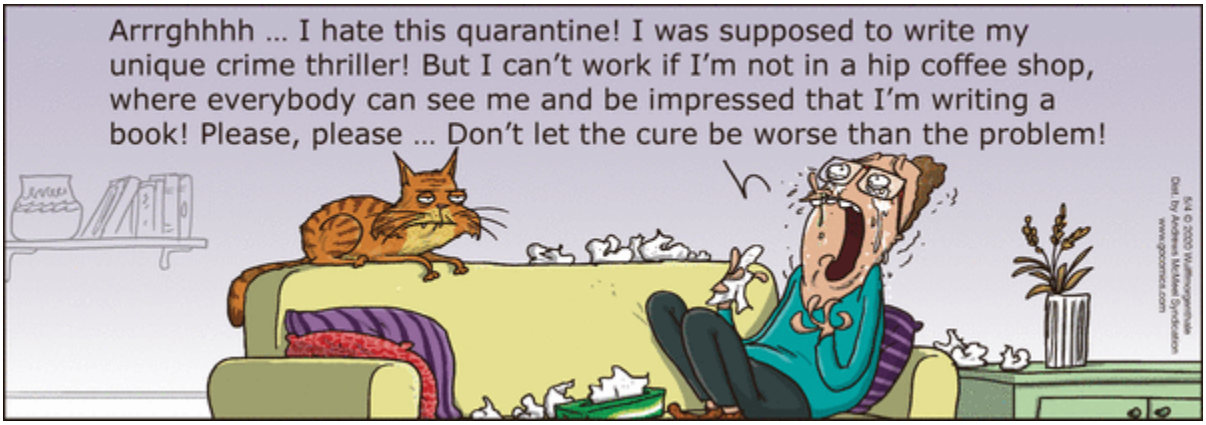
2020-05-04

COVID-19 Colleagues – here we are into week 7 of the newsletter. Let's hope for some encouraging news this week!! Best of all WuMo is back on the COVID case!!



I first heard Keith Jarrett when he played with Charles Lloyd at the Monterrey Jazz festival in 1966 (I was there!!). I thought he was a very special pianist and was not proven wrong. He went on to play a number solo concerts, the most famous of which was in Cologne Germany where they forgot to bring the correct piano in. Jarrett adapted and that evening's recording became one of the bestselling in jazz history. Here is Jarrett in Tokyo with some encores of popular songs: https://www.youtube.com/watch?v=Eqr0ZmgmDr4 There is a good podcast by Tim Harford about the Cologne recording. You cannot say I am not keeping you all entertained with all types of arcana.

A light reading day!!!

MODELING

- Background: While the mechanisms of adaptive immunity to pandemic coronavirus SARS-CoV-2 are still unknown, the immune response to the widespread endemic coronaviruses HKU1, 229E, NL63 and OC43 provide a useful reference for understanding repeat infection risk. Methods: Here we used data from proactive sampling carried out in New York City from fall 2016 to spring 2018. We combined weekly nasal swab collection with self-reports of respiratory symptoms from 191 participants to investigate the profile of recurring infections with endemic coronaviruses. Results: During the study, 12 individuals tested positive multiple times for the same coronavirus. We found no significant difference between the probability of testing positive at least once and the probability of a recurrence for the beta-coronaviruses HKU1 and OC43 at 34 weeks after enrollment/first infection. We also found no significant association between repeat infections and symptom severity but strong association between symptom severity and belonging to the same family. Conclusion: This study provides evidence that re-infections with the same endemic coronavirus are not atypical in a time window shorter than 1 year and that the genetic basis of innate immune response may be a greater determinant of infection severity than immune memory acquired after a previous infection. [**note: it is a small data set, but an**

interesting finding regarding coronavirus reinfection.]
https://www.medrxiv.org/content/10.1101/2020.04.27.20082032v1

NEWLY REGISTERED CLINICAL TRIALS

- Here is a small trial of duvelisib. The exceedingly high mortality rates of severe and critical COVID-19 warrant the identification and evaluation of novel therapies that could potentially mitigate the advanced disease manifestations. Based on preclinical data from this institution and others, the investigators hypothesize that PI3K inhibition with duvelisib could potentially quell aberrant hyperactivtation of the innate immune system, preferentially polarize macrophages, reduce pulmonary inflammation, and limit viral persistence, thereby improving patient outcomes. NCT04372602
- The Biontech SE/Pfizer vaccine trial has been registered. This is a Phase 1/2, randomized, placebo-controlled, observer-blind, dose-finding, and vaccine candidate-selection study in healthy adults. NCT04368728
- Why not try doxycycline? This is just what a French group is doing. The aim of the study is to compare a treatment with doxycycline vs a placebo as soon as the patient is confirmed COVID-19 + and before the onset of oxygen dependence with the aim of reducing or even abolishing the cytokine explosion and thus the evolution towards a serious form of the disease which can lead to death. Three criteria support the rational use of tetrcycline in COVI-19 (1) The coronaviruses is known to bind to metalloproteases (MMPs) of the host, in particular to ensure viral survival. Tetracyclines are known to chelate zinc from MMPs. Their chelating activity may help inhibit COVID19 infection by limiting its ability to replicate in the host. (2) Tetracyclines may also be able to inhibit the replication of positive-polarity single-stranded RNA viruses, such as COVID19 (demonstrated on the dengue virus). (3) In addition, tetracyclines are modulators of innate immunity (anti-inflammatory activity), a property used in the treatment of inflammatory skin diseases for many years. These modulating effects are noted on several targets of innate immunity: They can decrease the expression of NFKB, the release of inflammatory cytokines such as TNF-α, IL-1β and IL-6, inhibit granulomas inflammatory and free radical release. [**note: I like this drug as it's a good prophylactic treatment following a tick bite when one lives in a Lyme Disease endemic area. One dose of 200mg is all you need!!!**] NCT04371952
- The severe acute respiratory syndrome caused by COVID-19 is now a global catastrophic event. Currently there is no approved drug or vaccine for the disease. Methylene blue (MB, oxidized form, blue color) has been used in many different areas of clinical medicine, ranging from malaria to orthopedics. Leucomethylene Blue (reduced form of MB, colorless) may be applied for the treatment of COVID-19 according to the scientific evidences. [**note: this meets with my criteria, "throw a lot of stuff at the wall and see what sticks!"** NCT04370288
- Will aspirin work?? COVID-19 has a high infection rate and mortality, and serious complications such as heart injury cannot be ignored. Cardiac dysfunction occurred in COVID-19 patients, but the law and mechanism of cardiac dysfunction remains unclear. The occurrence of progressive inflammatory factor storm and coagulation dysfunction in severe and fatal cases of NCP points out a new direction for reducing the incidence of severe and critically ill patients, shortening the length of duration in severe and critically ill patients and reducing the incidence of complications of cardiovascular diseases. Aspirin has the triple effects of inhibiting virus replication, anticoagulant and anti-inflammatory, but it has not received attention in the treatment and

prevention of NCP. Although Aspirin is not commonly used in the guidelines for the treatment of NCP, it was widely used in the treatment and prevention of a variety of human diseases after its first synthesis in 1898. Subsequently, aspirin has been confirmed to have antiviral effect on multiple levels. Moreover, one study has confirmed that aspirin can inhibit virus replication by inhibiting prostaglandin E2 (PGE2) in macrophages and upregulation of type I interferon production. Subsequently, pharmacological studies have found that aspirin as an anti-inflammatory and analgesic drug by inhibiting cox-oxidase (COX). Under certain conditions, the platelet is the main contributor of innate immune response, studies have found that in the lung injury model in dynamic neutrophil and platelet aggregation. [**note: this might be the cheapest pharmaceutical intervention under study**] NCT04365309

- And yet another aspirin trial, this time with Vitamin D!! Although the novel SARS-CoV-2 virus (COVD-19) is classified as an acute respiratory infection, emerging data show that morbidity and mortality are driven by disseminated intravascular coagulopathy. Untreated CAC leads to microangiopathic thromboses, causing multiple systems organ failure and consuming enormous healthcare resources. Identifying strategies to prevent CAC are therefore crucial to reducing COVID-19 hospitalization rates. The pathogenesis of CAC is unknown, but there are major overlaps between severe COVID-19 and vitamin D insufficiency (VDI). We hypothesize that VDI is a major underlying contributor to CAC. Preliminary data from severe COVID-19 patients in New Orleans support this hypothesis. The purpose of the proposed multi-center, prospective, randomized controlled trial is to test the hypothesis that low-risk, early treatment with aspirin and vitamin D in COVID-19 can mitigate the prothrombotic state and reduce hospitalization rates. [**note: this trial uses low dose aspirin. We now have trials of all three major OTC anti-inflammatory drugs.**] NCT04363840

CLINICAL TRIAL RESULTS

- Nothing new.

DRUG DEVELOPMENT

- Will the Chinese be the first to develop a therapeutic mAb? Neutralizing antibody is one of the most effective interventions for acute pathogenic infection. Currently, over three million people have been identified for SARS-CoV-2 infection but SARS-CoV-2-specific vaccines and neutralizing antibodies are still lacking. SARS-CoV-2 infects host cells by interacting with angiotensin converting enzyme-2 (ACE2) via the S1 receptor-binding domain (RBD) of its surface spike glycoprotein. Therefore, blocking the interaction of SARS-CoV-2-RBD and ACE2 by antibody would cause a directly neutralizing effect against virus. In the current study, we selected the ACE2 interface of SARS-CoV-2-RBD as the targeting epitope for neutralizing antibody screening. We performed site-directed screening by phage display and finally obtained one IgG antibody (4A3) and several domain antibodies. Among them, 4A3 and three domain antibodies (4A12, 4D5, and 4A10) were identified to act as neutralizing antibodies due to their capabilities to block the interaction between SARS-CoV-2-RBD and ACE2-positive cells. The domain antibody 4A12 was predicted to have the best accessibility to all three ACE2-interfaces on the spike homotrimer. Pseudovirus and authentic SARS-CoV-2 neutralization assays showed that all four antibodies could potently protect host cells from virus infection. Overall, we isolated multiple formats of SARS-CoV-2-neutralizing antibodies via site-directed antibody screening, which could

be promising candidate drugs for the prevention and treatment of COVID-19. [**note: I wonder how this compares with the Regeneron approach?**]
https://www.biorxiv.org/content/10.1101/2020.05.03.074914v1

DIAGNOSTIC DEVELOPMENT

- Nothing much to offer here other than to note FDA has now issued 10 Emergency Use Authorizations for serology tests. All are for laboratory use and not for use in the field.

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2020-05-05

Did I miss posting a music video of out Nobel Laureate poet of the people? Yes, none other than the iconic Bob Dylan!! Before you ask, I was indeed at one of the 1974 performances of Bob and The Band, a truly memorable evening in a packed Assembly Hall at Indiana University (IIRC, it was about 2-3 weeks after I saw Luciano Pavarotti in recital, what a pair of artists). Here is a youthful Dylan on Canadian TV: https://www.youtube.com/watch?v=IJCmgKRszYM Also, Dylan's Nobel Prize acceptance speech is well worth listening to. I finally understand Moby Dick now. **It's time for another contest!!!! Who is the mysterious girl he is singing about? She might have even been on an album cover! I really want to give away prizes!**

It looks like FDA has finally decided to take a hard line on the distribution of serology tests that have not been given an Emergency Use Authorization.

Finally some REALLY GOOD NEWS! No, we still don't have a vaccine or cure for SARS-CoV-2 but we now have some evidence that filtered coffee may be good for heart health!!! A lengthy 20 year Norwegian study of over 500K men and women showed drinking filtered coffee was associated with a 15 percent reduction in the risk of dying prematurely. Rates were lower for unfiltered coffee. [**note: Good think I have three years supply of Melita filters as I expect there to be a huge run on these! Here is a handy household tip. Are you tired of your flimsy Melita coffee pot always cracking or breaking? Not to worry, You can use a 4 Cup Pyrex measuring cup. The lids for large Ziploc containers are just the right size to fit over the measuring cup. Just cut out a hole big enough to hold the filter cone and you are all set. You will also find that the lid to the broken coffee pot can also be repurposed here. Just invert it and it should fit nicely in the hole you cut. You now have a coffee pot that will last almost forever! Maybe I need to rethink things and start writing a kitchen advice column.**]

Oh dear, another world leader is speaking out about an unproven drug for SARS-CoV-2. This time it is favipiravir which has the same nasty safety profile as thalidomide. Favipiravir is a last line treatment against influenza and is being studied in a number of trials including a few in the United States.

MODELING

- We develop a multi-risk SIR model (MR-SIR) where infection, hospitalization and fatality rates vary between groups—in particular between the "young", "the middle-aged" and the "old". Our MR-SIR model enables a tractable quantitative analysis of optimal policy similar to those already

developed in the context of the homogeneous-agent SIR models. For baseline parameter values for the COVID-19pandemic applied to the US, we find that optimal policies differentially targeting risk/age groups significantly outperform optimal uniform policies and most of the gains can be realized by having stricter lockdown policies on the oldest group. For example, for the same economic cost (24.3% decline in GDP), optimal semi–targeted or fully-targeted policies reduce mortality from 1.83% to 0.71% (thus, saving 2.7 million lives) relative to optimal uniform policies. Intuitively, a strict and long lockdown for the most vulnerable group both reduces infections and enables less strict lockdowns for the lower-risk groups. We also study the impacts of social distancing, the matching technology, the expected arrival time of a vaccine, and testing with or without tracing on optimal policies. Overall, targeted policies that are combined with measures that reduce interactions between groups and increase testing and isolation of the infected can minimize both economic losses and deaths in our model. [**note: I include this paper as I have great respect for Daron Acemoglu having read several of his books.**] https://www.nber.org/papers/w27102.pdf

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- Here is some interesting data from the Michigan epicenter. The disease progression associated with the pro-inflammatory host response prompted us to examine the role of early corticosteroid therapy in patients with moderate to severe COVID-19. Methods: We conducted a single pre-test, single post-test quasi-experiment in a multi-center health system in Michigan from March 12 to March 27, 2020. Adult patients with confirmed moderate to severe COVID were included. A protocol was implemented on March 20, 2020 using early, short-course, methylprednisolone 0.5 to 1 mg/kg/day divided in 2 intravenous doses for 3 days. Outcomes of pre- and post-corticosteroid groups were evaluated. A composite endpoint of escalation of care from ward to ICU, new requirement for mechanical ventilation, and mortality was the primary outcome measure. All patients had at least 14 days of follow-up. Results: *We analyzed 213 eligible subjects, 81 (38%) and 132 (62%) in pre-and post-corticosteroid groups, respectively. The composite endpoint occurred at a significantly lower rate in post-corticosteroid group compared to pre-corticosteroid group (34.9% vs. 54.3%, p=0.005). This treatment effect was observed within each individual component of the composite endpoint. Significant reduction in median hospital length of stay was observed in the post-corticosteroid group (8 vs. 5 days, p < 0.001).* Multivariate regression analysis demonstrated an independent reduction in the composite endpoint at 14-days controlling for other factors (aOR: 0.45; 95% CI [0.25-0.81]). Conclusion: An early short course of methylprednisolone in patients with moderate to severe COVID-19 reduced escalation of care and improved clinical outcomes. [**note: yes it's small trial but the reduction in hospital stay is the same as in the widely touted remdesivir study!!! This drug is much cheaper! Should this be the new standard of care??**] https://www.medrxiv.org/content/10.1101/2020.05.04.20074609v1
- More on Vitamin D!! If vitamin D deficiency is associated to incidence or severity of SARS-CoV-2 infection, a global call could be made for vitamin D supplementation to mitigate the pandemic. Objective: to determine if lower serum 25-hydroxyvitamin D (25(OH)D) levels are correlated to

the risk for COVID-19 and its severity as measured by CT Design: single-center observational study Setting: AZ Delta general hospital Participants: 186 consecutive patients with PCR-confirmed SARS-CoV-2 infection hospitalized for COVID-19 from March 1, 2020 to April 7, 2020 Main outcome and measures: comparative analysis of 25(OH)D levels in patients hospitalized for COVID-19 at various radiological stages and a season/age/sex-matched diseased control population Results: we report on 186 SARS-CoV-2 infected patients requiring hospitalization for severe COVID-19: 109 males (median age 68 years, IQR 53-79 years) and 77 females (median age 71 years, IQR 65-74 years). At admission patients were screened by CT to determine temporal changes of COVID-19 lung disease and classified as stage 1 (ground glass opacities), 2 (crazy paving pattern) and 3 (consolidation). At intake, 25(OH)D levels were measured and compared to a season-matched population of 2717 diseased controls, consisting of 999 males (median age 69 years, IQR 53-81 years) and 1718 females (median age 68 years, IQR 43-83 years). Male and female COVID-19 patients combined showed lower median 25(OH)D than controls (18.6 ng/mL, IQR 12.6-25.3, versus 21.5 ng/mL, IQR 13.9-30.8; P=0.0016) and a higher fraction of vitamin D deficiency (58.6% versus 45.2%, P=0.0005). A strong sexual dimorphism was found: female patients had comparable vitamin D status as control females. Male COVID-19 patients, however, showed markedly higher percentage of vitamin D deficiency than controls (67.0% versus 49.2%, P=0.0006) and this effect was more pronounced with advanced radiological stage ranging from 55.2% in stage 1 to 74% in stage 3. Conclusions and relevance: vitamin D deficiency is a possible risk factor for severe SARS-CoV-2 infection in males. Vitamin D supplementation might be an inexpensive, accessible and safe mitigation for the SARS-CoV-2 pandemic.
https://www.medrxiv.org/content/10.1101/2020.05.01.20079376v1

- Anti-cytokine treatments are being evaluated but optimal patient selection remains unclear. Methods Between February 29 to April 6, 2020, 111 consecutive hospitalized patients with COVID-19 pneumonia were evaluated in a single centre retrospective study. Patients were divided in two groups: 42 severe cases (TOCI) with adverse prognostic features including raised CRP and IL-6 levels, who underwent anti-cytokine treatments, mostly tocilizumab, and 69 standard of care patients (SOC). Findings In the TOCI group, all received anti-viral therapy and 40% also received glucocorticoids. In TOCI, 62% of cases were ventilated and there were 3 deaths (17.8+/-10.6 days, mean follow up) with 7/26 cases remaining on ventilators, without improvement, and 17/26 developed bacterial superinfection. One fatality occurred in the 15 TOCI cases treated on noninvasive ventilation and 1 serious bacterial superinfection. Of the 69 cases in SOC, there was no fatalities and no bacterial complications. The TOCI group had higher baseline CRP and IL-6 elevations ($p<0.0001$ for both) and higher neutrophils and lower lymphocyte levels ($p= 0.04$ and $p=0.001$, respectively) with the TOCI ventilated patients having higher markers than non-ventilated TOCI patients. Interpretation Higher inflammatory markers, more superimposed infections and worse outcomes characterized ventilated TOCI cases compared to ward based TOCI therapy. Despite the confounding factors, this study suggests that therapy time in anti-cytokine randomized clinical trials will be key. [**note: this report is a real mess! I don't think much can be concluded because of the co-administration of other therapies. We still await good data on tocilizumab**.]
https://www.medrxiv.org/content/10.1101/2020.05.01.20078360v1
- Here is the first report on chloroquine from China. Background Effective therapies are urgently needed for the SARS-CoV-2 pandemic. Chloroquine has been proved to have antiviral effect

against coronavirus in vitro. In this study, we aimed to assess the efficacy and safety of chloroquine with different doses in COVID-19. Method In this multicenter prospective observational study, we enrolled patients older than 18 years old with confirmed SARS-CoV-2 infection excluding critical cases from 12 hospitals in Guangdong and Hubei Provinces. Eligible patients received chloroquine phosphate 500mg, orally, once (half dose) or twice (full dose) daily. Patients treated with non-chloroquine therapy were included as historical controls. The primary endpoint is the time to undetectable viral RNA. Secondary outcomes include the proportion of patients with undetectable viral RNA by day 10 and 14, hospitalization time, duration of fever, and adverse events. Results A total of 197 patients completed chloroquine treatment, and 176 patients were included as historical controls. The median time to achieve an undetectable viral RNA was shorter in chloroquine than in non-chloroquine (absolute difference in medians -6.0 days; 95% CI -6.0 to -4.0). The duration of fever is shorter in chloroquine (geometric mean ratio 0.6; 95% CI 0.5 to 0.8). No serious adverse events were observed in the chloroquine group. Patients treated with half dose experienced lower rate of adverse events than with full dose. Conclusions Although randomised trials are needed for further evaluation, this study provides evidence for safety and efficacy of chloroquine in COVID-19 and suggests that chloroquine can be a cost-effective therapy for combating the COVID-19 pandemic. [**note: I had to go and read the paper to find out that the treatment group reduced hospitalization by ONE day (20->19). Not a wonder drug in my mind.**]
https://www.medrxiv.org/content/10.1101/2020.04.26.20081059v1

