2020-06-01

Happy June and welcome to Week 11 of the newsletter!!

Let's spend some time on soul music and for me there was nobody like Sam Cooke. The tragedy of his early death was never adequately explained and remains controversial to this day. Unfortunately, his best composition 'A Change is Gonna Come' was never performed on video that I could find so we have to settle for a short appearance on the old Jerry Lewis show: https://www.youtube.com/watch?v=eltCoCoR2Yg There is a very funny back story about 'A Change is Gonna Come' on a Drunk History episode and here is the original Sam Cooke recording that always melts my heart: https://www.youtube.com/watch?v=wEBlaMOmKV4 The film clips accompanying this video are apt for reflection today!

Trying to decipher who will be at risk for long term illness will be difficult. Here is a nice Washington Post story on two people who suffered after affects from viral illnesses. So much that we don't know about our body's response to infection.

This came across my newsfeed yesterday afternoon. New coronavirus losing potency, top Italian doctor says. Is this too good to be true? Is there a seasonality to SARS-CoV-2. I'm not throwing my masks away just yet. And there is this report on farm workers where there is a high incidence of infections.

Lilly are the first company to start a mAb clinical trial which is good news.

STAT have an interview with Tony Fauci.

Here is a very thoughtful editorial commentary on the ethics of human challenge trials for COVID-19 vaccine development. As I noted yesterday, a better knowledge and assay method for neutralizing antibody might be a good and 'safer' substitute for such studies. Antibody characterization is approaching a level of sensitivity to look hard at that approach.

If you wanted to know how to do nasopharyngeal swabs, have no fear as I have you covered!! This can turn into an interesting DIY home project with you and your partner! It goes without saying that Q-Tips are not suitable for practicing (I'm still amazed how many people use them to clean out ear wax). What a treasure trove this newsletter is!

MODELING

- Bats are presumed reservoirs of diverse coronaviruses (CoVs) including progenitors of Severe Acute Respiratory Syndrome (SARS)-CoV and SARS-CoV-2, the causative agent of COVID-19. However, the evolution and diversification of these coronaviruses remains poorly understood. We used a Bayesian statistical framework and sequence data from all known bat-CoVs (including 630 novel CoV sequences) to study their macroevolution, cross-species transmission, and dispersal in China. We find that host-switching was more frequent and across more distantly related host taxa in alpha- than beta-CoVs, and more highly constrained by phylogenetic distance for beta-CoVs. We show that inter-family and -genus switching is most common in Rhinolophidae and the genus Rhinolophus. Our analyses identify the host taxa and geographic regions that define hotspots of CoV evolutionary diversity in China that could help target bat-CoV discovery for proactive zoonotic disease surveillance. Finally, we present a phylogenetic analysis suggesting a likely origin for SARS-CoV-2 in Rhinolophus spp. bats. [**note: fascinating**

paper on the phylogenetic analysis of SAR-CoV-2 This type of work will aid in field ecology studies.] https://www.biorxiv.org/content/10.1101/2020.05.31.116061v1

