intensive care unit (ICU) patients with COVID19 pneumonia complicated by acute hypoxemic respiratory failure (AHRF). Methods: This was a single center retrospective cohort study, comprising of 205 patients admitted to the general wards with COVID19 pneumonia. The primary outcome was a composite of ICU transfer, intubation, or inhospital mortality. Cox proportional hazard regression was implemented. Result: Among 205 patients, 60 (29.27%) were treated with corticosteroid. The mean age was ~57 years, and ~75% were men. Thirteen patients (22.41%) developed a primary composite outcome in the corticosteroid cohort vs. 54 (37.5%) patients in the non-corticosteroid cohort (P=0.039). The adjusted hazard ratio (HR) for the development of the composite primary outcome was 0.15 (95% CI, 0.07 to 0.33; P <0.001). The adjusted hazard ratio for ICU transfer was 0.16 (95% CI, 0.07 to 0.34; P < 0.001), intubation was 0.31 (95% CI, 0.14 to 0.70; P=0.005), death was 0.53 (95% CI, 0.22 to 1.31; P=0.172), and discharge was 3.65 (95% CI, 2.20 to 6.06; P<0.001). The corticosteroid cohort had increasing SpO2/FiO2 over time compared to the non-corticosteroid cohort who experience decreasing SpO2/FiO2 over time. Conclusion: Among non-ICU patients hospitalized with COVID-19 pneumonia complicated by AHRF, treatment with corticosteroid was associated with a significantly lower risk of the primary composite outcome of ICU transfer, intubation, or inhospital death. [**note: this is a single hospital retrospective study with 60 out of 205 patients treated with corticosteroids showing benefit.**] https://www.medrxiv.org/content/10.1101/2020.07.02.20145565v1
- The inflammatory response to COVID-19 infection in children remains poorly characterised. Methods We retrospectively analysed the medical records of 127 laboratory-confirmed COVID-19 patients aged 1 month to 16 years from Wuhan and Jingzhou of Hubei Province. Patients presented between January 25th and March 24th 2020. Information on clinical features, laboratory results, plasma cytokines/chemokines and lymphocyte subsets were analysed. Findings Children admitted to hospital with COVID-19 were more likely to be male (67.7%) and the median age was 7.3 [IQR 4.9] years. All but one patient with severe disease was aged under 2 and the majority (5/7) had significant co-morbidities. Despite 53% having viral pneumonia on CT scanning only 2 patients had low lymphocyte counts and no differences were observed in the levels of plasma proinflammatory cytokines, including interleukin (IL)-2, IL-4, IL-6, tumour necrosis factor (TNF)-alpha; and interferon (IFN)-gamma; between patients with mild, moderate or severe disease. Interpretations We demonstrated that the immune responses of children to COVID-19 infection is significantly different from that seen in adults. *Our evidence suggests that SARS-CoV-2 does not trigger a robust inflammatory response or "cytokine storm" in children with COVID-19, and this may underlie the generally better outcomes seen in children with this disease. These data also imply anti-cytokine therapies may not be effective in children with moderate COVID-19*. [**note: useful information from Hubei province on the different inflammatory response observed in children versus adults.]**
https://www.medrxiv.org/content/10.1101/2020.07.02.20145110v1
- With the progress of COVID-19 studies, it became evident that SARS-CoV-2 infection is often associated with thrombotic complications. The goal of our present study was to evaluate which component of clot formation process including endothelial function, platelets aggregation and plasma coagulation, as well as endogenous fibrinolysis in patients with COVID-19 correlates with

the severity of the disease. We prospectively included 58 patients with COVID-19 and 47 healthy volunteers as a control group that we recruited before the pandemic started. It turns out that plasma coagulation with subsequent platelet aggregation, but not endothelial function, correlates with the severity of the COVID-19. IL-6 blockade may play a beneficial role in COVID-19 induced coagulopathy. [**note: a small sample from Russia looking at coagulation problems in COVID-19, IL-6 blockade may prove advantageous in treating this problem.**] https://www.medrxiv.org/content/10.1101/2020.07.02.20145284v1

DRUG DEVELOPMENT

- Nothing today.