- Here is a large cohort study of patients in the Mt. Sinai Health System. To assess association of clinical features on patient outcomes. Design, Setting, and Participants: In this observational case series, patient-level data were extracted from electronic medical records for 28,336 patients tested for SARS-CoV-2 at the Mount Sinai Health System from 2/24/ to 4/15/2020, including 6,158 laboratory-confirmed cases. Exposures: Confirmed COVID-19 diagnosis by RT-PCR assay from nasal swabs. Main Outcomes and Measures: Effects of race on positive test rates and mortality were assessed. Among positive cases admitted to the hospital (N = 3,273), effects of patient demographics, hospital site and unit, social behavior, vital signs, lab results, and disease comorbidities on discharge and death were estimated. Results: Hispanics (29%) and African Americans (25%) had disproportionately high positive case rates relative to population base rates (p<2e-16); however, no differences in mortality rates were observed in the hospital. Outcome differed significantly between hospitals (Gray's T=248.9; p<2e-16), reflecting differences in average baseline age and underlying comorbidities. Significant risk factors for mortality included age (HR=1.05 [95% CI, 1.04-1.06]; p=1.15e-32), oxygen saturation (HR=0.985 [95% CI, 0.982-0.988]; p=1.57e-17), care in ICU areas (HR=1.58 [95% CI, 1.29-1.92]; p=7.81e-6), and elevated creatinine (HR=1.75 [95% CI, 1.47-2.10]; p=7.48e-10), alanine aminotransferase (ALT) (HR=1.002, [95% CI 1.001-1.003]; p=8.86e-5) and body-mass index (BMI) (HR=1.02, [95% CI 1.00-1.03]; p=1.09e-2). Asthma (HR=0.78 [95% CI, 0.62-0.98]; p=0.031) was significantly associated with increased length of hospital stay, but not mortality. Deceased patients were more likely to have elevated markers of inflammation. Baseline age, BMI, oxygen saturation, respiratory rate, white blood cell (WBC) count, creatinine, and ALT were significant prognostic indicators of mortality. Conclusions and Relevance: While race was associated with higher risk of infection, we did not find a racial disparity in inpatient mortality suggesting that outcomes in a single tertiary care health system are comparable across races. We identified clinical features

associated with reduced mortality and discharge. These findings could help to identify which COVID-19 patients are at greatest risk and evaluate the impact on survival. [**note: we are learning more about the markers that lead to a poor outcome.**] https://www.medrxiv.org/content/10.1101/2020.04.28.20075788v1

- More data from Italy on tocilizumab. Tocilizumab is used for treating moderate-severe Covid-19 pneumonia by targeting IL-6 receptors (IL-6R) and reducing cytokine release, but the pooled rate ratio among diabetic patients with adverse vs those with the more favorable course was 2.26. To date, the hyperglycemia has been shown to increase IL-6 and IL-6R, which has been suggested as a severity predictor in lung diseases of Covid-19 patients. However, there are no data about the effects of tocilizumab therapy on outcomes of hyperglycemic Covid-19 patients with pneumonia. To investigate this unsolved need, 475 Covid-19 positive patients were retrospectively studied since March 1st, 2020. Among them, 78 patients with pneumonia disease and treated with tocilizumab were further evaluated for a severe outcome (encompassing both the use of mechanical ventilation and/or death). Thirty-one (39.7%) hyperglycemic and 47 (60.3%) normoglycemic Covid-19 positive patients (blood glucose levels >140 mg/dl, at admission and/or during hospital stay) were evaluated. Noteworthy, 20 (64%) of hyperglycemic and 11 (23.4%) of normoglycemic patients were also diabetics (P<0.01). At admission, more elevated IL-6 levels in hyperglycemic patients were found and persists even after Tocilizumab administration. In a risk adjusted Cox-regression analysis, Tocilizumab in hyperglycemic did not attenuate the risks of severe outcome as did in normoglycemic patients (p<0.009). Therefore, we could conclude that reduced effects of Tocilizumab in hyperglycemic patients may due to the higher plasma IL-6 levels. Interestingly, when we added IL-6 levels in a Cox regression model the significance for the tocilizumab effect was lost (p<0.07). In this context, our observations evidence that optimal Covid-19 infection management with tocilizumab is not achieved during hyperglycemia both in diabetic and non-diabetic patients. [**note: looks like we need to wait for the conclusive clinical trials!**] https://www.medrxiv.org/content/10.1101/2020.04.29.20076570v1

DRUG DEVELOPMENT

- Don't know if this is the right category for this one, but it is an important finding if blood for transfusion comes from a donor who might be carrying SARS-CoV-2. The ability of this agent to be transmitted by blood transfusion has not been documented, although viral RNA has been detected in serum. Exposure to treatment with riboflavin and ultraviolet light (R + UV) reduces blood-borne pathogens while maintaining blood product quality. Here, we report on the efficacy of R + UV in reducing SARS-CoV-2 infectivity when tested in human plasma and whole blood products. STUDY DESIGN AND METHODS: SARS-CoV-2 (isolate USA-WA1/2020) was used to inoculate plasma and whole blood units that then underwent treatment with riboflavin and UV light (Mirasol Pathogen Reduction Technology System, Terumo BCT, Lakewood, CO). The infectious titers of SARS-CoV-2 in the samples before and after R + UV treatment were determined by plaque assay on Vero cells. Each plasma pool (n=9) underwent R + UV treatment performed in triplicate using individual units of plasma and then repeated using individual whole blood donations (n=3). RESULTS: Riboflavin and UV light reduced the infectious titer of SARS-CoV-2 below the limit of detection for plasma products at 60-100% of the recommended energy dose. At the UV light dose recommended by the manufacturer, the mean log reductions in the

viral titers were greater than or equal to 4.79 +/- 0.15 Logs in plasma and 3.30 +/- 0.26 in whole blood units. CONCLUSION: Riboflavin and UV light effectively reduced the titer of SARS-CoV-2 to the limit of detection in human plasma and by 3.30 +/- 0.26 on average in whole blood. Two clades of SARS-CoV-2 have been described and questions remain about whether exposure to one strain confers strong immunity to the other. Pathogen-reduced blood products may be a safer option for critically ill patients with COVID-19, particularly those in high-risk categories. https://www.biorxiv.org/content/10.1101/2020.05.03.074971v1

- Here we present the crystal structure of SARS-CoV-2 main protease (Mpro) covalently bound to 2-methyl-1-tetralone. This complex was obtained by co-crystallization of Mpro with HEAT (2-(((4-hydroxyphenethyl)amino)methyl)-3,4-dihydronaphthalen-1(2H)-one) in the framework of a large X-ray crystallographic screening project of Mpro against a drug repurposing library, consisting of 5632 approved drugs or compounds in clinical phase trials. Further investigations showed that HEAT is cleaved by Mpro in an E1cB-like reaction mechanism into 2-methylene-1-tetralone and tyramine. The catalytic Cys145 subsequently binds covalently in a Michael addition to the methylene carbon atom of 2-methylene-1-tetralone. According to this postulated model HEAT is acting in a pro-drug-like fashion. It is metabolized by Mpro, followed by covalent binding of one metabolite to the active site. The structure of the covalent adduct elucidated in this study opens up a new path for developing non-peptidic inhibitors. [**note: a lot of authors on this paper and I'm always a sucker for some good chemistry. They find the comound covalently binds to the Mpro and might serve to inform about new drug approaches.**] https://www.biorxiv.org/content/10.1101/2020.05.02.043554v1
- The main protease (Mpro) in SARS-CoV-2 is a viable drug target because of its essential role in the cleavage of the virus polypeptide and subsequent viral replication. Feline infectious peritonitis, a fatal infection in cats caused by a coronavirus, was successfully treated previously with a dipeptide-based protease inhibitor. Here we show this drug, GC376, and its analog GC373, are effective inhibitors of the Mpro from both SARS-CoV and SARS-CoV-2 with IC50 values in the nanomolar range. Crystal structures of the SARS-CoV and SARS-CoV-2 Mpro with these inhibitors have a covalent modification of the nucleophilic Cys145. NMR analysis reveals that inhibition proceeds via reversible formation of a hemithioacetal. GC373 and GC376 are potent inhibitors of SARS-CoV-2 in cell culture, with EC50 values near one micromolar and little to no toxicity. These protease inhibitors are soluble, non-toxic, and bind reversibly. They are strong drug candidates for the treatment of human coronavirus infections because they have already been successful in animals (cats). The work here lays the framework for their use in human trials for the treatment of COVID-19. [**note: maybe this cat drug will help out. It seems to have a good profile.**] https://www.biorxiv.org/content/10.1101/2020.05.03.073080v1

DIAGNOSTIC DEVELOPMENT

- We have developed a reverse-transcriptase loop mediated amplification (RT-LAMP) method targeting genes encoding the Spike (S) protein and RNA-dependent RNA polymerase (RdRP) of SARS-CoV-2. The LAMP assay achieves the same limit of detection as commonly used RT-PCR protocols based on artificial targets, recombinant Sindbis virus, and clinical samples. Clinical validation of single target LAMP (N=108) showed a positive percent agreement (PPA) of 33/34 (97.1%) and negative percent agreement (NPA) of 73/74 (98.6%) compared to reference RT-PCR. Dual target RT-LAMP achieved a PPA of 11/11 (100%) and NPA 13/13 (100%) when including

discrepant samples. The assay can be performed without a formal extraction procedure, with lyophilized reagents that do need cold chain, and is amenable to point-of-care application with visual detection. https://www.medrxiv.org/content/10.1101/2020.04.29.20075747v1

- Background Serological testing for SARS-CoV-2 IgG antibodies is needed to document the community prevalence and distribution of the virus, particularly since many individuals have mild symptoms and cannot access molecular diagnostic testing of naso-pharyngeal swabs. However, the requirement for serum/plasma limits serological testing to clinical settings where it is feasible to collect and process venous blood. To address this problem we developed a serological test for SARS-CoV-2 IgG antibodies that requires only a single drop of capillary whole blood, collected from a simple finger prick and dried on filter paper (dried blood spot, DBS). Methods Enzyme linked immunosorbent assay (ELISA) was optimized to detect SARS-CoV-2 IgG antibodies against the receptor-binding domain (RBD) of the spike protein. DBS samples were eluted overnight and transferred to a 96-well plate coated with antigen, and anti-human IgG-HRP was used to generate signal in proportion to bound antibody. DBS samples spiked with anti-SARS IgG antibody, and samples from known positive and negative cases, were compared to evaluate assay performance. Results Analysis of samples with known concentrations of anti-SARS IgG produced the expected pattern of dose-response. Optical density (OD) values were significantly elevated for known positive cases in comparison with samples from unexposed individuals. Discussion DBS ELISA provides a minimally-invasive alternative to venous blood collection that combines the convenience of sample collection in the home or non-clinical setting with the accuracy of ELISA in the lab. Serological testing for SARS-CoV-2 IgG antibodies in DBS samples should facilitate research across a wide range of community- and population-based settings on seroprevalence, predictors and duration of antibody responses, as well as correlates of protection from reinfection, each of which is critically important for pandemic control. [**note: of course this approach is not a good one for making critical policy decisions but it at lease is amenable to large scale community testing, something we are not doing right now.**] https://www.medrxiv.org/content/10.1101/2020.04.28.20081844v1
- We design a procedure (the complete Python code may be obtained at: https://github.com/abhishta91/antibody_montecarlo) using Monte Carlo (MC) simulation to establish the point estimators described below and confidence intervals for the base rate of occurrence of an attribute (e.g., antibodies against Covid-19) in an aggregate population (e.g., medical care workers) based on a test. The requirements for the procedure are the test's sample size (N) and total number of positives (X), and the data on test's reliability. The modus is the prior which generates the largest frequency of observations in the MC simulation with precisely the number of test positives (maximum-likelihood estimator). The median is the upper bound of the set of priors accounting for half of the total relevant observations in the MC simulation with numbers of positives identical to the test's number of positives. Our rather preliminary findings are: The median and the confidence intervals suffice universally; The estimator X/N may be outside of the two-sided 95% confidence interval; Conditions such that the modus, the median and another promising estimator which takes the reliability of the test into account, are quite close; Conditions such that the modus and the latter estimator must be regarded as logically inconsistent; Conditions inducing rankings among various estimators relevant for issues concerning over- or underestimation. [**note: I only include this one as a marker for myself. I haven't done a Monte Carlo calculation in years but am an aspiring Python programmer.**

Anyone that posts code to *github* gets top marks in my book.]
https://www.medrxiv.org/content/10.1101/2020.04.28.20075036v1

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2020-05-06

Alas, there was no winner of yesterday's contest. There is no unanimity of thinking about who 'girl of the north country' is, and of course Bob Dylan isn't talking. The song was on Dylan's second album, "The Freewheelin' Bob Dylan" and also on "Nashville Skyline" where he sings it as a duet with Johnny Cash. Most people believe it is Suze Rotolo who is on the iconic album cover with Dylan. If you are looking for a good book to read about Dylan and his time in New York, I can give a major recommendation for David Hajdu's "Positively 4th Street: The Lives and Times of Joan Baez, Bob Dylan, Mimi Baez Fariña, and Richard Fariña." Trivia note: Richard Fariña was a good friend and Cornell classmate of the reclusive American writer, Thomas Pynchon.

Here is a vivid memory of my almost misspent youth. Loyal readers had no way of knowing that your correspondent was a beach bum at one point. Anyone who goes to school at UC Santa Barbara had the beach in his/her system (do click on the link to see some wonderful panoramic photography of my alma mater). When the Beach Boys hit the scene we were all stoked up and ready to hit the waves!!! It was so rad!! Here is the iconic Surfin USA (co-written by none other than Chuck Berry): https://www.youtube.com/watch?v=sNypbmPPDco yes, the video is grainy and audio cloudy but all the other filmed versions are lip synch and we cannot have that!

Another good New Yorker article, this time an interview with former CDC honcho and now at University of Nebraska med school. He is a fascinating character who chased pandemic diseases all over the world.

At least Israel understands what needs to be done. They are going to do massive antibody testing using high quality testing approaches to see what the background level of infection is. 100,000 blood tests will be done. My own feeling is this is the way to go. I would be doing antibody testing In the US on everyone who goes in for routine blood draws (but I am most likely just a voice in the wilderness).

More on hydroxychloroquine from Derek Lowe who discusses the positive Wuhan data and some Italian information that seems not to be real.

Put on your reading glasses, lots of abstracts today!!

MODELING

- There have been some press reports about mutations in the SARS-CoV-2 spike protein causing increased viral infectivity. Here is the first preprint I've see on this. COVID-19 is a highly contagious disease caused by a novel coronavirus SARS-CoV-2. The interaction between SARS-CoV-2 spike protein and the host cell surface receptor ACE2 is responsible for mediating SARS-CoV-2 infection. By analyzing the spike-hACE2 interacting surface, we predicted many hot spot

residues that make major contributions to the binding affinity. Mutations on most of these residues are likely to be deleterious, leading to less infectious virus strains that may suffer from negative selection. Meanwhile, several residues with mostly advantageous mutations have been predicted. It is more probable that mutations on these residues increase the transmission ability of the virus by enhancing spike-hACE2 interaction. So far, only a limited number of mutations has been reported in this region. However, the list of hot spot residues with predicted downstream effects from this study can still serve as a tracking list for SARS-CoV-2 evolution studies. Coincidentally, one advantageous mutation, p.476G>S, started to surge in the last couple of weeks based on the data submitted to the public domain, indicating that virus strains with increased transmission ability may have already spread. [**note: Darwinian evolution at work. We are in a wait and see mode regarding the vaccine testing. If the newer mRNA vaccines work, this becomes a non-issue as the vaccine is easily changed.**] https://www.biorxiv.org/content/10.1101/2020.05.04.077842v1

- COVID-19 pandemic is a major human tragedy. Worldwide, SARS-CoV-2 has already infected over 3 million and has killed about 230,000 people. SARS-CoV-2 originated in China and, within three months, has evolved to an additional 10 subtypes. One particular subtype with a non-silent (Aspartate to Glycine) mutation at 614th position of the Spike protein (D614G) rapidly outcompeted other pre-existing subtypes, including the ancestral. We assessed that D614G mutation generates an additional serine protease (Elastase) cleavage site near the S1-S2 junction of the Spike protein. We also identified that a single nucleotide deletion (delC) at a known variant site (rs35074065) in a cis-eQTL of TMPRSS2, is extremely rare in East Asians but is common in Europeans and North Americans. The delC allele facilitates entry of the 614G subtype into host cells, thus accelerating the spread of 614G subtype in Europe and North America where the delC allele is common. The delC allele at the cis-eQTL locus rs35074065 of TMPRSS2 leads to overexpression of both TMPRSS2 and a nearby gene MX1. The cis-eQTL site, rs35074065 overlaps with a transcription factor binding site of an activator (IRF1) and a repressor (IRF2). IRF1 activator can bind to variant delC allele, but IRF2 repressor fails to bind. Thus, in an individual carrying the delC allele, there is only activation, but no repression. On viral entry, IRF1 mediated upregulation of MX1 leads to neutrophil infiltration and processing of 614G mutated Spike protein by neutrophil Elastase. The simultaneous processing of 614G spike protein by TMPRSS2 and Elastase serine proteases facilitates the entry of the 614G subtype into host cells. Thus, SARS-CoV-2, particularly the 614G subtype, has spread more easily and with higher frequency to Europe and North America where the delC allele regulating expression of TMPRSS2 and MX1 host proteins is common, but not to East Asia where this allele is rare. [**note: this is pretty provocative! Perhaps it explains the lower rate of mortality and morbidity in some Asian nations.**] https://www.biorxiv.org/content/10.1101/2020.05.04.075911v1

- **A Simple Method** for Estimating the Number of Unconfirmed COVID-19 Cases in a Local Area that Includes a Confidence Interval: A Case Study of Whatcom County, Washington. Along with many other data problems affecting the unfolding of the COVID-10 pandemic in the United States, virtually nothing is known about the number of positive, unconfirmed cases, especially in local areas. We show that it is possible to estimate the number of positive, unconfirmed COVID-19 cases using a simple, long-established method employed by demographers to estimate a population in the absence of a census count. We go on to show how a confidence interval can be constructed around an estimate of positive, unconfirmed COVID-19 cases constructed from

this method, using Whatcom County, Washington as a case study. [**note: as a firm believer in Occam's Razor, any study that purports to use a 'simple method' will always get a citation in this newsletter!!!**] https://www.medrxiv.org/content/10.1101/2020.04.30.20086181v1

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NEWLY REGISTERED CLINICAL TRIALS

- The exceedingly high mortality rates of severe and critical COVID-19 warrant the identification and evaluation of novel therapies that could potentially mitigate the advanced disease manifestations. Based on preclinical data from this institution and others, the investigators hypothesize that **PI3K inhibition with duvelisib** could potentially quell aberrant hyperactivtation of the innate immune system, preferentially polarize macrophages, reduce pulmonary inflammation, and limit viral persistence, thereby improving patient outcomes. NCT04372602
- This is a trial for an experimental drug that has never really shown much progress in treating lupus or RA. It's a mixture of two peptides: metenkefalin + tridecactide NCT04374032
- Ibrutinib is an investigational drug being developed for the treatment of COVID-19. Participants are assigned 1 of 2 groups, called treatment arms. Each group receives a different treatment. There is a 1 in 2 chance that participants will be assigned to placebo. Around 46 adult participants with a diagnosis of COVID-19 will be enrolled at multiple sites in Unites States. [**note: this is sponsored by AbbVie with Janssen listed as a collaborator.**] NCT04375397
- I think this is the first trial for GSK!!! This is a multi-center, double-blind, randomized, placebo-controlled trial to assess the efficacy and safety of otilimab for the treatment of severe pulmonary COVID-19 related disease. Otilimab is a human monoclonal anti-GM-CSF antibody that has not previously been tested in participants with severe pulmonary COVID-19 related disease. The aim of this study is to evaluate the benefit-risk of a single infusion of otilimab in the treatment of patients with severe COVID-19 related pulmonary disease. The study population will consist of hospitalized participants with new onset hypoxia requiring significant oxygen support or requiring early invasive mechanical ventilation (less than or equal to [<=] 48 hours before dosing). Participants will be randomized to receive a single intravenous (IV) infusion of otilimab or placebo, in addition to standard of care. NCT04376684
- Evaluating the use of Polymyxin B Cartridge Hemoperfusion for patients with septic shock and COVID-19. [**note: not sure how many patients fall into this category.**] NCT04352985