- Background Healthcare authorities have generally advised against wearing glove by the general population. However, the use of gloves has become a common sight in public places raising the question of the necessity of glove wearing practice by the general population Objective This study aims to investigate the prevalence and types of glove used as well as the acceptance of the glove practice by individuals visiting the high-risk area during Covid-19 pandemic. Setting This prospective observational study was conducted among individuals visiting a wet market and district specialist hospital During Covid-19 pandemic. The required data was recorded based on observation by trained data collectors who were stationed at the strategic entry point. Methods Individuals entering through dedicated entry point were observed for the type, category and practice of wearing personal protective equipment. Inclusion criteria for this study were any individuals entering the facilities from entry points without respiratory symptoms. Exclusion criteria for this study were individuals less than 2 years old, visiting the emergency department, facility staff, individuals who are suspected of multiple entry and individuals who are exiting the treatment facility entrance. Patients were categorized into two groups of acceptable and unacceptable glove practice. The Pearson chi-square was used to test for differences in investigated variables in the univariate setting. Main outcome measure Prevalence, acceptance of glove wearing practice. Results A total of 75 individuals (2.3%) compromising of 45 (60.0%) individuals from hospitals and 30 (40.0%) individuals from wet markets were seen wearing glove amongst 3322 individuals observed during the data collection period. A higher proportion of individuals visiting wet market (30.0%) were observed with unacceptable glove practice compared to individuals visiting the hospital (8.9%), $\chi 2$ (1) = 5.60, p=.018. Similarly, a Higher proportion of glove use among non-Malay (53.3%) compared to Malay (46.7%) was observed in hospital compared to a higher proportion of glove use among Malay compared to non-Malay (16.7%) visiting wet market, $\chi 2$ (1) = 10.20, p=.001. As for glove use, we found that male were using more medical-grade glove (78.8%) compared to non-medical grade glove (21.2%) while an equal amount of medical (50.0%) and non-medical grade glove (50.0%) was used among female, $\chi 2$ (1) = 6.546, p=.011. Besides, we found that higher proportion of individual using medical-grade glove was using medical grade facemask (68.3%) which was similar to the proportion of individuals using non-medical glove was using non-medical facemask (66.7%), $\chi 2$ (1) = 5.25, p=.022. Conclusion We present the prevalence and characteristics of glove wearing practice in high-risk location during the current COVID-19 outbreak in Malaysia. Facing a worldwide public health emergency with limited effective clinical treatment, the role of glove-wearing in mitigating COVID-19 transmission is questionable. If needed, the compliance to proper glove-wearing could be improved through targeted public health education [**note: this Malaysian study looks at glove wearing. At present, I don't know of any public health agency that recommends this as the data on environmental transmission of SARS-CoV-2 is scant. As long as one doesn't touch one's face when out shopping and frequently washes hands or uses sanitizer the use of gloves is not needed. Still, it's useful to post these abstracts out of interest.**] https://www.medrxiv.org/content/10.1101/2020.05.30.20117564v1

NEWLY REGISTERED CLINICAL TRIALS

- In this study, defined cases of COVID-19 with mild, moderate or severe pneumonia will be treated with standard treatment regimens in combination with oral administration of Colchicine Plus Herbal phenolic monoterpene Fractions. Improvement in clinical, laboratory and radiological manifestations will be evaluated in treated patient compared to control group. [**note: nothing in the submission about what class of terpenes to be used. This is an Iranian trial.]** NCT04392141
- The virus SARS-CoV-2 causes severe pneumonia which, in a proportion of patients progresses towards an Acute Respiratory Distress Syndrome (ARDS) mainly related to the antiviral immune response. To date, there is no available treatment that significantly improves outcome of patients with COVID-19 pneumonia. Sphingosine-1-phosphate receptor 1 (S1P1) ligands control vascular leakage in the airways and sphingosine-1-phosphate (S1P) receptor ligands devoid of activity on sphingosine-1-phosphate receptor 3 (S1P3) show an excellent safety profile, including ozanimod. Critically, S1P1 ligands mildly impact, but do not compromise viral clearance and they reduced lung injury in the highly pathogenic H1N1-ferret model, even without concomitant use of antivirals and with a synergistic effect when associated to antiviral agents. Ozanimod was approved by the FDA for the treatment of relapsing multiple sclerosis at the end of March 2020. The investigators believe that this immune modulator is at the top of the list of agents that should be trialed in order to mitigate the morbidity and mortality of COVID-19. The primary objective is to substantiate the impact of ozanimod on key outcomes of COVID-19 patient progression, which will guide decision making around sample size and the choice of endpoints for future clinical trial. [**note: this is a Canadian study of a BMS drug by way of Celgene. The drug recently was approved for relapsing MS]** NCT04405102
- Recent information appearing from different countries suggest that treatment of Coronavirus disease 2019 (COVID-19) with hydroxychloroquine or with a combination of hydroxychloroquine and azithromycin has either an indifferent effect on viral replication or substantial cardiotoxicity. This is a clinical trial aiming to prove that addition of oral clarithromycin to treatment regimen of COVID-19 is associated with early clinical improvement and attenuation of the high inflammatory burden of the host. The study will not comprise a placebo-comparator group since this is considered inappropriate in an era of a pandemic with substantial global mortality. [**note: yet another antibiotic enters clinical trials! This one is from Greece**] NCT04398004
- Combination Therapy with Isotretinoin and Tamoxifen may provide Complete Protection against Severe Acute Respiratory Syndrome Coronavirus [**note: this is an Egyptian trial with a provocative title. If anyone is interested there is a lengthy abstract supporting this approach (personally I think it is looney but what do I know. Full trial is at https://Clinicaltrials.gov and just punch in:** NCT04389580