VIRUS BIOCHEMISTRY

- Virus genome sequence variants that appear over the course of an outbreak can be exploited to map the trajectory of the virus from one susceptible host to another. While such variants are usually of no functional significance, in some cases they may allow the virus to transmit faster, change disease severity, or confer resistance to antiviral therapies. Since the discovery of SARS-CoV-2 as the cause of COVID-19, the virus has spread around the globe, and thousands of SARS-CoV-2 genomes have been sequenced. The rate of sequence variation among SARS-CoV-2 isolates is modest for an RNA virus but the enormous number of human-to-human transmission events has provided abundant opportunity for selection of sequence variants. Among these, the SARS-CoV-2 Spike protein variant, D614G, was not present in the presumptive common ancestor of this zoonotic virus, but was first detected in late January in Germany and China. The D614G variant steadily increased in frequency and now constitutes >97% of isolates world-wide, raising the question whether D614G confers a replication advantage to SARS-CoV-2. Structural models predict that D614G would disrupt contacts between the S1 and S2 domains of the Spike protein and cause significant shifts in conformation. Using single-cycle vectors we showed that D614G is three to nine-fold more infectious than the ancestral form on human lung and colon cell lines, as well as on other human cell lines rendered permissive by ectopic expression of human ACE2 and TMPRSS2, or by ACE2 orthologues from pangolin, pig, dog, or cat. Nonetheless, monoclonal antibodies targeting the receptor binding domain of the SARS-CoV-2 Spike protein retain full neutralization potency. These results suggest that D614G was selected for increased human-to-human transmission, that it contributed to the rapidity of SARS-CoV-2 spread around the world, and that it does not confer resistance to antiviral therapies targeting the receptor binding domain. [**note: more information on the current dominant SARS-CoV-2 mutation. It may be more infectious, but the genetic change does not alter the immune epitope(s).**] https://www.biorxiv.org/content/10.1101/2020.07.04.187757v1
- SARS-CoV-2 has emerged as a global pathogen, sparking urgent vaccine development efforts with the trimeric spike. However, the inability of antibodies like CR3022, which binds a cryptic spike epitope with nanomolar affinity, to neutralize virus, suggests a spike-based means of neutralization escape. Here, we show the SARS-CoV-2 spike to have 10% the unfolding enthalpy of a globular protein at physiological pH, where it is recognized by antibodies like CR3022, and up to 10-times more unfolding enthalpy at endosomal pH, where it sheds such antibodies, suggesting that the spike evades potentially neutralizing antibody through a pH-dependent mechanism of conformational masking. To understand the compatibility of this mechanism with

ACE2-receptor interactions, we carried out binding measurements and determined cryo-EM structures of the spike recognizing up to three ACE2 molecules at both physiological and endosomal pH. In the absence of ACE2, cryo-EM analyses indicated lower pH to reduce conformational heterogeneity. Single-receptor binding domain (RBD)-up conformations dominated at pH 5.5, resolving into a locked all-down conformation at lower pH through lowering of RBD and refolding of a pH-dependent switch. Notably, the emerging Asp614Gly strain partially destabilizes the switch that locks RBD down, thereby enhancing functional interactions with ACE2 while reducing evasion by conformational masking. [**note: this is a very complex paper. 'I think' the issue is that shifting to a lower pH environment such as is in the endosome allows the virus to shed bound antibody. They note many recently identified potent neutralizing antibodies appear to recognize this conformational change and suggest that this also be a spike vaccine target. This explanation does not do justice to the paper and you should read it for the details.**]
https://www.biorxiv.org/content/10.1101/2020.07.04.187989v1

DIAGNOSTIC DEVELOPMENT

- Nothing really novel.