CLINICAL TRIAL RESULTS

- Large cohort study of all tested individuals in Ontario, Canada. In this population-wide study in Ontario, Canada we report on all 194,372 unique residents who received testing for SARS-CoV-2 between January 23, 2020 and April 28, 2020. We found that while more women than men were tested for SARS-CoV-2, men had a higher rate of laboratory-confirmed COVID-19 infection, hospitalization, ICU admission and death. These findings were consistent even with age adjustment, suggesting that the observed differences in outcomes between women and men were not explained by age or systematic differences in testing by sex. Instead, they may be due to sex-based immunological or other gendered differences, such as higher rates of smoking leading to cardiovascular disease.
  https://www.medrxiv.org/content/10.1101/2020.04.30.20086975v1

- It is a small sample, but at this one UK hospital there was no difference in outcome based on ethnicity. During the current COVID-19 pandemic, anecdotal reports suggest that BAME background patients may be disproportionately affected compared to White but few objective data are available. We took advantage of near real-time hospital data access and analysis pipelines to look at the impact of ethnicity in 437 consecutive patients admitted during March to Kings College Hospital NHS Trust in London. Our key findings are firstly that BAME patients are significantly younger and have different co-morbidity profiles than White individuals. Secondly, there is no significant effect of ethnicity itself on severe outcomes (death or ITU admission) within 14-days of symptom onset, with adjustment for age, sex, comorbidities. https://www.medrxiv.org/content/10.1101/2020.05.02.20078642v1
- Large study at UCSF on patients presenting to the ER. We examined all patients presenting to an emergency department in San Francisco, California between February 3 and March 31, 2020 with an acute respiratory illness who were tested for SARS-CoV-2. We determined COVID-19 status by PCR and metagenomic next generation sequencing (mNGS). We compared demographics, comorbidities, symptoms, vital signs, and laboratory results including viral diagnostics using PCR and mNGS. Among those hospitalized, we determined differences in treatment (antibiotics, antivirals, respiratory support) and outcomes (ICU admission, ICU interventions, acute respiratory distress syndrome, cardiac injury). Findings: In a cohort of 316 patients, 33 (10%) tested positive for SARS-CoV-2; 31 patients, all without COVID-19, tested positive for another respiratory virus (16%). Among patients with additional viral testing, no co-infections with SARS-CoV-2 were identified by PCR or mNGS. Patients with COVID-19 reported longer symptoms duration (median 7 vs. 3 days), and were more likely to report fever (82% vs. 44%), fatigue (85% vs. 50%), and myalgias (61% vs 27%); $p<0.001$ for all comparisons. Lymphopenia (55% vs 34%, $p=0.018$) and bilateral opacities on initial chest radiograph (55% vs. 24%, $p=0.001$) were more common in patients with COVID-19. Patients with COVID-19 were more often hospitalized (79% vs. 56%, $p=0.014$). Of 186 hospitalized patients, patients with COVID-19 had longer hospitalizations (median 10.7d vs. 4.7d, $p<0.001$) and were more likely to develop ARDS (23% vs. 3%, $p<0.001$). Most comorbidities, home medications, signs and symptoms, vital signs, laboratory results, treatment, and outcomes did not differ by COVID-19 status. Interpretation: While we found differences in clinical features of COVID-19 compared to other acute respiratory illnesses, there was significant overlap in presentation and comorbidities. Patients with COVID-19 were more likely to be admitted to the hospital, have longer hospitalizations and develop ARDS, and were unlikely to have co-existent viral infections. These findings enhance understanding of the clinical characteristics of COVID-19 in comparison to other acute respiratory illnesses. https://www.medrxiv.org/content/10.1101/2020.05.02.20082461v1
- FINALLY, AN OBSERVATIONAL LOOK AT PATIENTS ON ANTI-RHEUMATIC DRUGS. This one is from Barcelona and we need much more of this to determine correlations regarding disease progression. OBJECTIVES: To investigate the incidence of COVID-19 in a cohort of adult and paediatric patients with rheumatic diseases receiving targeted biologic and synthetic disease modifying anti-rheumatic drugs (tDMARDs) and to explore the possible effect of these treatments in the clinical expression of COVID-19. METHODS: A cross-sectional study comprising of a telephone survey and electronic health records review was performed including all adult and paediatric patients with rheumatic diseases treated with tDMARDs in a large rheumatology

tertiary centre in Barcelona, Spain. Demographics, disease activity, COVID-19 related symptoms and contact history data were obtained from the start of the 2020 pandemic. Cumulative incidence of confirmed cases (SARS-CoV-2 positive PCR test) was compared to the population estimates for the same city districts from a governmental COVID-19 health database. Suspected cases were defined following WHO criteria and compared to those without compatible symptoms. RESULTS: 959 patients with rheumatic diseases treated with tDMARDs were included. We identified 11 confirmed SARS-CoV-2 positive cases in the adult cohort and no confirmed positive cases in the paediatric cohort. All patients had a successful recovery and only one patient required admission in the intensive care unit. When using the same classification criteria (only COVID-19 positive cases with pneumonia), COVID-19 incidence rates of the rheumatic patient cohort were very similar to that of the general population [(0.48% (95% CI 0.09 to 8.65%)] and [0.58% (95% CI 5.62 to 5.99%)], respectively. We found significant differences in tDMARDs proportions between the suspected and non-suspected cases (p=0.002). CONCLUSION: Adult and paediatric patients with rheumatic diseases on tDMARDs do not seem to present a higher risk of COVID-19 or a more severe disease outcome when compared to general population. Our exploratory analysis suggests that the proportion of COVID-19 suspected cases differs between tDMARDs.
https://www.medrxiv.org/content/10.1101/2020.04.30.20086090v1

- Objectives: **To investigate whether people who think they have had COVID-19 are less likely to engage in social distancing measures compared with those who think they have not had COVID-19**. Design: On-line cross-sectional survey. Setting: Data were collected between 20th and 22nd April. Participants: 6149 participants living in the UK aged 18 years or over. Main outcome measures: Perceived immunity to COVID-19, self-reported adherence to social distancing measures (going out for essential shopping, nonessential shopping, and meeting up with friends/family; total out-of-home activity), worry about COVID-19 and perceived risk of COVID-19 to oneself and people in the UK. Knowledge that cough and high temperature / fever are the main symptoms of COVID-19. Results: In this sample, 1493 people (24.3%) thought they had had COVID-19. Only 245 (4.0%) reported receiving a test result saying they had COVID-19. Reported test results were often incongruent with participants' belief that they had had COVID-19. People who believed that they had had COVID-19 were: more likely to agree that they had some immunity to COVID-19; less likely to report adhering to social distancing measures; less worried about COVID-19; and less likely to know that cough and high temperature / fever are two of the most common symptoms of COVID-19. Conclusions: The number of people in the UK who think they have already had COVID-19 is about twice the rate of current prevalence estimates. People who think that they have had COVID-19 may contribute to transmission of the virus through non-adherence to social distancing measures. Clear communications to this growing group are needed to explain why protective measures continue to be important and to encourage sustained adherence. [**note: I guess someone had to do this and who better than the Brits?**] https://www.medrxiv.org/content/10.1101/2020.04.30.20086223v1

DRUG DEVELOPMENT

- Here is an interesting finding on Alpha I Antitrypsin. The transmembrane serine protease TMPRSS2 is indispensable for S protein priming of the MERS, SARS-Cov, and SARS-CoV2 coronaviruses, a process that is necessary for entry of the virus into host cells. Therefore,

inhibiting TMPRSS2 holds promise as an approach toward preventing transmission of coronaviruses. Herein, we developed an in vitro system to measure TMPRSS2 activity and tested the inhibition of TMPRSS2 by several synthetic and natural protease inhibitors. Camostat mesylate and bromhexine hydrochloride (BHH) inhibited TMPRSS2 proteolytic function. In addition, we identified the small molecule 4-(2-aminomethyl)benzenesulfonyl fluoride (AEBSF) and the human, anti-inflammatory protein alpha 1 antitrypsin (A1AT) as inhibitors of TMPRSS2. AEBSF and A1AT inhibited TMPRSS2 activity in a dose-dependent manner. AEBSF and A1AT inhibited TMPRSS2 in the same range of concentrations (100-0.1 uM). We suggest that treatment with these inhibitors, particularly A1AT, which is an FDA-approved drug, might be effective in limiting SARS-CoV and SARS-CoV2 transmissibility and as a COVID-19 treatment. [**note: the paper is short and worth reading on the role of A1AT**] https://www.biorxiv.org/content/10.1101/2020.05.04.077826v1

- Background: Effective antiviral drugs for COVID-19 are still lacking. This study aims to evaluate the clinical outcomes and plasma concentrations of baloxavir marboxil and favipiravir in COVID-19 patients. Methods: Favipiravir and baloxavir acid were evaluated for their antiviral activity against SARS-CoV-2 in vitro before the trial initiation. We conducted an exploratory trial with 3 arms involving hospitalized adult patients with COVID-19. Patients were randomized assigned in a 1:1:1 ratio into baloxavir marboxil group, favipiravir group, and control group. The primary outcome was the percentage of subjects with viral negative by Day 14 and the time from randomization to clinical improvement. Virus load reduction, blood drug concentration and clinical presentation were also observed. The trial was registered with Chinese Clinical Trial Registry (ChiCTR 2000029544). Results: Baloxavir showed antiviral activity in vitro with the half-maximal effective concentration (EC50) of 5.48 μM comparable to arbidol and lopinavir, but favipiravir did not demonstrate significant antiviral activity up to 100 μM. Thirty patients were enrolled. The percentage of patients who turned viral negative after 14-day treatment was 70%, 77%, and 100% in the baloxavir, favipiravir, and control group respectively, with the medians of time from randomization to clinical improvement was 14, 14 and 15 days, respectively. One reason for the lack of virological effect and clinical benefits may be due to insufficient concentrations of these drugs relative to their antiviral activities. Conclusions: Our findings do not support that adding either baloxavir or favipiravir under the trial dosages to the existing standard treatment. [**note: this trial is of little use other than the PK study of the antivirals. Patients were on multiple drug therapies and all one can say is favipiravir is not useful as an adjunct to therapy.]** https://www.medrxiv.org/content/10.1101/2020.04.29.20085761v1

DIAGNOSTIC DEVELOPMENT

- I have not seen pre-prints about this but this NY Times story on how Crispr can be used for Coronavirus is very interesting. The nice thing about the technology is that it can be quickly change to accommodate any mutations in the virus.
- More on group testing from Harvard/MIT researchers. The ongoing pandemic of SARS-CoV-2, a novel coronavirus, caused over 3 million reported cases of coronavirus disease 2019 (COVID-19) and 200,000 reported deaths between December 2019 and April 2020. Cases and deaths will increase as the virus continues its global march outward. In the absence of effective pharmaceutical interventions or a vaccine, wide-spread virological screening is required to inform where restrictive isolation measures should be targeted and when they can be lifted.

However, limitations on testing capacity have restricted the ability of governments and institutions to identify individual clinical cases, appropriately measure community prevalence, and mitigate transmission. Group testing offers a way to increase efficiency, by combining samples and testing a small number of pools. Here, we evaluate the effectiveness of group testing designs for individual identification or prevalence estimation of SARS-CoV-2 infection when testing capacity is limited. To do this, we developed mathematical models for epidemic spread, incorporating empirically measured individual-level viral kinetics to simulate changing viral loads in a large population over the course of an epidemic. We used these to construct representative populations and assess pooling strategies for community screening, accounting for variability in viral load samples, dilution effects, changing prevalence and resource constraints. We confirmed our group testing framework through pooled tests on de-identified human nasopharyngeal specimens with viral loads representative of the larger population. We show that group testing designs can both accurately estimate overall prevalence using a small number of measurements and substantially increase the identification rate of infected individuals in resource-limited settings.
https://www.medrxiv.org/content/10.1101/2020.05.01.20086801v1

- Some good work from a commercial lab and academic collaborators. Developing high throughput diagnostics which does not involve handling of infectious material becomes imperative in the case of pandemics such as the recent outbreak of SARS-CoV2. Methods: A protein microarray technology was used to detect the plurality of antibody response to four novel antigens namely S1 glycoprotein, Receptor binding domain (RBD), S2 glycoprotein and Nucleoprotein of the novel coronavirus named SARS-CoV2 using serum samples. A DBS card was additionally used to compare its performance with a venipuncture-based serum separator tube (SST) draw. Results: The three main subclasses of antibodies IgM, IgA and IgG were analyzed to see the variations in immune responses in the affected population and compared to their microbial RT-PCR based NP swab results. The clinical sensitivity and specificity were determined to be 98.1% and 98.6%. In the matrix comparison study, which would enable patients to test without risk of transmitting the virus, DBS matched with higher than 98% accuracy to a venipuncture-based SST collection. Conclusion: Multiplex testing enables higher sensitivity and specificity which is essential while establishing exposure on a population scale. This flexible platform along with a discrete collection methodology would be crucial and broadly useful to scale up testing in current and future pandemics. Minimum sample volume that can be collected using DBS cards can be processed in this multiplex pillar plate format enabling the capacity to provide the reliability of high throughput analyzers while having the ease of collection similar to rapid tests. Keywords: COVID19, SARS-CoV2, Antibody, Multiplex, serology, biomarker, immune modulation, prevalence, high sensitivity, high specificity, Dried blood spot, pandemic, venipuncture https://www.medrxiv.org/content/10.1101/2020.04.29.20085068v1

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2020-05-07

This has to be one of the best classical-pop fusion songs. The great Freddie Mercury and Montserrat Caballe singing Barcelona: https://www.youtube.com/watch?v=pPG5DP2RQZk it doesn't get any

better than this!! Caballe sang one of the most magnificent 'Casta Diva' that I've seen. It was a windswept evening at an open-air performance in France and the voice is just in prime shape. The YouTube clip is nowhere as good as the DVD. I'll look for a good clip of Queen's 'We are the Champions.'

Derek Lowe discusses the current state of antibody research. I posted the abstract to the Dutch paper when it came out. They look to have the best characterized mAb around. No word on whether they have a partner for scale-up production. Here is Jon Cohen, who wrote a lot about the HIV research 30 years ago, on the race for antibodies to stop coronavirus.

I'm old enough to remember the advertisements on TV about setting up a chinchilla farm at home and selling the pelts to furriers. My mother quickly disabused me of this get rich quick scheme. But wait, there's more --- it's time to get into llama farming!!! Yes, llamas are well known for their fine wool and now, they may be a cure for SARS-CoV-2 . I'll need to invest in some farm land as my yard can only support one or two.

Our state just announced the closing of schools for the remainder of this school year. Here is a story from Science on the conundrum policy makers face. We still need more data on this cohort.

First and last political rant; feel free to skip! I don't mean to offend anyone. As my loyal readers know, I have tried to keep this newsletter as fee from politics as possible. My own feeling is that irrespective of country's politics we are all in this fight together. I have been impressed by how scientists from all over are working feverishly (OK, maybe that's the wrong word 😊) to define the disease, look for drugs and find a good vaccine. As you will see in one of the papers below, I found the statements from the Chinese scientists out of line. Most of you read the mainstream press here in the US and know the lay of the land. My own prejudice is for more testing and a return to normalcy as quickly as we can while protecting the public health. I have no control over the politics of all this. *End of rant.*

Lots of abstracts today and I didn't look at the Clinical Trials Database.

MODELING

- In recent weeks, several seroprevalence studies have appeared which attempt to determine the prevalence of antibodies against SARS-CoV-2 in the population of certain European and American locations. Many of these studies find an antibody prevalence comparable to the false positive rate of their respective serology tests and the relatively low statistical power associated with each study has invited criticism. To determine the strength of the signal, we perform a meta-analysis on the publicly available seroprevalence data based on Bayesian hierarchical modelling with Markov Chain Monte Carlo and Generalized Linear Mixed Modelling with prediction sampling. We examine studies with results from Santa Clara County (CA), Los Angeles County (CA), San Miguel County (CO), Chelsea (MA), the comte de Geneve (Switzerland), and Gangelt (Germany). Our results are in broad agreement with the conclusions of the studies; we find that there is evidence for non-trivial levels of antibody prevalence across all study locations. However, we also find that a significant probability mass exists for antibody prevalence at levels

lower than the reported figures. The results of our meta-analysis on the recent seroprevalence studies point to an important and strongly suggestive signal. [**note: this is a very decent analysis of the large field serology studies. The analysis finds that the study conclusions are reasonable and that such surveillance needs to continue so that we can be better informed about viral spread. Not only do they use Monte Carlo analysis but also Markov Chain! I need to find my old applied math textbook now.**]
https://www.medrxiv.org/content/10.1101/2020.05.03.20089201v1

- Background: A significant proportion of patients with COVID-19 generate negative pharyngeal swab viral nucleic acid test results but test positive using fecal samples. However, fecal-oral transmission of COVID-19 has not been established to date. The purpose of this study was to evaluate the duration of fecal swab positivity in COVID-19 patients after pharyngeal swab nucleic acid test turned negative and to explore its potential for fecal-oral transmission. Methods: A retrospective analysis of clinical records, laboratory results, and chest computed tomography (CT) findings of 17 COVID-19 patients confirmed by laboratory tests from January 22 to February 7, 2020 at a tertiary hospital was performed. The potential of fecal-oral transmission was assessed by detecting the presence of SARS-CoV-2 nucleic acid in fecal swab samples. Results: A total of 16 patients (94.1%) had fever; other symptoms included dry cough, dyspnea, nausea, diarrhea, sore throat, fatigue, and muscle pain. Three patients had decreased white blood cell counts, 7 had decreased lymphocyte numbers, and 7 had increased C-reactive protein levels. Fecal samples of 11 patients tested positive for SARS-CoV-2 nucleic acid, of whom the time for the fecal samples to become SARS-CoV-2 nucleic acid-negative was longer in 10 patients than that for pharyngeal swab samples, and only one case exhibited a shorter time for his fecal sample to become SARS-CoV-2 nucleic acid-negative compared to his pharyngeal swab sample. The remaining 6 patients were negative for SARS-CoV-2 nucleic acid in fecal samples. Conclusion: In COVID-19 patients who tested positive for SARS-CoV-2 nucleic acid in both pharyngeal swab and fecal samples, the time for the fecal samples to become SARS-CoV-2 nucleic acid-negative was generally longer than that in pharyngeal swab samples. However, there is currently no evidence demonstrating that the virus can be transmitted through the fecal-oral route. [**note: there have been a number of proposals regarding testing of sewage samples in select areas to look for viral spread. This small study shows that fecal shedding of virus goes on even after nasal swabs are negative. It's certainly worth considering this as a public health approach. Unsaid and obvious is WASH YOUR HANDS after attending to your bathroom needs!**] https://www.medrxiv.org/content/10.1101/2020.05.02.20089094v1 and here are some Turkish results on wastewater monitoring: https://www.medrxiv.org/content/10.1101/2020.05.03.20089417v1 showing that it can be a useful tool to give an early warning.
- The case fatality rate (CFR) can be used to predict the number of potential deaths in the epidemic and thus can reflect the appropriateness and quality of medical measures developed by public health. When a new disease breaks out, it is particularly important to accurately estimate the CFR. However, while the epidemic is still developing, the crude CFR is often lower than the true value and the hospital CFR is often higher than the true value due to differences in occurrence time, patient number, and treatment plans. Therefore, this study proposes a bi-directional correction method to estimate the CFR. COVID-19 data from China were used to evaluate this method. The results show that this method provides more accurate results than

both the crude CFR and hospital CFR. Additionally, this method was used to estimate the CFR of COVID-19 in other countries, with an aim to provide a reference for prevention and control decisions for the COVID-19 epidemic and for the evaluation of medical efforts. [**note: getting an accurate CFR has been problematic because of the lack of knowledge of the true infection background rate. Perhaps this analytic approach is valid, but who really knows? I did read the paper and the authors get a little snarky at the end when they discuss CFR in foreign countries, "…the United Kingdom first proposed the strategy of herd immunity. The US President also believes that the COVID-19 CFR will not exceed 1% which is lower than the ordinary flu. Unfortunately these original judgement were wrong and those leaders missed the effective time for epidemic containment…..This information may remind policy makers to be vigilant and take appropriate epidemic prevention measures to reduce the impact on society." I note this to emphasize that scientific papers should be free of political statements and I'll leave it at that.**] https://www.medrxiv.org/content/10.1101/2020.05.02.20089144v1