- Researchers think that people s mental health and behavior will be very affected by the steps taken to slow the spread of COVID-19, such as social distancing. Also, the threat of disease and death, to people and their loved ones, can cause much stress. Researchers want to learn more about these stressors and how they are affecting people.

  This online study will include both new participants and those who have taken part in past National Institute of Mental Health studies. All will complete the same surveys and tasks. Participants will give their name and email address. They will get a username and password. The file that links their username to their personal data will be kept secure. Participants will complete a set of surveys about the following:

- Sociodemographic data, such age, race, and income
- Education and work status
- Mental and medical illness and treatment
- Family medical history
- Mood
- COVID-19 experience
- Anxiety
- Substance and alcohol use
- Attention control
- Other mental health related topics.

Participants will complete a finger-tapping task. For this, they will press a key a certain number of times in a limited period. They will get to practice the task. After the task, they will complete a survey about it.

It will take about 1 hour to complete the surveys and the task. About 8 months later, participants will be contacted to repeat the surveys and task. *Compensation is provided*. [**note: this is no joke but a trial being run by NIMH! I think all my loyal readers are eligible for this trial as the only criteria are over 18 years of age and an English speaker. I wonder what the compensation is.**] NCT04377100

CLINICAL TRIAL RESULTS

- From March 24 to April 6, 2020, 52 consecutive patients were included in the anakinra group and 44 historical patients were identified in the Groupe Hospitalier Paris Saint-Joseph COVID cohort study. Admission to the ICU for invasive mechanical ventilation or death occurred in 13 (25%) patients in the anakinra group and 32 (73%) patients in the historical group (hazard ratio [HR] 0·22 [95% CI 0·11–0·41; p<0·0001). The treatment effect of anakinra remained significant in the multivariate analysis (HR 0·22 [95% CI 0·10–0·49]; p=0·0002). An increase in liver aminotransferases occurred in seven (13%) patients in the anakinra group and four (9%) patients in the historical group. [**note: there are some controlled trials of this drug going on. This is the first report that I have seen showing activity and it is interesting that it is an IL-1 blocker where much of the trial work is focusing on IL-6.**] https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(20)30164-8/fulltext and a more lengthy overview https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(20)30096-5/fulltext
- This study anonymously examined 2,734 psychiatric patients worldwide for worsening of their pre-existing psychiatric condition during the COVID-19 pandemic. Valid responses mainly from 12 featured countries indicated self-reported worsening of psychiatric conditions in 2/3rd of the patients assessed that was validated through their significantly higher scores on scales for general psychological disturbance, post- traumatic stress disorder, and depression. Female gender, feeling no control of the situation and reporting dissatisfaction with the response of the state during the COVID- 19 pandemic, and reduced interaction with family and friends increased

the worsening of pre-existing psychiatric conditions, whereas optimism, ability to share concerns with family and friends and using social media like usual were associated with less worsening. An independent clinical investigation from the USA confirmed worsening of psychiatric conditions during the COVID-19 pandemic based on identification of new symptoms that necessiated clinical interventions such as dose adjustment or starting new medications in more than half of the patients. [**note: a multi-country study of worsening psychiatric symptoms that is unsurprising in its results.**]
https://www.medrxiv.org/content/10.1101/2020.05.28.20116178v1