- Population-based serosurveys provide one avenue for estimating infection rates and monitoring the progression of the epidemic, overcoming many of these limitations. Methods: Taking advantage of a pool of adult participants from population-representative surveys conducted in Geneva, Switzerland, we implemented a study consisting of 8 weekly serosurveys among these participants and their household members older than 5 years. We tested each participant for anti-SARS-CoV-2-IgG antibodies using a commercially available enzyme-linked immunosorbent assay (Euroimmun AG, Lubeck, Germany). We estimated seroprevalence using a Bayesian regression model taking into account test performance and adjusting for the age and sex of Geneva's population. Results: In the first three weeks, we enrolled 1335 participants coming from 633 households, with 16% <20 years of age and 53.6% female, a distribution similar to that of Geneva. In the first week, we estimated a seroprevalence of 3.1% (95% CI 0.2-5.99, n=343). This increased to 6.1% (95% CI 2.6-9.33, n=416) in the second, and to 9.7% (95% CI 6.1-13.11, n=576) in the third week. We found that 5-19 year-olds (6.0%, 95% CI 2.3-10.2%) had similar seroprevalence to 20-49 year olds (8.5%, 95%CI 4.99-11.7), while significantly lower seroprevalence was observed among those 50 and older (3.7%, 95% CI 0.99-6.0, p=0.0008). Interpretation: Assuming that the presence of IgG antibodies is at least in the short-term associated with immunity, these results highlight that the epidemic is far from burning out simply due to herd immunity. Further, no differences in seroprevalence between children and middle age adults are observed. These results must be considered as Switzerland and the world look towards easing restrictions aimed at curbing transmission. [**note: this meets the Rumsfeld threshold of knowns/unknowns. Much more work needs to be done in this area.**] https://www.medrxiv.org/content/10.1101/2020.05.02.20088898v1
- OBJECTIVE: To examine the associations of stay-at-home order and face-masking recommendation with trends in daily new cases and deaths of laboratory-confirmed coronavirus disease 2019 (COVID-19) in the United States DESIGN: Piecewise log-linear modelling of temporal trends with turning-points, followed by quasi-experimental study on trend turning-point. Simulation studies were carried out to understand the outcomes under the scenarios if early-implementation and removal of stay-at-home order occurred. SETTING: Population data in the United States PARTICIPANTS: Residents in the U.S., who were affected by the stay-at-home and face-masking policies MAIN OUTCOME MEASURES: Turning-points of the daily new cases and deaths of COVID-19, and COVID-19 time-varying reproduction numbers (Rt) in the U.S.

RESULTS: The number and the proportion of U.S. residents under SAHO increased between March 19 and April 7, and plateaued at 29,0829,980 and 88.6%, respectively. The trend in COVID-19 daily cases reduced after March 23 (P<0.001) and further reduced on April 3 (P<0.001), which was associated with implementation of SAHO by 10 states on March 23, and the Centers for Disease Control and Preventions recommendation of face-masking, respectively. Similar turning points were identified in the trends of daily deaths with a lag time. The estimates of Rt based on the 3 reported mean serial-intervals of COVID-19 all started to decline on March 19, when SAHO was first implemented in the U.S. and declined faster after March 23. After a short plateau, Rt continued to decline after April 3 and fell below/around 1.0 on April 13. CONCLUSIONS: There were 2 turning points of COVID-19 daily new cases or deaths in the U.S., which appeared to associate with implementation of SAHO and the CDC face-masking recommendation. Simulation on early-implementation and removal of SAHO reveals considerable impact on COVID-19 daily new cases and deaths. These findings may inform decision-making of lifting SAHO and face. [**note: I guess we'll see what happens as states begin to open for business and social distancing and mask wearing practices are not followed.**] https://www.medrxiv.org/content/10.1101/2020.05.01.20088237v1

- Wait, there's more! Re-opening societies and economies across the globe following the initial wave of the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) pandemic requires scientifically-guided decision processes and policy development. Public health authorities now consider it highly likely that transmission of SARS-CoV-2 and COVID-19 will follow a pattern of seasonal circulation globally. To guide mitigation strategies and tactics in a location-specific manner, accurate simulation of prolonged or intermittent patterns of social/physical distancing is required in order to prevent healthcare systems and communities from collapsing. It is equally important to capture the stochastic appearance of individual transmission events. Traditional epidemiological/statistical models cannot make predictions in a geospatial temporal manner based on human individuals in a community. Thus, the challenge is to conduct spatio-temporal simulations of transmission chains with real-world geospatial and georeferenced information of the dynamics of the disease and the effect of different mitigation strategies such as isolation of infected individuals or location closures. Here, we present a stochastic, geospatially referenced and demography-specific agent-based model with agents representing human beings and include information on age, household composition, daily occupation and schedule, risk factors, and other relevant properties. Physical encounters between humans are modeled in a time-dependent georeferenced network of the population. The model (GERDA-1) can predict infection dynamics under normal conditions and test the effect of different mitigation scenarios such as school closures, reduced social contacts as well as closure or reopening of public/work spaces. Specifically, it also includes the fate and influence of health care workers and their access to protective gear. Key predictions so far entail: (i) the effect of specific groups on the spreading, specifically that children in school contribute substantially to distribution. (ii) the result of reopening society depends crucially on how strict the measures have been during lock-down. (iii) the outcome of reopening is a stochastic process - in the majority of cases, we must expect a second wave, in some cases not. To the best of our best knowledge, the GERDA-1 model is the first model able to predict a bimodal behavior of SARS-Cov-2 infection dynamics. Given the criticality of the global situation, informing the scientific community, decision makers and the general public seems prudent. Therefore, we here provide a pre-print of the GERDA-1

model together with a first set of predictions and analyses as work in progress. [**note: This is a GREAT paper for all you public health people. It has some very good diagrams and is well written. Download this one as it is a keeper!**]
https://www.medrxiv.org/content/10.1101/2020.05.03.20089235v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow, my vision is getting blurry from reading too many abstracts this AM.

CLINICAL TRIAL RESULTS

- Wow! Large sampling of patients in the NHS data system. Background Establishing who is at risk from a novel rapidly arising cause of death, and why, requires a new approach to epidemiological research with very large datasets and timely data. Working on behalf of NHS England we therefore set out to deliver a secure and pseudonymised analytics platform inside the data centre of a major primary care electronic health records vendor establishing coverage across detailed primary care records for a substantial proportion of all patients in England. The following results are preliminary. Data sources Primary care electronic health records managed by the electronic health record vendor TPP, pseudonymously linked to patient-level data from the COVID-19 Patient Notification System (CPNS) for death of hospital inpatients with confirmed COVID-19, using the new OpenSAFELY platform. Population 17,425,445 adults. Time period 1st Feb 2020 to 25th April 2020. Primary outcome Death in hospital among people with confirmed COVID-19. Methods Cohort study analysed by Cox-regression to generate hazard ratios: age and sex adjusted, and multiply adjusted for co-variates selected prospectively on the basis of clinical interest and prior findings. Results There were 5683 deaths attributed to COVID-19. In summary after full adjustment, death from COVID-19 was strongly associated with: being male (hazard ratio 1.99, 95%CI 1.88-2.10); older age and deprivation (both with a strong gradient); uncontrolled diabetes (HR 2.36 95% CI 2.18-2.56); severe asthma (HR 1.25 CI 1.08-1.44); and various other prior medical conditions. Compared to people with ethnicity recorded as white, black people were at higher risk of death, with only partial attenuation in hazard ratios from the fully adjusted model (age-sex adjusted HR 2.17 95% CI 1.84-2.57; fully adjusted HR 1.71 95% CI 1.44-2.02); with similar findings for Asian people (age-sex adjusted HR 1.95 95% CI 1.73-2.18; fully adjusted HR 1.62 95% CI 1.43-1.82). Conclusions We have quantified a range of clinical risk factors for death from COVID-19, some of which were not previously well characterised, in the largest cohort study conducted by any country to date. People from Asian and black groups are at markedly increased risk of in-hospital death from COVID-19, and contrary to some prior speculation this is only partially attributable to pre-existing clinical risk factors or deprivation; further research into the drivers of this association is therefore urgently required. Deprivation is also a major risk factor with, again, little of the excess risk explained by co-morbidity or other risk factors. The findings for clinical risk factors are concordant with policies in the UK for protecting those at highest risk. Our OpenSAFELY platform is rapidly adding further NHS patients' records; we will update and extend these results regularly. Keywords COVID-19, risk factors, ethnicity, deprivation, death, informatics. [**note: wouldn't it be nice to have a large EMR system in the US where this type of research could be more easily done?**]
https://www.medrxiv.org/content/10.1101/2020.05.06.20092999v1

- French researchers on the functional alteration of innate T cells. Covid-19 can induce lung infection ranging from mild pneumonia to life-threatening acute respiratory distress syndrome (ARDS). Dysregulated host immune response in the lung is a key feature in ARDS pathophysiology. However, cellular actors in Covid-19-driven ARDS are poorly understood. Here, we dynamically analyzed the biology of innate T cells, a heterogeneous class (MAIT, γδT and iNKT cells) of T lymphocytes, presenting potent anti-infective and regulatory functions. Patients presented a compartmentalized lung inflammation paralleled with a limited systemic inflammation. Circulating innate T cells of critically ill Covid-19 patients presented a profound and persistent phenotypic and functional alteration. Highly activated innate T cells were detected in airways of patients suggesting a recruitment to the inflamed site and a potential contribution in the regulation of the local inflammation. Finally, the expression of the CD69 activation marker on blood iNKT and MAIT cells at inclusion was predictive of disease severity. Thus, patients present an altered innate T cell biology that may account for the dysregulated immune response observed in Covid-19-related acute respiratory distress syndrome. [**note: this is an interesting paper and we really need to figure out what governs this out of whack immune response.**] https://www.medrxiv.org/content/10.1101/2020.05.03.20089300v1
- The COVID-19 patients with severe symptoms account for a majority of mortality of this disease. However, early detection and effective prediction of patients with mild to severe symptoms remains challenging. In this study, we performed proteomic profiling of urine samples from 32 healthy control individuals and 6 COVID-19 positive patients (3 mild and 3 severe). We found that urine proteome samples from the mild and severe COVID-19 patients with comorbidities can be clearly differentiated from healthy proteome samples based on the clustering analysis. Multiple pathways have been compromised after the COVID-19 infection, including the dysregulation of immune response, complement activation, platelet degranulation, lipoprotein metabolic process and response to hypoxia. We further validated our finding by directly comparing the same patients' urine proteome after recovery. This study demonstrates the COVID-19 pathophysiology related molecular alterations could be detected in the urine and the potential application of urinary proteome in auxiliary diagnosis, severity determination and therapy development of COVID-19. [**note: urine sampling should also be done on patients and correlated with serological testing.**]
https://www.medrxiv.org/content/10.1101/2020.05.02.20088666v1
- Background The most severely COVID-19 patients need intensive care and show increased risk of thromboembolic events. Although some patients meet the diagnostic criteria for the Disseminated Intravascular Coagulation, the pathogenesis of the diffuse thrombotic status remains unclear. The aim of the present study is to evaluate the presence of antiphospholipid antibodies (aPL) in sera of deceased patients with autoptic proven thrombotic microangiopathy to evaluate if some patients may have developed Catastrophic Antiphospholipid Syndrome (CAPS). Methods Thirty-five patients were enrolled. The available medical history, comorbidities, therapies, laboratory and autopsy findings were collected post-mortem from clinical records. IgA, IgG and IgM anti cardiolipin (ACA) and anti β2 glycoprotein 1 (β2GP1) antibodies, IgG and IgM anti phosphatidylserine/prothrombin (PS/PT) antibodies were tested for all the patients. Results 3/35 (8.6%) patients were slightly positive for aPL: one for ACA IgG and two for ACA IgM but values were low (< 3X the cut off). No patients tested positive for ACA IgA neither for β2GP1 isotypes. 3/35 (8.6%) patients were positive for PS/PT, one for IgG and two for IgM, but values

were less than 2X the cut off. No patients showed simultaneous positivity for ACA and PS/ PT. Conclusions It is difficult to categorize the vascular events into a conventional disease: we did not find significant association with anti-phospholipid antibodies. It is most likely that several factors contribute to trigger the hypercoagulability status and the thromboembolism but, on the basis our results, CAPS is probably not involved into the pathogenesis of these phenomena. [**note: these Italian researches rule out at least one possible pathogenic pathway to the observed lung blood clots.**]
https://www.medrxiv.org/content/10.1101/2020.04.30.20086397v1

- A novel strain of coronavirus appeared in December 2019. Over the next few months, this novel coronavirus spread throughout the world, being declared a pandemic by the World Health Organization on March 11, 2020. As of this writing (March 28, 2020) over one hundred thousand individuals in the United States of America were confirmed cases. One way of treating the associated disease, COVID-19, is to reuse existing FDA-approved medications. One medication that has shown promise is hydroxychloroquine (HCQ). However, the utility and safety of HCQ among pregnant COVID-19 patients remains a concern. [**note: this is a very small number of patients but it appears that there were no adverse drug events on pregnant women in terms of delivery as compared to the larger non HCQ cohort. I wonder if a pregnancy registry was ever set up for this drug given the broad spread use for malaria prophylaxis?]**
https://www.medrxiv.org/content/10.1101/2020.04.29.20085621v1
- It is suggested that potential acquired immunity against SARS-CoV-2 from prior exposure may be determined by detecting the presence of circulating IgG antibodies against viral antigens, such as the spike glycoprotein and its receptor binding domain (RBD). Testing our asymptomatic population for evidence of COVID-19 immunity would also offer valuable epidemiologic data to aid health care policies and health care management. Currently, there are over 100 antibody tests that are being used around the world without approval from the FDA or similar regulatory bodies, and they are mostly for rapid and qualitative assessment, with different degrees of error rates. ELISA-based testing for sensitive and rigorous quantitative assessment of SARS-CoV-2 antibodies can potentially offer mechanistic insights into the COVID-19 disease and aid communities uniquely challenged by limited financial resources and access to commercial testing products. Employing recombinant SARS-CoV-2 RBD and spike protein generated in the laboratory, we devised a quantitative ELISA for the detection of circulating serum antibodies. Serum from twenty SARS-CoV-2 RT-PCR confirmed COVID-19 hospitalized patients were used to detect circulating IgG titers against SARS-CoV-2 spike protein and RBD. Quantitative detection of IgG antibodies to the spike glycoprotein or the RBD in patient samples was not always associated with faster recovery, compared to patients with borderline antibody response to the RBD. One patient who did not develop antibodies to the RBD completely recovered from COVID-19. In surveying 99 healthy donor samples (procured between 2017-February 2020), we detected RBD antibodies in one donor from February 2020 collection with three others exhibiting antibodies to the spike protein but not the RBD. Collectively, our study suggests that more rigorous and quantitative analysis, employing large scale sample sets, is required to determine whether antibodies to SARS-CoV-2 spike protein or RBD is associated with protection from COVID-19 disease. It is also conceivable that humoral response to SARS-CoV-2 spike protein or RBD works in association with adaptive T cell response to determine clinical sequela and severity of COVID-19 disease. [**note: more confounding information on the immune**

response! I guess with lots more testing of this type we will finally figure this out.]
https://www.medrxiv.org/content/10.1101/2020.05.01.20087684v1

DRUG DEVELOPMENT

- Good work from China isolating specific SARS-CoV-2 antibodies. Neutralizing antibodies could be antivirals against COVID-19 pandemics. Here, we report the isolation of four human-origin monoclonal antibodies from a convalescent patient in China. All of these isolated antibodies display neutralization abilities in vitro. Two of them (B38 and H4) block the binding between RBD and vial cellular receptor ACE2. Further competition assay indicates that B38 and H4 recognize different epitopes on the RBD, which is ideal for a virus-targeting mAb-pair to avoid immune escape in the future clinical applications. Moreover, therapeutic study on the mouse model validated that these two antibodies can reduce virus titers in the infected mouse lungs. Structure of RBD-B38 complex revealed that most residues on the epitope are overlapped with the RBD-ACE2 binding interface, which explained the blocking efficacy and neutralizing capacity. Our results highlight the promise of antibody-based therapeutics and provide the structural basis of rational vaccine design. [**note: it did not escape my attention that the scientists were quick to file a patent on this!**]
https://www.medrxiv.org/content/10.1101/2020.05.01.20077743v1
- The pandemic of COVID-19, which is caused by the SARS-CoV-2 virus infection, has posed a threat to global healthcare system. The repurposing drug is one of the feasible ways for the emergency treatment. As the low-molecular-weight drugs have a higher possibility to fully match the interactions with essential targets of SARS-CoV-2, we propose a strategy to uncover such drugs using the fragment-based approach.Here, using the ligand-observed and protein-observed fragment screening approach, we identified niacin and hit 1 binding to the catalytic pocket of the main protease of SARS-CoV-2 (Mpro), thus modestlyinhibited the enzymatic activity of Mpro.The chemical shift perturbations induced by niacin and hit 1 indicates a partial overlap of their binding sites, i.e., the catalytic pocket of Mpro may accommodate derivatives with larger molecular size. We hence searched drugs containing the niacin or hit 1 pharmocophore,and uncovered carmofur, bendamustine, triclabendazole and emedastine, which demonstrated higher potency of inhibiting protease activity than the fragment screening hits. Our work demonstrated that fragment-based approach is a feasible way to uncover low-molecular-weight drugs against SARS-CoV-2, and potentially other targets without specific drug yet. [**note: what a weird group of drugs to come up in a hit. I've been on fexofenadine for seven weeks now and am still SARS-CoV-2 free (I think, only a serology test will affirm this). Of course it could also be the AREDS-2 vitamins I'm taking (love that Zn and Cu).**]
https://www.biorxiv.org/content/10.1101/2020.05.05.079848v1
- There are as yet no licenced therapeutics for the COVID-19 pandemic. The causal coronavirus (SARS-CoV-2) binds host cells via a trimeric Spike whose receptor binding domain (RBD) recognizes angiotensin-converting enzyme 2 (ACE2), initiating conformational changes that drive membrane fusion. We find that monoclonal antibody CR3022 binds the RBD tightly, neutralising SARS-CoV-2 and report the crystal structure at 2.4 angstrom of the Fab/RBD complex. Some crystals are suitable for screening for entry-blocking inhibitors. The highly conserved, structure-stabilising, CR3022 epitope is inaccessible in the prefusion Spike, suggesting that CR3022 binding would facilitate conversion to the fusion-incompetent post-fusion state. Cryo-EM analysis

confirms that incubation of Spike with CR3022 Fab leads to destruction of the prefusion trimer. Presentation of this cryptic epitope in an RBD-based vaccine might advantageously focus immune responses. Binders at this epitope may be useful therapeutically, possibly in synergy with an antibody blocking receptor attachment. [**note: this is an interesting approach to the design of a therapeutic. Most efforts target enzyme inhibition.**]
https://www.biorxiv.org/content/10.1101/2020.05.05.079202v1

- Urgent action is needed to fight the ongoing COVID-19 pandemic by reducing the number of infected people along with the infection contagiousness and severity. Chlorpromazine (CPZ), the prototype of typical antipsychotics from the phenothiazine group, is known to inhibit clathrin-mediated endocytosis and acts as an antiviral, in particular against SARS-CoV-1 and MERS-CoV. In this study, we describe the in vitro testing of CPZ against a SARS-CoV-2 isolate in monkey and human cells. We evidenced an antiviral activity against SARS-CoV-2 with an IC50 of ~10µM. Because of its high biodistribution in lung, saliva and brain, such IC50 measured in vitro may translate to CPZ dosage used in clinical routine. This extrapolation is in line with our observations of a higher prevalence of symptomatic and severe forms of COVID-19 infections among health care professionals compared to patients in psychiatric wards. These preclinical findings support the repurposing of CPZ, a largely used drug with mild side effects, in COVID-19 treatment. [**note: anyone want to guess how many clinical trials are being carried out with chlorpromazine? TWO! One is in Egypt and the second in France where this paper came from. Chlorpromazine came up in some drug repurposing hits before. It goes to show that we need to have more imagination in conducting trials. The PK and PD of this drug seem to point to its utility here. Of course everyone will also achieve some mental health benefits.**]
https://www.biorxiv.org/content/10.1101/2020.05.05.079608v1
- The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2) has resulted in an unprecedented public health crisis. There are currently no SARS-CoV-2-specific treatments or vaccines available due to the novelty of the virus. Hence, rapid development of effective vaccines against SARS-CoV-2 are urgently needed. Here we developed a pilot-scale production of a purified inactivated SARS-CoV-2 virus vaccine candidate (PiCoVacc), which induced SARS-CoV-2-specific neutralizing antibodies in mice, rats and non-human primates. These antibodies neutralized 10 representative SARS-CoV-2 strains, suggesting a possible broader neutralizing ability against SARS-CoV-2 strains. Three immunizations using two different doses (3 µg or 6 µg per dose) provided partial or complete protection in macaques against SARS-CoV-2 challenge, respectively, without observable antibody-dependent enhancement of infection. These data support clinical development of SARS-CoV-2 vaccines for humans. [**note: this is the published paper and I think I linked to the preprint several weeks ago. This is a traditional inactivated virus approach to vaccine development. We have a long history of this being a sound approach. I just want to get a good vaccine ASAP.]**
[https://science.sciencemag.org/content/early/2020/05/05/science.abc1932
- The pandemic of Corona Virus Disease 2019 (COVID-19) caused by SARS-CoV-2 has become a global crisis. The replication of SARS-CoV-2 requires the viral RNA-dependent RNA polymerase (RdRp), a target of the antiviral drug, Remdesivir. Here we report the cryo-EM structure of the SARS-CoV-2 RdRp either in the apo form at 2.8 Å resolution or in complex with a 50-base template-primer RNA and Remdesivir at 2.5 Å resolution. The complex structure reveals that the

partial double-stranded RNA template is inserted into the central channel of the RdRp where Remdesivir is covalently incorporated into the primer strand at the first replicated base pair and terminates chain elongation. Our structures provide critical insights into the mechanism of viral RNA replication and a rational template for drug design to combat the viral infection. [**note: more good work from China showing how remdesivir works on the RNA polymerase enzyme.**] https://science.sciencemag.org/content/early/2020/04/30/science.abc1560

DIAGNOSTIC DEVELOPMENT

- Aims: To validate the diagnostic accuracy of a Euroimmun SARS-CoV-2 IgG and IgA immunoassay for COVID-19 disease. Methods: In this unmatched (1:1) case-control validation study, we used sera of 181 laboratory-confirmed SARS-CoV-2 cases and 176 negative controls collected before the emergence of SARS-CoV-2. Diagnostic accuracy of the immunoassay was assessed against a whole spike protein-based recombinant immunofluorescence assay (rIFA) by receiver operating characteristic (ROC) analyses. Discrepant cases between ELISA and rIFA were further tested by pseudo-neutralization assay. Results: COVID-19 patients were more likely to be male and older than controls, and 50.3% of them were hospitalized. ROC curve analyses indicated that IgG and IgA had a high diagnostic accuracy with AUCs of 0.992 (95% Confidence Interval [95%CI]: 0.986-0.996) and 0.977 (95%CI: 0.963-0.990), respectively. IgG assays outperformed IgA assays (p=0.008). Considering optimized cut-offs taking the 15% inter-assay imprecision assessed into account, an IgG ratio cut-off > 1.5 displayed a 100% specificity (95%CI: 98-100) and a 100% positive predictive value (95%CI: 97-100). A 0.5 cut-off displayed a 97% sensitivity (95%CI: 93-99) and a 97% negative predictive value (95%CI: 93-99). Adopting these thresholds, rather than those of the manufacturer, improved assay performance, leaving 12% of IgG ratios ranging between 0.5-1.5 as indeterminate. Conclusions: The Euroimmun assay displays a nearly optimal diagnostic accuracy using IgG against SARS-CoV-2 in a samples of patients, without any obvious gains from considering IgA serology. The optimized cut-offs are fit for rule-in and rule-out purposes, allowing determination of whether individuals have been exposed to SARS-CoV-2 or not in our study population. They should however not be considered as a surrogate of protection at this stage. [**note: good lab directors always validate tests which should be SOP. While serology tests can be variable, so can RT-PCR tests. There is no such thing as 100% accuracy all the time. We need to use the tools we have to make informed decisions.**] https://www.medrxiv.org/content/10.1101/2020.05.02.20080879v1
- From hard hit Ecuador! Several qPCR kits are available for SARS-CoV-2 diagnosis, mostly lacking of evaluation due to covid19 emergency. We evaluated nCoV-QS (MiCo BioMed) kit using CDC kit as gold standard. We found limitations for nCoV-QS: 1) lower sensibility 2) lack of RNA quality control probe 3) no capacity to quantify viral load. [**note: see above comment!**] https://www.medrxiv.org/content/10.1101/2020.05.01.20081034v1
- With most diagnostic infrastructure dependent on specialised instruments, their exclusive reagent supplies quickly become bottlenecks in times of peak demand, creating an urgent need for novel approaches to boost testing capacity. We address this challenge by refocusing the full synthetic biology stack available at the London Biofoundry onto the development of alternative patient sample testing pipelines. We present a reagent-agnostic automated SARS-CoV-2 testing platform that can be quickly deployed and scaled, and that accepts a diverse range of reagents. Using an in-house-generated, open-source, MS2-virus-like-particle-SARS-CoV-2 standard, we

validate RNA extraction and RT-qPCR workflows as well as two novel detection assays based on CRISPR-Cas and Loop-mediated isothermal Amplification (LAMP) approaches. In collaboration with an NHS diagnostic testing lab, we report the performance of the overall workflow and benchmark SARS-CoV-2 detection in patient samples via RT-qPCR, CRISPR-Cas, and LAMP against clinical test sets. The validated RNA extraction and RT-qPCR platform has been installed in NHS diagnostic labs and now contributes to increased patient sample processing in the UK while we continue to refine and develop novel high-throughput diagnostic methods. Finally, our workflows and protocols can be quickly implemented and adapted by members of the Global Biofoundry Alliance and the wider scientific and medical diagnostics community. https://www.medrxiv.org/content/10.1101/2020.05.02.20088344v1

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2020-05-08

I came upon the Swiss soprano, Regula Mühlemann, pretty much by mistake the other day. What a wonderful singer she is. Here she sings Mozart's Exsultate Jubilate: https://www.youtube.com/watch?v=o_fA-Ls4RXk I am constant surprised by Mozart's compositions.

A large cohort study was released by Columbia University yesterday in the New England Journal of Medicine showing **hydroxychloroquine had no effect in hospitalized patients with SARS-CoV-2**. Money quote, "*Clinical guidance at our medical center has been updated to remove the suggestion that patients with Covid-19 be treated with hydroxychloroquine. In our analysis involving a large sample of consecutive patients who had been hospitalized with Covid-19, hydroxychloroquine use was not associated with a significantly higher or lower risk of intubation or death (hazard ratio, 1.04; 95% CI, 0.82 to 1.32). The study results should not be taken to rule out either benefit or harm of hydroxychloroquine treatment, given the observational design and the 95% confidence interval, but the results do not support the use of hydroxychloroquine at present, outside randomized clinical trials testing its efficacy*." Will other hospitals follow this lead? Will chlorpromazine be the new standard of care as remdesivir is in and will always be in short supply? Tough decisions!!

Moderna, first to test a SARS-CoV-2 vaccine in the US is moving into larger trials with an FDA blessing.

FDA may be moving closer to approving a home testing collection method for SARS-CoV-2. Guidelines allow companies to develop and market test kits with tools to swab noses and mail the specimens to any lab in the country that does testing. [**note: good article and worth reading.**]

Here is nice editorial in Science on a combination approach to COVID-19.

MODELING

- Background: The COVID-19 pandemic is the greatest public health crisis of the last 100 years. Countries have responded with various levels of lockdown to save lives and stop health systems from being overwhelmed. At the same time, lockdowns entail large socio-economic costs. One exit strategy under consideration is a mobile phone app that traces close contacts of those infected with COVID- 19. Recent research has demonstrated the theoretical effectiveness of this solution in different disease settings. However, concerns have been raised about such apps

because of the potential privacy implications. This could limit the acceptability of app-based contact tracing among the general population. As the effectiveness of this approach increases strongly with app take-up, it is crucial to understand public support for this intervention. Objectives: The objective of this study is to investigate user acceptability of a contact-tracing app in five countries hit by the pandemic. Methods We conducted a multi-country, large-scale (N = 5995) study to measure public support for digital contact tracing of COVID-19 infections. We ran anonymous online surveys in France, Germany, Italy, the UK and the US. We measured intentions to use a contact-tracing app across different installation regimes (voluntary installation vs. automatic installation by mobile phone providers), and studied how these intentions vary across individuals and countries. Results: We found strong support for the app under both regimes, in all countries, across all sub-groups of the population, and irrespective of regional-level COVID-19 mortality rates. We inves- tigated the main factors that may hinder or facilitate take-up and found that concerns about cyber security and privacy, together with lack of trust in government, are the main barriers to adoption. Conclusions: Epidemiological evidence shows that app-based contact-tracing can suppress the spread of COVID-19 if a high enough proportion of the population uses the app and that it can still reduce the number of infections if take-up is moderate. Our findings show that the willingness to install the app is very high. The available evidence suggests that app-based contact tracing may be a viable approach to control the diffusion of COVID-19. [**note: if more people would adopt this new technology some very good data can be acquired. Privacy issues can be addressed by appropriate encryption. https://www.medrxiv.org/content/10.1101/2020.05.05.20091587v1]**
https://www.medrxiv.org/content/10.1101/2020.05.05.20091587v1

- The first case of COVID-19 was confirmed in Israel on February 21, 2020. Within approximately 30 days, the total number of confirmed cases climbed up to 1,000, accompanied by a doubling period of less than 3 days. About one week later, after this number exceeded 4,000 cases, and following some extremely strict measures taken by the Israeli government, the daily detection rate started a sharp decrease from the peak value of 1,131 down to slightly more than 100 new confirmed cases on April 30. Motivated by this encouraging data, similar to the trends observed in many other countries, along with the growing economic pressures, the Israeli government started relaxing its emergency regulations as part of an "exit strategy". This article attempts to analyze the currently available data on Israel and a country of similar size (Sweden) in order to understand the local dynamics of COVID-19, assess the effect of the implemented intervention measures, and discuss some plausible scenarios for the foreseeable future. [**note: Israel enforced a tough lockdown and seems to have succeeded in controlling the outbreak.**]
https://www.medrxiv.org/content/10.1101/2020.05.05.20091645v1
- Background: Although recognised as effective measures to curb the spread of the COVID-19 outbreak, social distancing and self-isolation, have been suggested to generate burden throughout the population. To provide scientific data to help identify risk-factors for the psychosocial strain during the COVID-19 outbreak, an international cross-disciplinary online survey was circulated in April 2020. This report outlines the mental, emotional and behavioural consequences of COVID-19 home confinement. Method: Thirty-five research organisations from four continents promoted the survey through their networks to the general society, in Ten different languages. Questions were presented in a differential format with questions related to responses before and during confinement period. Results: 1047 replies (54% women) from

Western-Asia (36%), North-Africa (40%), Europe (21%) and other countries (3%) were analysed. The COVID-19 home confinement evoked a negative effect on mental wellbeing and emotional status ($P < 0.001$; $0.43 \leq d \leq 0.65$) with a greater proportion of individuals experiencing psychosocial and emotional disorders (10% to 16.5%). These psychosocial tolls were associated with unhealthy lifestyle behaviours with a greater proportion of individuals experiencing (i) physical (+15.2%) and social (71.2%) inactivity, (ii) poor sleep quality (12.8%), (iii) unhealthy diet behaviours (10%), and (iv) unemployment (6%). Conversely, participants demonstrated a greater use (15%) of technology solutions during the confinement period. Conclusion: These findings elucidate the risk of psychosocial strain during the current home confinement period and provide a clear remit for the urgent implementation of technology-based intervention to foster an Active and Healthy Confinement Lifestyle (AHCL). [**note: somebody had to do this research and a big shout out to this huge group of multi-country researchers!!!! You can put me in the 'poor sleep quality' category!!**]
https://www.medrxiv.org/content/10.1101/2020.05.04.20091017v1 and here for a look at the motional consequences: https://www.medrxiv.org/content/10.1101/2020.05.05.20091058v1

- Background A COVID-19 outbreak occurred in a cruise ship with 3711 passengers and crew in 2020. This study is to test the hypothesis that environmental surfaces played important roles in transmission for SARS-CoV-2 during this outbreak. Methods We sampled environmental surfaces including air from common areas in the cruise ship and cabins in which confirmed COVID-19 cases and non-cases had stayed after they left the cabins. We tested the samples for SARS-CoV-2 by rt-PCR and conducted viral isolation. Findings Of 601 samples tested, SARS-CoV-2 RNA was detected from 58 samples (10%) from case-cabins from which they left 1-17 days before sampling, but not from non-case-cabins. Except for one sample from an air hood in a corridor, SARS-CoV-2 RNA was not detected from samples in common areas. SARS-CoV-2 RNA was not detected from all 14 air samples. RNA was most often detected on the floor around toilet in the bathroom (39%, 13/33, cycle quantification (Cq): 26.21-37.62) and bed pillow (34%, 11/32, Cq: 34.61-38.99). There was no difference in the detection proportion between cabins for symptomatic (15%, 28/189, Cq: 29.79-38.86) and asymptomatic cases (21%, 28/131, Cq: 26.21-38.99). No SARS-CoV-2 virus was isolated from any of the samples. Interpretation The environment around the COVID-19 cases was extensively contaminated from SARS-CoV-2 during COVID-19 outbreak in the cruise ship. Transmission risk of SARS-CoV-2 from symptomatic and asymptomatic patients seems to be similar and the environmental surface could involve viral transmission through direct contact. [**note: I'm not sure which cruise ship this was as the paper did not mention it. This industry is going to have a tough time coming back.**]
https://www.medrxiv.org/content/10.1101/2020.05.02.20088567v1

NEWLY REGISTERED CLINICAL TRIALS

- Time to go out and buy an Apple watch!!! You can then get into this German trial. In this study, the investigators aim to observe patients with COVID-19 via SmartWatches on top of their clinical routine. The investigators aim to determine, whether the addition of SmartWatches enhances risk stratification, early detection of complications and prognostics in patients with COVID-19, who have cardiovascular diseases or receive medication with arrhythmogenic risk. [**note: actually this is a good thing, particularly if you are given HCQ+hydroxychloroquine!**]
NCT04376853

- YES, the first US-based ivermectin trial (maybe they didn't read the UK paper on the poor PK of the drug relative to the *in vitro* testing) and it's at Johns Hopkins right up the road from me. They also studying bicalutamide in this trial. NCT04374279
- Here is a trial to see if IL-7 works. Comparison of the effects of CYT107 vs Placebo administered IM at 10µg/kg twice a week for two weeks on immune reconstitution of lymphopenic COVID-19 patients. NCT04379076
- Here is a drug I've never heard of before but I'm not all-knowing. At present there is no approved drug treatment for Covid-19. In this study we plan to investigate if an experimental drug called IMU-838 (vidofludimus calcium) can improve your symptoms, prevent worsening that would initiate further treatments such as ventilation, and can lower your virus number if given in addition to your doctor's choice of standard therapy. We will also test if IMU-838 has any side effects and measure the level of IMU 838 in your blood. NCT04379271
- WOW, I think this is the first gene therapy protocol that I've seen! DeltaRex-G is a safe, non-pathogenic, replication incompetent, RNA virus-based gene vector. DeltaRex-G nanoparticles (~100 nm) can mimic RNA virus SARS-CoV-2 by binding to viral receptors in human cells and may serve as a decoy to prevent SARSCoV-2 cell entry by crowding/neutralizing the SARS-CoV-2 even where the receptors may be different. DeltaRex-G is a disease-seeking retrovector encoding a cytocidal dominant negative human cyclin G1 as genetic payload). When injected intravenously, the DeltaRex-G nanoparticles has a navigational system that targets exposed collagenous proteins (XC proteins) in injured tissues (e.g. inflamed lung, kidney, etc.), thus increasing the effective drug concentration at the sites of injury, in the vicinity of activated/proliferative T cells evoked by COVID-19. The DeltaRex-G then enters the rapidly dividing T cells and kills them by arresting the G1cell division cycle, hence, reducing cytokine release and ARDS; NCT04378244

CLINICAL TRIAL RESULTS

- BACKGROUND: Estimates of pediatric morbidity and mortality from COVID-19 are vital for planning optimal use of human and material resources throughout this pandemic. METHODS: Government websites from countries with minimum 1000 cases in adults and children on April 13, 2020 were searched to find the number of cases confirmed in children, the age range, and the number leading to hospitalization, intensive care unit (ICU) admission or death. A systematic literature search was performed April 13, 2020 to find additional data from cases series. RESULTS: Data on pediatric cases were available from government websites for 23 of the 70 countries with minimum 1000 cases by April 13, 2020. Of 424 978 cases in these 23 countries, 8113 (1.9%) occurred in children. Nine publications provided data from 4251 cases in 4 additional countries. Combining data from the websites and the publications, 330 of 2361 cases required admission (14%). The ICU admission rate was 2.2 % of confirmed cases (44 of 2031) and 7.2% of admitted children (23 of 318). Death was reported for 15 cases. CONCLUSION: Children accounted for 1.9% of confirmed cases. The true incidence of pediatric infection and disease will only be known once testing is expanded to individuals with less severe or no symptoms. Admission rates vary from 0.3 to 10% of confirmed cases (presumably varying with the threshold for testing) with about 7% of admitted children requiring ICU care. Death is rare in middle and high income countries. [**note: lots of parents of kids in our neighborhood are concerned about this issue. This report is reassuring but more data is needed.**] https://www.medrxiv.org/content/10.1101/2020.05.05.20091751v1