DRUG DEVELOPMENT

- Early clinical care mainly focused on respiratory illnesses. However, a variety of neurological manifestations in both adults and newborns are also emerging. To determine whether SARS-CoV-2 could target the human brain, we infected iPSC-derived human brain organoids. Our findings show that SARS-CoV-2 was able to infect and kill neural cells, including cortical neurons. This phenotype was accompanied by impaired synaptogenesis. Finally, Sofosbuvir, an FDA-approved antiviral drug, was able to rescue these alterations. Given that there are currently no vaccine or antiviral treatments available, urgent therapies are needed. Our findings put Sofosbuvir forward as potential treatments to alleviate COVID-19-related neurological symptoms. [**note: no registered clinical trials on this one, perhaps Gilead should throw it into the fray.**] https://www.biorxiv.org/content/10.1101/2020.05.30.125856v1

DIAGNOSTIC DEVELOPMENT

- Nothing new after the plethora of abstracts yesterday!

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2020-06-02

Let's turn to another wonderful soul tune today, composed and first sung by Otis Redding, Sitting on the Dock of the Bay. This was one of the last pieces Redding composed as he died in an airplane crash shortly after this was recorded and there is no video performance available. However, the American singer/songwriter Sara Bareilles provides a soulful cover for us: https://www.youtube.com/watch?v=DTvnL1jWNao Redding's original recording is here: https://www.youtube.com/watch?v=rTVjnBo96Ug

A good Lancet piece on physical distancing, face masks, and eye protection for the prevention of COVID-19.

Is optimism and not evidence driving America's return to work strategy?

There are a bunch of good articles in The New York Times today. I don't know how many are behind their paywall as we have a subscription. I am providing the links for those of you who have access and maybe some of them are freebies (I have no way of telling): some lessons learned, a profile of coronavirus at six months (has it only been this long?), and some mysteries and gaps sill remain. How a Belgian gin distillery repurposed itself to make hand sanitizer.

American appear to be receptive for a COVID-19 vaccine.

MODELING

- Airborne transmission is a recognized pathway of contagion; however, it is rarely quantitatively evaluated. This study presents a novel approach for quantitative assessment of the individual infection risk of susceptible subjects exposed in indoor microenvironments in the presence of an asymptomatic infected SARS-CoV-2 subject. The approach allowed the maximum risk for an exposed healthy subject to be evaluated or, starting from an acceptable risk, the maximum exposure time. We applied the proposed approach to four distinct scenarios for a prospective assessment, highlighting that, in order to guarantee an acceptable individual risk of 10^{-3} for exposed subjects in naturally ventilated indoor environments, the exposure time should be shorter than 20 min. The proposed approach was used for retrospective assessment of documented outbreaks in a restaurant in Guangzhou (China) and at a choir rehearsal in Mount Vernon (USA), showing that, in both cases, the high attack rate values can be justified only assuming the airborne transmission as the main route of contagion. *Moreover, we shown that such outbreaks are not caused by the rare presence of a superspreader, but can be likely explained by the co-existence of conditions, including emission and exposure parameters, leading to a highly probable event, which can be defined as a superspreading event*. [**note: this is one of the rare models that is quite interesting to read. I find their conclusion about a 'superspreader' somewhat persusasive.**]
  https://www.medrxiv.org/content/10.1101/2020.06.01.20118984v1
- This explorative monocentric study shows IgA and IgG antibody profiles from 110 patients with self-reported mild to moderate, or no COVID-19 related symptoms after laboratory-confirmed infection with SARS-CoV-2. The study region is in an urban and well-defined environment in a low-incidence region in Northern Germany. We found that approx. 70 % of the patients developed sustainable antibodies 3 weeks or later after the infection. In about 30 % of the patients with mild to moderate symptoms, no significant antibodies could be detected in two consecutive analyses. Conversely, out of ten patients without symptoms, four were repeatedly positive. Expectedly, six had no specific antibodies. The data indicate that antibody-positivity is a useful indicator of a previous SARS-CoV-2 infection. Negative antibodies do not rule out SARS-CoV-2 infection. Future studies need to determine the functionality of the antibodies in terms of personal protection and ability to transmit the virus. [**note: serology testing from a low incidence region in Germany.]**
  https://www.medrxiv.org/content/10.1101/2020.05.30.20111393v1
- Background: Early detection and risk mitigation efforts are essential for averting large outbreaks of SARS-CoV-2. Active surveillance for SARS-CoV-2 can aid in early detection of outbreaks, but the testing frequency required to identify an outbreak at its earliest stage is unknown. We assess what testing frequency is required to detect an outbreak before there are 10 detectable infections. Methods: A dynamic compartmental transmission model of SARS-CoV-2 was developed to simulate spread among a university community. After introducing a single infection into a fully susceptible population, we calculate the probability of detecting at least one case on each succeeding day with various NAT testing frequencies (daily testing achieving 25%, 50%, 75%, and 100% of the population tested per month) assuming an 85% test sensitivity.