- COVID-19, caused by SARS-CoV-2, is an acute self-resolving disease in most of the patients, but some patients can develop a severe illness or even death. To characterize the host responses and identify potential biomarkers during disease progression, we performed a longitudinal transcriptome analysis for peripheral blood mononuclear cells (PBMCs) collected from 4 COVID-19 patients at 4 different time points from symptom onset to recovery. We found that PBMCs at different COVID-19 disease stages exhibited unique transcriptome characteristics. SARS-CoV-2 infection dysregulated innate immunity especially type I interferon response as well as the disturbed release of inflammatory cytokines and lipid mediators, and an aberrant increase of low-density neutrophils may cause tissue damage. Activation of cell death, exhaustion and migratory pathways may lead to the reduction of lymphocytes and dysfunction of adaptive immunity. COVID-19 induced hypoxia may exacerbate disorders in blood coagulation. Based on our analysis, we proposed a set of potential biomarkers for monitoring disease progression and predicting the risk of severity. [**note: more good stuff from China with more possible biomarkers for disease progression.**]
  https://www.medrxiv.org/content/10.1101/2020.05.05.20091355v1
- Numerous observational studies have suggested that the neutrophil-to-lymphocyte ratio (NLR) and lymphocyte proportion and the platelet-to-lymphocyte ratio (PLR) are inflammatory markers. Our study aimed to detect the role of NLR, PLR in predicting the prognosis of COVID-19. Methods: Four hundred and fifteen consecutive patients were enrolled in Shanghai Public Health Clinical Center affiliated to Fudan University, between 20 January and 11 April 2020 with confirmed COVID-19. Epidemiology, symptoms, signs, and laboratory examinations during the hospital stay were collected and compared between non-severe and severe patients. Statistical analysis was performed by SPSS 25.0 software. Results: Four hundred and fifteen laboratory-confirmed COVID-19 patients were included in our study, among which 386 (93%) patients were not severe, and 27 (7%) were severe. The proportion of males in severe cases is higher than in non-severe cases (75.86% vs. 50.52%, P=0.008). The age between the two groups is different (p=0.022). Compared with non-severe patients, severe patients exhibited more comorbidities, including hypertension (48.28% vs. 19.43%, p<0.001), diabetes (20.69% vs. 6.99%, p=0.009), chronic obstructive pulmonary disease (51.72% vs. 6.22%, p<0.001), and fatty liver (37.93% vs. 15.8%, p=0.002), respectively. NLR and PLR showed significant difference (p<0.001). Diabetes (OR 0.28; 95% CI 15.824-187.186), fatty liver (OR 21.469; 95% CI 2.306-199.872), coronary heart disease (OR 18.157; 95% CI 2.085-158.083), NLR (OR 1.729; 95% CI 1.050-2.847) were significantly associated with severe cases with COVID-19. The NLR of patients in severe group had a 1.729-fold higher than that of no-severe group (OR 1.729; 95% CI 1.050-2.847, P=0.031). Conclusions: NLR is an independent risk factor of severe COVID-19 patients. PLR, NLR were significantly different between severe and non-severe patients, so assessment of NLR, PLR may help identify high risk cases with COVID-19. Key words: Neutrophil-to-lymphocyte ratio, Platelet-to-lymphocyte ratio, COVID-19, Severity [**note: more good stuff about clinical markers!!**]
  https://www.medrxiv.org/content/10.1101/2020.05.04.20090431v1
- SARS-CoV-2 is currently causing a devastating pandemic and there is a pressing need to understand the dynamics, specificity, and neutralizing potency of the humoral immune response during acute infection. Herein, we report the dynamics of antibody responses to the receptor-binding domain (RBD) of the spike protein and virus neutralization activity in 44 COVID-19 patients. RBD-specific IgG responses were detectable in all patients 6 days after PCR

confirmation. Using a clinical isolate of SARS-CoV-2, neutralizing antibody titers were also detectable in all patients 6 days after PCR confirmation. The magnitude of RBD-specific IgG binding titers correlated strongly with viral neutralization. In a clinical setting, the initial analysis of the dynamics of RBD-specific IgG titers was corroborated in a larger cohort of PCR-confirmed patients (n=231). These findings have important implications for our understanding of protective immunity against SARS-CoV-2, the use of immune plasma as a therapy, and the development of much-needed vaccines. [**note: detectable antibodies in a short period of time following identification of infection by PCR. We are learning more about immunity.]** https://www.medrxiv.org/content/10.1101/2020.05.03.20084442v1

- Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a global pandemic. The percentage of infected individuals who seroconvert is still an open question. In addition, it has been shown in some individuals that viral genome can still be detected at considerable time post symptom resolution. Here we investigated both seroconversion and PCR-positivity in a large cohort of convalescent serum donors in New York City. Methods: Individuals with confirmed or suspected SARS-CoV-2 infection were screened via PCR for presence of viral genome and via enzyme-linked immunosorbent assay for presence of anti SARS-CoV-2 spike antibodies. Results: All but three confirmed SARS-CoV-2 patients seroconverted to the SARS-CoV-2 spike while only 37.4% of suspected SARS-CoV-2 patients seroconverted. PCR-positivity was detected up to 28 days from symptom resolution. Conclusions: Here we show that the vast majority of confirmed COVID19 patients seroconvert, potentially providing immunity to reinfection. We also report that in a large proportion of individuals, viral genome can be detected via PCR in the upper respiratory tract for weeks post symptom resolution, but it is unclear if this signal represents infectious virus. [**note: more good stuff from the Mt. Sinai group!! This was also covered in today's NY Times if you want the lay explanation. Another interesting finding is the viral genome detection in the upper respiratory track for weeks post symptom infection (cough, cough; I was wondering why I still have this persistent cough!!).**] https://www.medrxiv.org/content/10.1101/2020.04.30.20085613v1

DRUG DEVELOPMENT

- Background: The SARS-CoV-2 pandemic has resulted in enormous morbidity and mortality worldwide, yet no medications to date are proven to improve clinical outcomes in hospitalized COVID-19 patients. Famotidine is commonly used for gastric acid suppression but also has recently gained attention as an antiviral that may inhibit SARS-CoV-2 replication. Objective: To determine whether famotidine use is associated with improved clinical outcomes in patients with COVID-19 initially hospitalized to a non-intensive care setting. Design: Retrospective cohort study. Setting: Inpatients at a single academic medical center. Participants: Consecutive hospitalized patients with COVID-19 infection from February 25 to April 13, 2020. Measurements: Famotidine use (exposure); intubation or death (primary outcome) Results: 1,620 hospitalized patients with COVID-19 were analyzed including 84 (5.1%) who received famotidine within 24 hours of hospital admission. There were no differences between famotidine users and non-users in age, body mass index, or comorbidities including diabetes or hypertension. 340 (21%) patients met the study composite outcome of death or intubation. Use of famotidine was associated with reduced risk for death or intubation (adjusted hazard ratio (aHR) 0.40, 95% CI 0.20-0.81) and also with reduced risk for death alone (aHR 0.29, 95% CI 0.11-

0.78). Proton pump inhibitors, which also suppress gastric acid, were not associated with reduced risk for death or intubation. In patients without COVID-19 hospitalized during the same time period, no association was observed between use of famotidine and death or intubation. Limitations: Retrospective analysis; non-randomized exposure. Conclusion: Famotidine use is associated with reduced risk of intubation or death in hospitalized COVID-19 patients. Randomized controlled trials are warranted to determine whether famotidine therapy improves outcomes in hospitalized COVID-19 patients. [**note: this is a paper worth reading. One of the Columbia U hosptials examined whether famotidine has clinical usefulness. They did find reduced risk of intubation or death. There are some mechanistic discussions n the paper. PPIs don't seem to have any clinical effect.**]
https://www.medrxiv.org/content/10.1101/2020.05.01.20086694v1

- The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease 2019 (COVID-19) has led to the rapid initiation of urgently needed clinical trials of repurposed drug combinations and monotherapies. These regimens were primarily relying on mechanism-of-action based selection of drugs, many of which have yielded positive in vitro but largely negative clinical outcomes. To overcome this challenge, we report the use of IDentif.AI, a platform that rapidly optimizes infectious disease (ID) combination therapy design using artificial intelligence (AI). In this study, IDentif.AI was implemented on a 12-drug candidate therapy search set representing over 530,000 possible drug combinations. IDentif.AI demonstrated that the optimal combination therapy against SARS-CoV-2 was comprised of remdesivir, ritonavir, and lopinavir, which mediated a 6.5-fold improvement in efficacy over remdesivir alone. Additionally, IDentif.AI showed hydroxychloroquine and azithromycin to be relatively ineffective. The identification of a clinically actionable optimal drug combination was completed within two weeks, with a 3-order of magnitude reduction in the number of tests typically needed. IDentif.AI analysis was also able to independently confirm clinical trial outcomes to date without requiring any data from these trials. The robustness of the IDentif.AI platform suggests that it may be applicable towards rapid development of optimal drug regimens to address current and future outbreaks. [**note: I'll have to double check but I think there is a trial or two with this combination. This is the kind of stuff that needs to be done as I have always felt that a single drug is not going to be optimal therapy. Of course this is just another in the many AI papers but still it is provocative.]**
https://www.medrxiv.org/content/10.1101/2020.05.04.20088104v1
- The SARS-CoV-2 pandemic that originated from Wuhan, China, in December 2019 has impacted public health, society and economy and the daily lives of billions of people in an unprecedented manner. There are currently no specific registered antiviral drugs to treat or prevent SARS-CoV-2 infections. Therefore, drug repurposing would be the fastest route to provide at least a temporary solution while better, more specific drugs are being developed. Here we demonstrate that the antiparasitic drug suramin inhibits SARS-CoV-2 replication, protecting Vero E6 cells with an EC_{50} of ~20 µM, which is well below the maximum attainable level in human serum. Suramin also decreased the viral load by 2-3 logs when Vero E6 cells or cells of a human lung epithelial cell line (Calu-3) were treated. Time of addition and plaque reduction assays showed that suramin acts on early steps of the replication cycle, possibly preventing entry of the virus. In a primary human airway epithelial cell culture model, suramin also inhibited the progression of infection. The results of our preclinical study warrant further investigation and

suggest it is worth evaluating whether suramin provides any benefit for COVID-19 patients, which obviously requires well-designed, properly controlled randomized clinical trials. [**note: it seems as though every anti-parasite drug has activity *in vitro*. This one is no different, but what is the mechanism here? This drug has some wicked side effects.**]
https://www.biorxiv.org/content/10.1101/2020.05.06.081968v1

DIAGNOSTIC DEVELOPMENT

- The recent outbreak of the novel coronavirus SARS-CoV-2, which causes COVID-19, can be diagnosed using RT-qPCR, but inadequate access to reagents and equipment has slowed disease detection and impeded efforts to mitigate viral spread. Alternative approaches based on combinations of isothermal amplification and CRISPR-mediated detection, such as the SHERLOCK (S pecific H igh S ensitivity E nzymatic R eporter Un LOCK ing) technique, offer reduced dependence on RT-qPCR equipment, but previously reported methods required multiple fluid handling steps, complicating their deployment outside clinical labs. Here we developed a simple test chemistry called STOP (SHERLOCK Testing in One Pot) for detecting SARS-CoV-2 in one hour that is suitable for point-of-care use. This simplified test, STOPCovid, provides sensitivity comparable to RT-qPCR-based SARS-CoV-2 tests and has a limit of detection of 100 copies of viral genome input in saliva or nasopharyngeal swabs per reaction. Using lateral flow readout, the test returns result in 70 minutes, and using fluorescence readout, the test returns result in 40 minutes. Moreover, we validated STOPCovid using nasopharyngeal swabs from COVID-19 patients and were able to correctly diagnose 12 positive and 5 negative patients out of 3 replicates. We envision that implementation of STOPCovid will significantly aid "test-trace-isolate" efforts, especially in low-resource settings, which will be critical for long-term public health safety and effective reopening of the society. [**note: This is GREAT stuff. From one of the inventors of Crispr, here is another way to test for the virus. Wait, there's more!! FDA has issued an Emergency Use Authorization for the test!!!**]
https://www.medrxiv.org/content/10.1101/2020.05.04.20091231v1
- Molecular testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the gold standard for diagnosis of coronavirus disease 2019 (COVID-19), but the test clinical performance is poorly understood. From 3/10/2020-5/1/2020 NewYork-Presbyterian laboratories performed 27,377 SARS-CoV-2 molecular assays from 22,338 patients. Repeat testing was performed in 3,432 patients, of which 2,413 had negative and 1,019 had positive first day results. Repeat-tested patients were more likely to be older, male, African-American or Hispanic, and to have severe disease. Among the patients with initially negative results, 18.6% became positive upon repeat-testing. Only 58.1% of any-time positive patients had a result of "detected" on the first test. The clinical sensitivity of COVID-19 molecular assays is estimated between 66.2% and 95.6%, depending on the unknown number of false negative results in single-tested patients. Conversion to a negative result is unlikely to occur before 15 to 20 days after initial testing or 20-30 days after the onset of symptoms, with 50% conversion occurring at 28 days after initial testing. Forty-nine initially-positive patients converted to negative and then back to positive in subsequent days. Conversion from first day negative to positive results increased linearly with each day of testing, reaching 25% probability in 20 days. In summary, our study provides estimates of the clinical performance of SARS-CoV-2 molecular assays and suggests time frames for appropriate repeat testing, namely 15 to 20 days after a positive test and the same or next 2

days after a negative test in a patient with high suspicion for COVID-19. [**note: lots of people assume that molecular tests are 100% accurate. Real-life results suggest otherwise and this paper from a large NYC hospital adds to that.**] https://www.medrxiv.org/content/10.1101/2020.05.06.20093575v1 and here is another report on this topic from Stanford and University of Washington: https://www.medrxiv.org/content/10.1101/2020.05.03.20089151v1

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2020-05-09

It occurs to me that we need to have a string quartet performance to fill out the chamber music repertoire! One of my favorites is Schubert's Quartet #14 D.810 aka "Death and the Maiden." Here is the fine Hagen Quartet in performance: https://www.youtube.com/watch?v=ovEYC-mFjvU

Wow, are things moving fast! On Friday FDA approved the first home saliva collection kit for SARS-CoV-2 testing. Don't go chewing slippery elm just yet! The test kit will cost $100, requires a doctor's prescription and can only be performed at the Rutgers U clinical laboratory.

On Thursday, Terri Gross interviewed chef Tom Colicchio about what restaurants need to do to survive the pandemic. As you know I'm a big fan of podcasts and listen a variety of them on my morning walks. 'Fresh Air' is always full of good interviews and Terri and Tom have a wonderful discussion on what restaurants might do to help out. This is well worth listening to. You should all be watching Top Chef as well where Tom is the chief judge!!

Here is a good article from Harvard researchers about how much testing is required (hint: more than we are presently doing. The Atlantic has set up The COVID Tracking Project to provide "…the most complete data available about COVID-19 in the US. A big shout out to groups doing what the CDC ought to!

Here is a lengthy policy piece on the ethics of controlled human infection to study COVID-19.

MODELING

- The concern about (socio-)economic consequences of collective lockdowns in the Covid-19 pandemic calls for alternative strategies. We consider a *divide and conquer strategy* in which a high risk group (HRG) is put on strict isolation, whereas the remainder of the population is exposed to the virus, building up immunity against Covid-19. The question is whether this strategy may suppress the effective reproduction number below the critical value of $R_{eff} < 1$

without further lockdown once the HRG is released from isolation. While this proposal appears already rather academic, we show that R_eff < 1 can only be obtained provided that the HRG is less than ~20-30% of the total population. Hence, this strategy is likely to fail in countries with a HRG larger than the given upper bound. In addition, we argue that the maximum infection rate occurring in this strategy is likely to exceed realistic capacities of most health care systems. While the conclusion is rather negative in this regard, we emphasise that the strategy of *stopping the curve* at an early stage of the Covid-19 pandemic has a chance to work out. The required duration of the lockdown is estimated to be $\tau \sim 14$ days/(1-R_eff) (up to some order one factor) for R_eff < 1, provided a systematic tracing strategy of new infections exists for the subsequent relaxation phase. In this context we also argue why R_eff remains the crucial parameter which needs to be accurately monitored and controlled. [**note: I am always fond of military metaphors and am an astute student of von Clausewitz, hence the posting of this link.**] https://www.medrxiv.org/content/10.1101/2020.05.05.20092155v1

- Italy was the first European country to experience sustained local transmission of COVID-19. As of 1st May 2020, the Italian health authorities reported 28,238 deaths nationally. To control the epidemic, the Italian government implemented a suite of non-pharmaceutical interventions (NPIs), including school and university closures, social distancing and full lockdown involving banning of public gatherings and non essential movement. In this report, we model the effect of NPIs on transmission using data on average mobility. We estimate that the average reproduction number (a measure of transmission intensity) is currently below one for all Italian regions, and significantly so for the majority of the regions. Despite the large number of deaths, the proportion of population that has been infected by SARS-CoV-2 (the attack rate) is far from the herd immunity threshold in all Italian regions, with the highest attack rate observed in Lombardy (13.18% [10.66%-16.70%]). Italy is set to relax the currently implemented NPIs from 4th May 2020. Given the control achieved by NPIs, we consider three scenarios for the next 8 weeks: a scenario in which mobility remains the same as during the lockdown, a scenario in which mobility returns to pre-lockdown levels by 20%, and a scenario in which mobility returns to pre-lockdown levels by 40%. The scenarios explored assume that mobility is scaled evenly across all dimensions, that behaviour stays the same as before NPIs were implemented, that no pharmaceutical interventions are introduced, and it does not include transmission reduction from contact tracing, testing and the isolation of confirmed or suspected cases. New interventions, such as enhanced testing and contact tracing are going to be introduced and will likely contribute to reductions in transmission; therefore our estimates should be viewed as pessimistic projections. We find that, in the absence of additional interventions, even a 20% return to pre-lockdown mobility could lead to a resurgence in the number of deaths far greater than experienced in the current wave in several regions. Future increases in the number of deaths will lag behind the increase in transmission intensity and so a second wave will not be immediately apparent from just monitoring of the daily number of deaths. Our results suggest that SARS-CoV-2 transmission as well as mobility should be closely monitored in the next weeks and months. To compensate for the increase in mobility that will occur due to the relaxation of the currently implemented NPIs, adherence to the recommended social distancing measures alongside enhanced community surveillance including swab testing, contact tracing and the early isolation of infections are of paramount importance to reduce the risk of resurgence in transmission. [**note: this is useful to look at given the comparisons that have been made**

between Italy and the US. It comes from the Imperial College in London who were the first to put forth a widely used model.]
https://www.medrxiv.org/content/10.1101/2020.05.05.20089359v1

- Objectives: Recent data suggest higher COVID-19 rates and severity in Black, Asian, and minority ethnic (BAME) communities. The mechanisms underlying such associations remain unclear. We aimed to study the association between ethnicity and risk of COVID-19 infection and disentangle any correlation with socioeconomic deprivation or previous comorbidity. Design: Prospective cohort. Setting: UK Biobank linked to Hospital Episode Statistics (HES) and COVID-19 tests until 14 April 2020. Participants: UK Biobank participants from England, excluding drop-outs and deaths. Main measures: COVID-19 infection based on a positive PCR test. Ethnicity was self-reported and classified using Office of National Statistics groups. Socioeconomic status was based on index of multiple deprivation quintiles. Comorbidities were self-reported and completed from HES. Analyses: Multivariable Poisson analysis to estimate incidence rate ratios of COVID-19 infection according to ethnicity, adjusted for socioeconomic status, alcohol drinking, smoking, body mass index, age, sex, and comorbidity. Results: 415,582 participants were included, with 1,416 tested and 651 positive for COVID-19. The incidence of COVID-19 was 0.61% (95% CI: 0.46%-0.82%) in Black/Black British participants, 0.32% (0.19%-0.56%) in other ethnicities, 0.32% (0.23%-0.47%) in Asian/Asian British, 0.30% (0.11%-0.80%) in Chinese, 0.16% (0.06%-0.41%) in mixed, and 0.14% (0.13%-0.15%) in White. Compared with White participants, Black/Black British participants had an adjusted relative risk (RR) of 3.30 (2.39-4.55), Chinese participants 3.00 (1.11-8.06), Asian/Asian British participants 2.04 (1.36-3.07), other ethnicities 1.93 (1.08-3.45), and mixed ethnicities 1.07 (0.40-2.86). Socioeconomic status (adjusted RR 1.93 (1.51-2.46) for the most deprived), obesity (adjusted RR 1.04 (1.02-1.05) per kg/m2) and comorbid hypertension, chronic obstructive pulmonary disease, asthma, and specific renal diseases were also associated with increased risk of COVID-19. Conclusions: COVID-19 rates in the UK are higher in BAME communities, those living in deprived areas, obese patients, and patients with previous comorbidity. Public health strategies are needed to reduce COVID-19 infections among the most susceptible groups. [**note: more data on ethnicity and co-morbidities, this time from the UK. This pretty much confirms what has been observed and unless there is something new, I'm not going to post on future studies of this type.**]
https://www.medrxiv.org/content/10.1101/2020.05.06.20092676v1 and
https://www.medrxiv.org/content/10.1101/2020.05.06.20092700v1
- Importance: The COVID-19 pandemic has resulted in more than 3.5 million cases and 245 thousand deaths worldwide as of May 6, 2020. Determining the extent of the presence of the virus on public surfaces is critical for understanding the potential risk of infection in these areas. Objective: To evaluate the presence of SARS-CoV-2 RNA on public surfaces in a densely populated urban area in Brazil. Design and Setting: A total of 101 samples were collected from different surfaces in public places in the region of Belo Horizonte with the highest number of COVID-19 cases. Samples were collected near the hospital and public transportation areas using sterile swabs, and then submitted to nucleic acid extraction and genomic detection and quantification by one-step qPCR. Results: Seventeen of the 101 samples tested positive (16.8%) for SARS-CoV-2 RNA, including samples from bus stations/terminals, public squares, and sidewalks, including those near hospitals. Conclusions and Relevance: Our data indicated the contamination of public surfaces by SARS-CoV-2, especially near hospital areas, highlighting the

risk of infection for the population. Constant monitoring of the virus in urban areas is required as a strategy to fight the pandemic and prevent further infections. [**note: I think this is the first field study looking for virus on environmental surfaces. I suggests exercising care when in crowded spaces along with frequent hand washing or use of sanitizer.**] https://www.medrxiv.org/content/10.1101/2020.05.07.20094631v1