A proportion of infected individuals (varied from 1-60%) are assumed to present to health services (HS) for symptomatic testing. We ascertain the expected number of detectable infections in the community when there is a >90% probability of detecting at least 1 case. Sensitivity analyses examine impact of transmission rates (Rt=0=2, 2.5,3), presentation to HS (1%/5%/30%/60%), and pre-existing immunity (0%/10%) Results: Assuming an 85% test sensitivity, identifying an outbreak with 90% probability when the expected number of detectable infections is 9 or fewer requires NAT testing of 100% of the population per month; this result holds for all transmission rates and all levels of presentation at health services we considered. . If 1% of infected people present at HS and Rt=0=3, testing 75%/50%/25% per month could identify an outbreak when the expected numbers of detectable infections are 12/17/30 respectively; these numbers decline to 9/11/12 if 30% of infected people present at HS . As proportion of infected individuals present at health services increases, the marginal impact of active surveillance is reduced. Higher transmission rates result in shorter time to detection but also rapidly escalating cases without intervention. Little differences were observed with 10% pre-existing immunity. Conclusions: Widespread testing of 100% of the campus population every month is required to detect an outbreak when there are fewer than 9 detectable infections for the scenarios examined, but high presentation of symptomatic people at HS can compensate in part for lower levels of testing. Early detection is necessary, but not sufficient, to curtail disease outbreaks; the proposed testing rates would need to be accompanied by case isolation, contact tracing, quarantine, and other risk mitigation and social distancing interventions. [**note: paging Mitch Daniels (he is the president of Purdue University who wrote an op-ed in the Washington Post about the need to open Purdue this fall) and other college and university presidents; this paper is required reading! Enough said.**] https://www.medrxiv.org/content/10.1101/2020.06.01.20118885v1 & right behind is this UK paper on the same topic! https://www.medrxiv.org/content/10.1101/2020.06.01.20100461v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow, BUT there is this abstract to ponder……
- The SOLIDARITY and DisCoVeRy trials were launched to facilitate the rapid worldwide comparison of the efficacy and safety of treatments against COVID-19. This study aimed to review the trial designs of SOLIDARITY and DisCoVeRy and their feasibility to generate high-quality evidence. Method: A systematic search of the European Clinical trial registry, the U.S. National Library of Medicine ClinicalTrials.gov, and the International Clinical Trials Registry Platform (ICTRP) created by the World Health Organization (WHO) was conducted on May 10th, 2020 to identify the study details of the SOLIDARITY and DisCoVeRy trials. A supplementary search of PubMed, the websites of the WHO and French authorities, and Google search engine was conducted. A critical review was performed on the findings. Results: The DisCoVeRy trial design was detailed consistently in both the European and the US clinical rial registries. SOLIDARITY was registered on ICTRP, with country-specific information reported on country-level registry platforms. The design of DisCoVeRy trial appears to be ideal from the methodological perspective. Both trials appear difficult to implement, impractical, and disconnected from the pandemic reality. This is consistent with the apparent failure of the trials to deliver conclusions before the end of the pandemic. Conclusion: Both trials constitute an interesting initiative yet may lack the resources to support a high-quality implementation. The