NEWLY REGISTERED CLINICAL TRIALS

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CLINICAL TRIAL RESULTS

- Background & Aims: New York is the current epicenter of Coronavirus disease 2019 (COVID-19) pandemic. The underrepresented minorities, where the prevalence of obesity is higher, appear to be affected disproportionately. Our objectives were to assess the characteristics and early outcomes of patients hospitalized with COVID-19 in the Bronx and investigate whether obesity is associated with worse outcomes. Methods: This retrospective study included the first 200 patients admitted to a tertiary medical center with COVID-19. The electronic medical records were reviewed at least three weeks after admission. The primary endpoint was in-hospital mortality. Results: 200 patients were included (female sex: 102, African American: 102). The median BMI was 30 kg/m2. The median age was 64 years. Hypertension (76%), hyperlipemia (46.2%), and diabetes (39.5%) were the three most common comorbidities. Fever (86%), cough (76.5%), and dyspnea (68%) were the three most common symptoms. 24% died during hospitalization (BMI <25 kg/m2: 31.6%, BMI 25-34 kg/m2: 17.2%, BMI≥35 kg/m2: 34.8%, p= 0.03). The multivariate analysis for mortality, demonstrates that BMI≥35 kg/m2 (OR: 3.78; 95% CI: 1.45 - 9.83; p=0.006), male sex (OR: 2.74; 95% CI: 1.25 - 5.98; p=0.011) and increasing age (OR: 1.73; 95% CI: 1.13 - 2.63; p=0.011) were independently associated with higher inhospital mortality. Similar results were obtained for the outcomes of increasing oxygen requirement and intubation. Conclusions: In this cohort of hospitalized patients with COVID-19 in a minority-predominant population, severe obesity, increasing age, and male sex were associated with higher in-hospital mortality and in general worse in-hospital outcomes. [**note: further substantiation of what we know, if you are an overweight, minority, male, you better make sure that your exposure to the virus is minimized as clinical prognosis is not good if you catch it.**] https://www.medrxiv.org/content/10.1101/2020.05.05.20091983v1
- From Boston - Abstract Background Coronavirus disease 2019 (COVID19) is an acute respiratory illness with a high rate of hospitalization and mortality. Prognostic biomarkers are urgently needed. Red blood cell distribution width (RDW), a component of complete blood counts that reflects cellular volume variation, has been shown to be associated with elevated risk for morbidity and mortality in a wide range of diseases. Methods We retrospectively studied the relationship between RDW and COVID19 mortality risk for 1198 adult patients diagnosed with SARS COV2 at 4 Partners Healthcare Network Hospitals between March 4, 2020, and April 28, 2020. Findings Elevated RDW (> 14.5%) was associated with increased mortality in patients of all ages with a risk ratio of 2.5 (95% CI, 2.3-2.8). Stratified by age, the risk ratio was 6.2 (4.4-7.9, N = 312) < 50 years, 3.2 (2.5-4.1, N = 230) 50-60, 2.3 (1.6-3.1, N = 236) 60-70, 1.2 (0.7-1.8, N = 203) 70-80, and 1.9 (1.5-2.3, N = 216) > 80 years. RDW was significantly associated with mortality in Cox proportional hazards models adjusted for age, D-Dimer, absolute lymphocyte count, and

common comorbidities (p < 1e-4 for RDW in all cases). Patients whose RDW increased during admission had a ~3-fold elevation in mortality risk compared to those whose RDW did not change. Interpretation Elevated RDW at diagnosis and an increase in RDW during admission are both associated with increased mortality risk for adult COVID19 patients at a large academic medical center network. [**note: another useful biomarker to poor clinical prognosis. I hope some enterprising young MD is compiling all of this into a treatment analysis and guideline!!**]
https://www.medrxiv.org/content/10.1101/2020.05.05.20091702v1

- Background COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was declared a global pandemic in March 2020. Electronic cigarette use (vaping) rapidly gained popularity in the US in recent years. Whether electronic cigarette users (vapers) are more susceptible to COVID-19 infection is unknown. Methods Using integrated data in each US state from the 2018 Behavioral Risk Factor Surveillance System (BRFSS), United States Census Bureau and the 1Point3Acres.com website, generalized estimating equation (GEE) models with negative binomial distribution assumption and log link functions were used to examine the association of weighted proportions of vapers with number of COVID-19 infections and deaths in the US. Results The weighted proportion of vapers who used e-cigarettes every day or some days ranged from 2.86% to 6.42% for US states. Statistically significant associations were observed between the weighted proportion of vapers and number of COVID-19 infected cases as well as COVID-19 deaths in the US after adjusting for the weighted proportion of smokers and other significant covariates in the GEE models. With every one percent increase in weighted proportion of vapers in each state, the number of COVID-19 infected cases increase by 0.3139 (95% CI: 0.0554 - 0.5723) and the number of COVID-19 deaths increase by 0.3705 (95% CI: 0.0623 - 0.6786) in log scale in each US state. Conclusions The positive associations between the proportion of vapers and the number of COVID-19 infected cases and deaths in each US state suggest an increased susceptibility of vapers to COVID-19 infections and deaths. [**note: DON'T SMOKE AND DON'T VAPE!**]
https://www.medrxiv.org/content/10.1101/2020.05.05.20092379v1
- The susceptibility of different populations to the SARS-CoV-2 infection is not yet understood. A deeper analysis of the genomes of individuals from different populations might explain their risk for infection. In this study, a combined analysis of ACE2 coding variants in different populations and computational chemistry calculations are conducted in order to probe the potential effects of ACE2 coding variants on SARS-CoV-2/ACE2 binding affinity. Our study reveals novel interaction data on the variants and SARS-CoV-2. We could show that ACE2-K26R; which is more frequent in the Ashkenazi Jewish population decrease the electrostatic attraction between SARS-CoV-2 and ACE2. On the contrary, ACE2-I468V, R219C, K341R, D206G, G211R were found to increase the electrostatic attraction and increase the binding to SARS-CoV-2; ordered by the strength of binding from weakest to strongest. I468V, R219C, K341R, D206G and G211R were more frequent in East Asian, South Asian, African and African American, European and European and South Asian populations, respectively. SARS-CoV-2/ACE2 interface in the WT protein and corresponding variants is showed to be a dominated by van der Waals (vdW) interactions. All the mutations except K341R induce an increase in the vdW attractions between the ACE2 and the SARS-CoV-2. The largest increase of is observed for the R219C mutant. [**note: We need much more of this type of analysis. IIRC, I linked to another paper several days ago that was looking for differences such as this. This will help explain why we see differential population**

infection rates.] https://www.biorxiv.org/content/10.1101/2020.05.08.084384v1 and this Chinese study shows the differences in ACE2 binding is a variety of different animals: https://www.biorxiv.org/content/10.1101/2020.05.08.084061v1

- Background: COVID-19 has rapidly emerged as a pandemic infection that has caused significant mortality and economic losses. Potential therapies and means of prophylaxis against COVID-19 are urgently needed to combat this novel infection. As a result of in vitro evidence suggesting zinc sulfate may be efficacious against COVID-19, our hospitals began using zinc sulfate as add-on therapy to hydroxychloroquine and azithromycin. We performed a retrospective observational study to compare hospital outcomes among patients who received hydroxychloroquine and azithromycin plus zinc versus hydroxychloroquine and azithromycin alone. Methods: Data was collected from electronic medical records for all patients being treated with admission dates ranging from March 2, 2020 through April 5, 2020. Initial clinical characteristics on presentation, medications given during the hospitalization, and hospital outcomes were recorded. Patients in the study were excluded if they were treated with other investigational medications. Results: The addition of zinc sulfate did not impact the length of hospitalization, duration of ventilation, or ICU duration. In univariate analyses, zinc sulfate increased the frequency of patients being discharged home, and decreased the need for ventilation, admission to the ICU, and mortality or transfer to hospice for patients who were never admitted to the ICU. After adjusting for the time at which zinc sulfate was added to our protocol, an increased frequency of being discharged home (OR 1.53, 95% CI 1.12-2.09) reduction in mortality or transfer to hospice remained significant (OR 0.449, 95% CI 0.271-0.744). Conclusion: This study provides the first in vivo evidence that zinc sulfate in combination with hydroxychloroquine may play a role in therapeutic management for COVID-19. [**note: I found this to be confusing. If they analyze the data one way there is no effect, another way there is an effect. I'm sure zinc sulfate is probably safe so one might as well use if HCQ is the drug of choice.**] https://www.medrxiv.org/content/10.1101/2020.05.02.20080036v1

DRUG DEVELOPMENT

- A series of epidemiological explorations have suggested a negative association between national BCG vaccination policy and the prevalence and mortality of COVID-19. Nevertheless, these comparisons are difficult to validate due to broad differences between countries such as socioeconomic status, demographic structure, rural vs. urban settings, time of arrival of the pandemic, number of diagnostic tests and criteria for testing, and national control strategies to limit the spread of COVID-19. We review evidence for the potential biological basis of BCG cross-protection from severe COVID-19 and refine the epidemiological analysis to mitigate effects of potentially confounding factors (e.g., stage of the COVID-19 epidemic, development, rurality, population density and age structure). Results fail to confirm the null hypothesis of no-association between BCG vaccination and COVID-19 mortality, and suggest that BCG could have a protective effect. Nevertheless, the analyses are restricted to coarse-scale signals and should be considered with caution. BCG vaccination clinical trials are required to corroborate the patterns detected here and to establish causality between BCG vaccination and protection from severe COVID-19. Public health implications of a plausible BCG cross-protection from severe COVID-19 are discussed. [**A wise analysis of the viability of BCG vaccination. There are about**

four clinical trials going on with healthcare workers and those should settle this once and maybe for all.] https://www.medrxiv.org/content/10.1101/2020.05.05.20091975v1

- Considering the massive amount of clinical trial registers aimed to find effective drugs for the prevention and treatment of COVID-19, it is challenging to have a comprehensive view of which drugs are being studied more extensively and when is expected that we will have consistent results regarding their effectiveness. This systematic review included all clinical trials on pharmacological therapy related to COVID-19 and SARS-CoV-2 registered at the International Clinical Trials Registry Platform (WHO-ICTRP) up to April 22, 2020. Clinical trials characteristics (country, design, sample size, main outcomes, expected completion data, type of participants, length of the interventions, main outcomes). How many trials and he accumulated sample size by drug or combination of drugs, and by month in 2020 was depicted. We identified 412 clinical trials registers addressing the effect of pharmacological treatments on COVID-19, predominantly from Asia and Europe (42.2% and 31.1% of clinical trials registers, respectively). The most main outcomes studied were clinical recovery (54.4% of the clinical trials registers, respiratory recovery (28.2%) mortality (27.4%), viral load/negativity (20.4%). During 2020, a huge amount of clinical trials are expected to be completed: 41 trials (60,366 participants) using hydroxychloroquine, 20 trials (1,588 participants) using plasma, 18 trials (6,830 participants) using chloroquine, 12 trials (9,938 participants using lopinavir/ritonavir, 11 trials (1,250 participants) using favipiravir, 10 trials (2,175 participants) using tocilizumab and 6 trials (13,540 participants) using Remdesivir. The distribution of the number of registered clinical trials among the different therapeutic options leads to an excess of sample size for some and a lack for others. Our data allow us to conclude that by the end of June we will have results of almost 20 trials involving 40000 patients for hydroxychloroquine and 5 trials with 4500 patients for remdesivir; however, low statistical power is expected from the 9 clinical trials testing the efficacy of favipiravir or the 5 testing tocilizumab, since they will recruit less than 1000 patients each one. [**note: I have not seen an aggregated data set such as this one. Interesting conclusion about whether the trials will be adequately powered to show efficacy.**] https://www.medrxiv.org/content/10.1101/2020.05.05.20091785v1
- In the absence of approved drugs or vaccines, there is a pressing need to develop tools for the therapy and prevention of Covid-19. Human monoclonal antibodies have very good probability of being safe and effective tools for the therapy and prevention of SARS-CoV-2 infection and disease. Here we describe the screening of PBMCs from seven people that survived Covid-19 infection to isolate human monoclonal antibodies against SARS-CoV-2. Over 1100 memory B cells were single cell sorted using the stabilized prefusion form of the spike protein and incubated for two weeks to allow natural production of antibodies. Supernatants from each cell were tested by ELISA for spike protein binding, and positive antibodies were further tested for neutralization of spike binding to the ACE2 receptor on Vero E6 cells and for virus neutralization in vitro. From the 1167 memory B specific for SARS-CoV-2, we recovered 265 B lymphocytes expressing human monoclonals recognizing the spike protein and 10 of these were able to inhibit the binding of the spike protein to the receptor. Finally, 17 mAbs were able to neutralize the virus when assessed for neutralization in vitro. Lead drug candidates will be selected from the panel of potent neutralizing antibodies identified with the procedure described in this study. [**The Italians enter the race for a therapeutic mAb!**
https://www.biorxiv.org/content/10.1101/2020.05.05.078154v1

DIAGNOSTIC DEVELOPMENT

- The SARS-CoV-2 (COVID-19) pandemic poses a significant public-health problem. In order to control the pandemic, rapid tests for detecting existing infections and assessing virus spread are critical. Approaches to detect viral RNA based on reverse transcription loop-mediated isothermal amplification (RT-LAMP) hold outstanding promise towards greatly simplified and broadly applicable testing methods. RT-LAMP assays appear more robust than qPCR-based methods and only require incubation at a constant temperature, thus eliminating the need for sophisticated instrumentation. Here, we tested a two-color RT-LAMP protocol using clinical SARS-CoV-2 samples and also established a protocol that does not require prior RNA isolation ("swab-to-RT-LAMP"). Our study is based on several hundred clinical patient samples with a wide range of viral loads, thus allowing, for the first time, to accurately determine the sensitivity and specificity of the RT-LAMP assay for the detection of SARS-CoV-2 in patients. We found that RT-LAMP can reliably detect SARS-CoV-2 samples with a qPCR threshold cycle number (CT value) of up to 30 in the standard RT-qPCR assay. We used both, either purified RNA or direct pharyngeal swab specimens and showed that RT-LAMP assays have, despite a decreased sensitivity compared to RT-qPCR, excellent specificity. We also developed a multiplexed LAMP-sequencing protocol as a validation and backup procedure to double-check the results of visual RT-LAMP assays. LAMP-sequencing is fully scalable and can assess the results of thousands of LAMP reactions in parallel. We propose decentralised COVID-19 testing as a routine to allow facilities and institutions to return to near-to-full functionality. [**note: more good work from Germany about coming up with a different diagnostic approach that is scalable.**] https://www.medrxiv.org/content/10.1101/2020.05.05.20092288v1
- From China - Objective: To investigate the performance of serological test and dynamics of serum antibody with the progress of SARS-CoV-2 infections. Methods: A total of 419 patients were enrolled including 19 confirmed cases and 400 patients from fever clinics. Their serial serum samples collected during the hospitalization were menstruated for IgM and IgG against SARS-CoV-2 using gold immunochromatographic assay and chemiluminescence immunoassay. We investigated whether thermal inactivation could affect the results of antibody detection. The dynamics of antibodies with the disease progress and false positive factors for antibody testing were also analyzed. Results: The positive rate of IgG detection was 91.67% and 83.33% using two CLIA, respectively. However, the IgM positive rate was dramatically declined might due to the lack of blood samples at early stages of the disease. The chemiluminescence immunoassay had a favorable but narrow linear range. Our work showed increased IgG values in serums from virus-negative patients and four negative samples were IgG weak-positive after thermal incubation. Our data showed the specificity of viral N+S proteins was higher than single antigen. Unlike generally thought that IgM appeared earlier than IgG, there is no certain chronological order of IgM and IgG seroconversion in COVID-19 patients. It was difficult to detect antibodies in asymptomatic patients suggesting that their low viral loads were not enough to cause immune response. Analysis of common interferent in three IgG false-positive patients, such as rheumatoid factor, proved that false positives were not caused by these interfering substances and antigenic cross-reaction. Conclusions: Viral serological test is an effective means for SARS-CoV-2 infect detection using both chemiluminescence immunoassay and gold immunochromatographic assay. Chemiluminescence immunoassay against multi-antigens has obvious advantages but still need improve in reducing false positives. [**note: everyone is**

working to improve serology testing.]
https://www.medrxiv.org/content/10.1101/2020.05.05.20092551v1

- The severe acute respiratory syndrome virus, SARS-CoV-2 (hereafter COVID-19), rapidly achieved global pandemic status, provoking large-scale screening programs in many nations. Their activation makes it imperative to identify methods that can deliver a diagnostic result at low cost. This paper describes an approach which employs sequence variation in the gene coding for its envelope protein as the basis for a scalable, inexpensive test for COVID-19. It achieves this by coupling a simple RNA extraction protocol with low-volume RT-PCR, followed by E-Gel screening and sequencing on high-throughput platforms to analyze 10,000 samples in a run. Slight modifications to the protocol could support screening programs for other known viruses and for viral discovery. Just as the $1,000 genome is transforming medicine, a $1 diagnostic test for viral and bacterial pathogens would represent a major advance for public health. [**note: A $1 test for SARS-CoV-19? Sign me up!!!**]
https://www.biorxiv.org/content/10.1101/2020.05.05.079400v1

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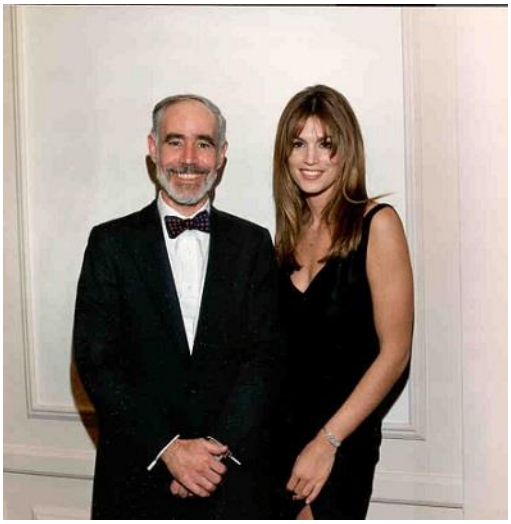
2020-05-10

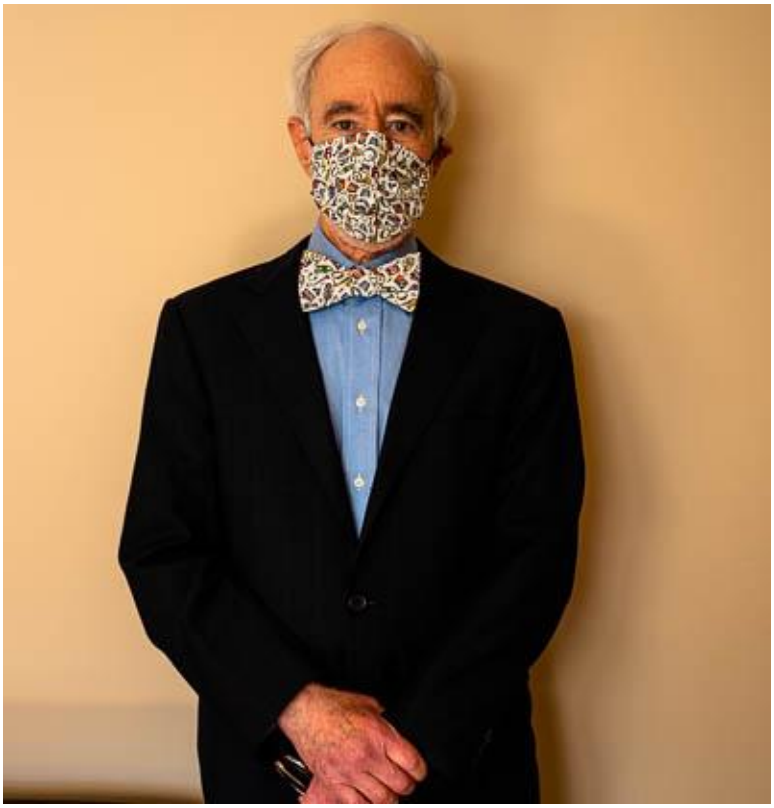
To all the mothers, Happy Mother's Day!!!!!

Sunday is usually a day of reflection and what better way than with this Verdi Requiem:
https://www.youtube.com/watch?v=Nlq9lJRElBk&t=11s

Dearest readers, I have a special treat for all of you. Most of you don't know that at one time in the long ago past, I did a stint as an Armani model. Yes, it is true and here is me and a certain super-model just off the runway.



I was thinking to myself there has to also be a way to revive my modeling career. What better way than to dress for success the COVID-19 way!!! With the help of my wonderful bowtie supplier, I now present business dress for the COVID-19 ERA!!



For you fashionistas, I was also featured in the Washington Post way back in 2005. I did get my famous tag line in the interview, "I'm a slave to fashion; we all must have standards!" Eat your heart out Tim Gunn. Unfortunately, my picture didn't make the front page they deferred to Mac Dunaway; it's hard to compete with the likes of Faye's brother! Enough fashion talk, on with the main business of the day. **Wait, let's run a contest! First person to correctly identify my partner in the Armani picture wins a prize! This one should be easy.**

Here is a thoughtful paper from ten top notch scientists about how to close the discovery gap.

I constantly look at lots of different sites with the goal of providing my readers with the best information. Here is something that popped up on the very good STAT website. I think I may have mentioned testing within the adult film industry in a prior newsletter and here is a more detailed analysis. Always learn from what has worked in the past!!!

There are some good modeling abstracts today and a scattering of new clinical trials. We still are waiting for some of the controlled clinical trial results.

MODELING

- This paper seeks to determine which workers affected by lockdown measures can return to work when a government decides to apply lockdown exit strategies. This system, which we call Sequential Selective Multidimensional Decision (SSMD), involves deciding sequentially, by

geographical areas, sectors of activity, age groups and immunity, which workers can return to work at a given time according to the epidemiological criteria of the country as well as that of a group of reference countries, used as a benchmark, that have suffered a lower level of lockdown de-escalation strategies. We apply SSMD to Spain, based on affiliation to the Social Security system prior to the COVID-19 pandemic, and conclude that 98.37% of the population could be affected. The proposed system makes it possible to accurately identify the target population for serological IgG antibody tests in the work field, as well as those affected by special income replacement measures due to lockdown being maintained over a longer period. [**note: the Spaniards are trying to figure out who can go back to work, let's hope the US is doing the same thing as we need to begin getting the economy back on track!**]
https://www.medrxiv.org/content/10.1101/2020.05.06.20093344v1

- Contact tracing is critical to limiting the spread of pandemics such as COVID-19, but most protocols only "forward-trace" to notify people who were recently exposed. Using a stochastic branching process model, we find that "bidirectional" tracing to identify infector individuals robustly outperforms forward-only approaches across a wide range of scenarios. The addition of rapid smartphone-based exposure notification offers few benefits over conventional manual tracing alone unless uptake of the digital system is near-universal. However, as long as exposure events can be detected by nearly all smartphones, the combination of manual and digital with bidirectional tracing more than doubles the probability of controlling outbreaks across three epidemiological scenarios. Implementing combined bidirectional tracing may be critical to controlling COVID-19 without more costly interventions. [**note: it is time to start hiring some of those whose jobs are lost and turn them into public health workers. America has a great can-do spirit and this is something we CAN DO.**]
https://www.medrxiv.org/content/10.1101/2020.05.06.20093369v1
- Countries with ambitious strategies to "crush the curve" of their epidemic trajectories, to promptly eliminate SARS-CoV-2 transmission at national level, include China, Korea, Japan, Taiwan, New Zealand and Australia. In stark contrast, many of the European countries hit hardest over the last two months, including Italy, Spain, France, Ireland and the United Kingdom, currently appear content to merely "flatten the curve" of their epidemic trajectories so that transmission persists at rates their critical care services can cope with. Here is presented a simple set of arithmetic modelling analyses that explain why preferable crush the "curve strategies", to eliminate transmission within months, would require only a modest amount of additional containment effort when compared to "flatten the curve" strategies that allow epidemics to persist at a steady, supposedly manageable level for years, decades or even indefinitely. [**note: from Ireland, more proof of the validity of Occam's Razor.**]
https://www.medrxiv.org/content/10.1101/2020.05.06.20093112v1
- Most countries are suffering severely from the ongoing covid-19 pandemic despite various levels of preventive measures. A common question is if and when a country or region will reach herd immunity h. The classical herd immunity level h_C is defined as h_C=1-1/R_0, where R_0 is the basic reproduction number, for covid-19 estimated to lie somewhere in the range 2.2-3.5 depending on country and region. It is shown here that the disease-induced herd immunity level h_D, after an outbreak has taken place in a country/region with a set of preventive measures put in place, is actually substantially smaller than h_C. As an illustration we show that if R_0=2.5 in an age-structured community with mixing rates fitted to social activity studies, and also categorizing

individuals into three categories: low active, average active and high active, and where preventive measures affect all mixing rates proportionally, then the disease-induced herd immunity level is h_D=43% rather than h_C=1-1/2.5=60%. Consequently, a lower fraction infected is required for herd immunity to appear. The underlying reason is that when immunity is induced by disease spreading, the proportion infected in groups with high contact rates is greater than that in groups with low contact rates. Consequently, disease-induced immunity is stronger than when immunity is uniformly distributed in the community as in the classical herd immunity level. [**note: this is a pretty provocative paper and would be welcome news *if* it does hold up.**] https://www.medrxiv.org/content/10.1101/2020.05.06.20093336v1

NEWLY REGISTERED CLINICAL TRIALS

- This study aims to examine the tolerability of high dose of leflunomide in patients with COVID-19 who are not yet hospitalized, but have risk factors for disease progression and complications. [**note: phase 1 trial of a rheumatoid arthritis drug that I have not seen previously mentioned in any of the repurposing studies.**] NCT04361214
- B38-CAP is a bacteria-derived ACE2-like enzyme that suppresses hypertension and cardiac dysfunction Angiotensin-converting enzyme 2 (ACE2) is critically involved in cardiovascular physiology and pathology, and is currently clinically evaluated to treat acute lung failure. Here we show that the B38-CAP, a carboxypeptidase derived from Paenibacillus sp. B38, is an ACE2-like enzyme to decrease angiotensin II levels in mice. In protein 3D structure analysis, B38-CAP homolog shares structural similarity to mammalian ACE2 with low sequence identity. In vitro, recombinant B38-CAP protein catalyzed the conversion of angiotensin II to angiotensin 1-7, as well as other known ACE2 target peptides. Treatment with B38-CAP suppressed angiotensin II-induced hypertension, cardiac hypertrophy, and fibrosis in mice. Moreover, B38-CAP inhibited pressure overload-induced pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction in mice. Our data identify the bacterial B38-CAP as an ACE2-like carboxypeptidase, indicating that evolution has shaped a bacterial carboxypeptidase to a human ACE2-like enzyme. Bacterial engineering could be utilized to design improved protein drugs for hypertension and heart failure.On the contraryTreatment with recombinant human ACE2 protein (rhACE2), which is devoid of its membrane-anchored domain thus soluble, has been demonstrated to exhibit beneficial effects in various animal models including heart failure, acute lung injury, and diabetic nephropathy, and so forth. rhACE2 is currently tested in the clinic to treat ARDS and COVID-19 infected patients . Using cell cultures and organoids, researchers from the Karolinska Institutet in Sweden and the University of British Columbia (UBC) in Canada, showed that by adding a genetically modified variant of ACE2, called human recombinant soluble angiotensin-converting enzyme 2 (hrsACE2), COVID-19 was prevented from entering cells.The paper, published in Cell, shows that hrsACE2 had a dose dependent effect of viral growth of SARS-CoV-2 and was able to reduce it by a factor of 1,000 to 5,000 in cell cultures. Despite its beneficial effects, rhACE2 is a glycosylated protein and thus its preparation requires time- and cost-consuming protein expression system with mammalian or insect cells, which may not be advantageous in drug development and medical economy Although it had been reported that an immune response is associated with the chronic infusion of rhACE2 resulting in the degradation of rhACE226, this was not observed for B38-CAP; there were no antibodies against B38-CAP detectable in the serum of mice infused with B38-CAP for 2 weeks. Implantation of B38-CAP-filled osmotic mini-

pumps significantly suppressed Ang II-induced hypertension in conscious mice .without affecting the heart rate. These results indicate that B38-CAP antagonizes the vasopressor effect of Ang II. So the principle investigator expects and suggests that treating with cloned Bacterial ACE2 receptors -like enzyme of B38-CAP could be promising COVID-19 infection- and lung injury preventing drug better than recombinant human ACE2 in addition to brsACE2, expected to lure the virus to attach itself to the copy instead of the actual cells… It distracts the virus from infecting the cells to the same degree and should lead to a reduction in the growth of the virus in the lungs and other organs. A study showed that recombinant B38-CAP protein downregulates Ang II levels in mice and antagonizes Ang II-induced hypertension, pathological cardiac hypertrophy, and myocardial fibrosis. We also show beneficial effects of B38-CAP on the pathology of pressure overload-induced heart failure in mice without overt toxicities. [**note: and from Egypt!! Out of the box thinking on this one.**] NCT04375046

- The global pandemic of novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began in Wuhan, China, in December 2019, and has since spread worldwide.1 As of April 14, 2020, there have been more than 1.5 million reported cases and 124 000 deaths in more than 200 countries. A recent open-label nonrandomized French study reporte that addition of azithromycin to hydroxychloroquine in 6 patients resulted in numerically superior viral clearance (6/6, 100%) compared with hydroxychloroquine monotherapy (8/14, 57%) or control (2/16, 12.5%). Azithromycin alone has never been tested, whereas azithromycin has immunomodulating and anti-inflammatory properties that could theoretically prevent or limit secondary worsening. Our hypothesis is that azithromycin combined with amoxicillin/clavulanate will be superior to amoxicillin/clavulanate alone to obtain viral clearance at Day 6 in COVID-19 patients with pneumonia and hospitalized in a non-intensive care unit ward. [**note: double the antibiotic, double the fun to paraphrase the old Wrigley Doublemint gum commercial and no HCQ in this trial.**] NCT04363060

CLINICAL TRIAL RESULTS

- IMPORTANCE How to appropriately care for patients who become PCR-negative for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still not known. Patients who have recovered from coronavirus disease 2019 (COVID-19) could profoundly impact the health care system if a subset were to be PCR-positive again with reactivated SARS-CoV-2. OBJECTIVE To characterize a single center COVID-19 cohort with and without recurrence of PCR positivity, and develop an algorithm to identify patients at high risk of retest positivity after discharge to inform health care policy and case management decision-making. DESIGN, SETTING, AND PARTICIPANTS A cohort of 414 patients with confirmed SARS-CoV-2 infection, at The Second Affiliated Hospital of Southern University of Science and Technology in Shenzhen, China from January 11 to April 23, 2020. EXPOSURES Polymerase chain reaction (PCR) and IgM-IgG antibody confirmed SARS-CoV-2 infection. MAIN OUTCOMES AND MEASURES Univariable and multivariable statistical analysis of the clinical, laboratory, radiologic image, medical treatment, and clinical course of admission/quarantine/readmission data to develop an algorithm to predict patients at risk of recurrence of PCR positivity. RESULTS 16.7% (95CI: 13.0%-20.3%) patients retest PCR positive 1 to 3 times after discharge, despite being in strict quarantine. The driving factors in the recurrence prediction model included: age, BMI; lowest levels of the blood laboratory tests during hospitalization for cholinesterase, fibrinogen, albumin, prealbumin,

calcium, eGFR, creatinine; highest levels of the blood laboratory tests during hospitalization for total bilirubin, lactate dehydrogenase, alkaline phosphatase; the first test results during hospitalization for partial pressure of oxygen, white blood cell and lymphocyte counts, blood procalcitonin; and the first test episodic Ct value and the lowest Ct value of the nasopharyngeal swab RT PCR results. Area under the ROC curve is 0.786. CONCLUSIONS AND RELEVANCE This case series provides clinical characteristics of COVID-19 patients with recurrent PCR positivity, despite strict quarantine, at a 16.7% rate. Use of a recurrence prediction algorithm may identify patients at high risk of PCR retest positivity of SARS-CoV-2 and help modify COVID-19 case management and health policy approaches. [**note: Stanford and Shenzhen researchers address an important problem here, those patients showing a recurrence of positive viral appearance.**] https://www.medrxiv.org/content/10.1101/2020.05.06.20089573v1

- Purpose: Conjunctival signs and symptoms are observed in a subset of patients with COVID-19, and SARS-CoV-2 has been detected in tears, raising concerns regarding the eye both as a portal of entry and carrier of the virus. The purpose of this study was to determine whether ocular surface cells possess the key factors required for cellular susceptibility to SARS-CoV-2 entry/infection. Methods: We analyzed human post-mortem eyes as well as surgical specimens for the expression of ACE2 (the receptor for SARS-CoV-2) and TMPRSS2, a cell surface-associated protease that facilitates viral entry following binding of the viral spike protein to ACE2. Results: Across all eye specimens, immunohistochemical analysis revealed expression of ACE2 in the conjunctiva, limbus, and cornea, with especially prominent staining in the superficial conjunctival and corneal epithelial surface. Surgical conjunctival specimens also showed expression of ACE2 in the conjunctival epithelium, especially prominent in the superficial epithelium, as well as the substantia propria. All eye and conjunctival specimens also expressed TMPRSS2. Finally, western blot analysis of protein lysates from human corneal epithelium obtained during refractive surgery confirmed expression of ACE2 and TMPRSS2. Conclusions: Together, these results indicate that ocular surface cells including conjunctiva are susceptible to infection by SARS-CoV-2, and could therefore serve as a portal of entry as well as a reservoir for person-to-person transmission of this virus. This highlights the importance of safety practices including face masks and ocular contact precautions in preventing the spread of COVID-19 disease. [**note: safety goggles for all? It's not clear what the viral titer is for ocular entry. Certainly, ER workers need face shields but does the general public. I'll have to find my old Speedo swim goggles!**] https://www.biorxiv.org/content/10.1101/2020.05.09.086165v1
- Background Amid the crisis of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), front-line clinicians in collaboration with backstage medical researchers analyzed clinical characteristics of COVID-19 patients and reported the prognosis using myocardial data records upon hospitalization. Methods We reported 135 cases of laboratory-confirmed COVID-19 patients admitted in The First People's Hospital of Jiangxia District in Wuhan, China. Demographic data, medical history, and laboratory parameters were taken from inpatient records and compared between patients at the Intensive Care Unit (ICU) and non-ICU isolation wards for prognosis on disease severity. In particular, survivors and non-survivors upon ICU admission were compared for prognosis on disease mortality. Results For COVID-19 patients, blood test results showed more significantly deranged values in the ICU group than those in non-ICU. Among those parameters for ICU patients, myocardial variables including troponin T, creatine kinase isoenzymes, myoglobin, were found

significantly higher in non-survivors than in survivors. Conclusions Upon hospitalization abnormal myocardial metabolism in COVID-19 patients could be prognostic indicators of a worsened outcome for disease severity and mortality. [**note: myocardial markers for poor prognosis.**] https://www.medrxiv.org/content/10.1101/2020.05.06.20068882v1

DRUG DEVELOPMENT

- The site of SARS-CoV-2 entry and replication critically impacts strategies for COVID-19 diagnosis, transmission mitigation, and treatment. We determined the cellular location of the SARS-CoV-2 target receptor protein, ACE2, in the human upper airway, finding striking enrichment (200-700 fold) in the olfactory neuroepithelium relative to nasal respiratory or tracheal epithelial cells. This cellular tropism of SARS-CoV-2 may underlie its high transmissibility and association with olfactory dysfunction, while suggesting a viral reservoir potentially amenable to intranasal therapy. [**note: honestly don't know where to put this interesting finding. I still wonder if those of us who have allergic rhinitis and constantly have buildup of mucous are somehow protected against nasal infection. I continue to believe someone should be testing for this and I'm happy to donate the sample even if it has to be done via an invasive procedure!**] https://www.biorxiv.org/content/10.1101/2020.05.08.084996v1

DIAGNOSTIC DEVELOPMENT

- Background: While the recommended laboratory diagnosis of COVID-19 is a molecular based assay, population-based studies to determine the prevalence of COVID-19 usually use serological assays. Objective: To evaluate the sensitivity and specificity of a rapid diagnostic test for COVID-19 compared to quantitative reverse transcription polymerase chain reaction (qRT-PCR). Methods: We evaluated the sensitivity using a panel of finger prick blood samples from participants >18 years of age that had been tested for COVID-19 by qRT-PCR. For assessing specificity, we used serum samples from the 1982 Pelotas (Brazil) Birth Cohort participants collected in 2012 with no exposure to SARS-CoV-2. Results: The sensitivity of the test was 77.1% (95% CI 66.6 - 85.6), based upon 83 subjects who had tested positive for qRT-PCR at least 10 days before the rapid diagnostic test (RDT). Based upon 100 sera samples, specificity was 98.0% (95% CI 92.9 - 99.8). There was substantial agreement (Kappa score 0.76) between the qRT-PCR results and the RDT. Interpretation. The validation results are well in line with previous assessments of the test, and confirm that it is sufficiently precise for epidemiological studies aimed at monitoring levels and trends of the COVID-19 pandemic. [**note: Make it work!! Good stuff from Brazil, validating a rapid serological test from China that can be used to do field epidemiology.**] https://www.medrxiv.org/content/10.1101/2020.05.06.20093476v1
- Currently, the presence of the virus in individual patients and at the population level is being monitored by testing symptomatic cases by PCR for the presence of viral RNA. There is an urgent need for SARS-CoV-2 serologic tests to identify all infected individuals, irrespective of clinical symptoms, to conduct surveillance and implement strategies to contain spread. As the receptor binding domain (RBD) of the viral spike (S) protein is poorly conserved between SARS-CoVs and other pathogenic human coronaviruses, the RBD represents a promising antigen for detecting CoV specific antibodies in people. Here we use a large panel of human sera (70 SARS-CoV-2 patients and 71 control subjects) and hyperimmune sera from animals exposed to zoonotic CoVs to evaluate the performance of the RBD as an antigen for accurate detection of SARS-CoV-2-

specific antibodies. By day 9 after the onset of symptoms, the recombinant SARS-CoV-2 RBD antigen was highly sensitive (98%) and specific (100%) to antibodies induced by SARS-CoVs. We observed a robust correlation between levels of RBD binding antibodies and SARS-CoV-2 neutralizing antibodies in patients. Our results, which reveal the early kinetics of SARS-CoV-2 antibody responses, strongly support the use of RBD-based antibody assays for population-level surveillance and as a correlate of neutralizing antibody levels in people who have recovered from SARS-CoV-2 infections. [**note: the binding domain of SARS-CoV-2 antibodies is not shared with other human and animal coronaviruses.**]
https://www.medrxiv.org/content/10.1101/2020.05.06.20093377v1

- FDA approves a new type of antigen test kit. The U.S. Food and Drug Administration has issued the first emergency use authorization (EUA) for a COVID-19 antigen test, a new category of tests for use in the ongoing pandemic. These diagnostic tests quickly detect fragments of proteins found on or within the virus by testing samples collected from the nasal cavity using swabs. The EUA was issued late Friday to Quidel Corporation for the Sofia 2 SARS Antigen FIA. This test is authorized for use in high and moderate complexity laboratories certified by Clinical Laboratory Improvement Amendments (CLIA), as well as for point-of-care testing by facilities operating under a CLIA Certificate of Waiver.