authors call for a pandemic task force, with various experts on the front-line of COVID-19, to inform policy-makers to make effective decisions that may not be based on traditional, methodological state-of-the-art evidence, but rather pragmatic and revisable decisions reflecting emerging evidence for the benefit of patients and society. [**note: hmmmmmm --- I think I wrote a paper about this exact topic over a month ago!!!** https://www.medrxiv.org/content/10.1101/2020.06.01.20118927v1

CLINICAL TRIAL RESULTS

- A key question in COVID-19 infection is why some previously healthy patients develop severe pulmonary failure and some ultimately die. Initial pulmonary failure does not exhibit classical features of ARDS; hypercoagulability is a common laboratory feature, and pulmonary thrombotic microangiopathy has been reported post mortem1,2,3. Biomarkers cannot robustly identify such patients pre-emptively and no specific interventions exist to mitigate clinical deterioration. Mononuclear phagocytic cells are key immune cells and bind fibrinogen through the CD11b/CD18 dimer CR3, whose activated form can initiate microthrombus formation. Accordingly, we profiled circulating monocyte CD11b/CD18 cell surface density from COVID-19 infected adults who were (i) symptomatic but breathless, (ii) requiring ventilatory support, and (iii) recovering following ICU care for hypoxia. [**note: another piece of evidence as to why some patients have worse outcomes. Increase monocyte expression of complement receptor 3 may be key.**] https://www.medrxiv.org/content/10.1101/2020.05.31.20118638v1
- We examined whether the greater severity of coronavirus disease 2019 (COVID-19) amongst men and non-White ethnicities is explained by cardiometabolic, socio-economic, or behavioural factors. Methods We studied 4,510 UK Biobank participants tested for COVID-19 (positive, n=1,326). Multivariate logistic regression models including age, sex, and ethnicity were used to test whether addition of: 1)cardiometabolic factors (diabetes, hypertension, high cholesterol, prior myocardial infarction, smoking, BMI); 2)25(OH)-vitamin D; 3)poor diet; 4)Townsend deprivation score; 5)housing (home type, overcrowding); or 6)behavioural factors (sociability, risk taking) attenuated sex/ethnicity associations with COVID-19 status. Results There was over-representation of men and non-White ethnicities in the COVID-19 positive group. Non-Whites had, on average, poorer cardiometabolic profile, lower 25(OH)-vitamin D, greater material deprivation, and were more likely to live in larger households and flats/apartments. Male sex, non-White ethnicity, higher BMI, Townsend deprivation score, and household overcrowding were independently associated with significantly greater odds of COVID-19. The pattern of association was consistent for men and women; cardiometabolic, socio-demographic and behavioural factors did not attenuate sex/ethnicity associations. Conclusions Sex and ethnicity differential pattern of COVID-19 is not adequately explained by variations in cardiometabolic factors, 25(OH)-vitamin D levels, or socio-economic factors. Investigation of alternative biological pathways and different genetic susceptibilities is warranted. [**note: this is another example of the Rumsfeld Paradigm. Puzzles continue to pop up regarding our knowledge of the virus. I continue to believe that there is an underlying human genetic component.**] https://www.medrxiv.org/content/10.1101/2020.06.01.20118943v1
- Background Machine learning can assist clinicians in forecasting patients with COVID-19 who develop respiratory failure requiring mechanical ventilation. This analysis aimed to determine a 48 hours prediction of moderate to severe respiratory failure, as assessed with PaO2/FiO2 < 150

mmHg, in hospitalized patients with COVID-19 pneumonia. Methods This was an observational study that comprised all consecutive adult patients with COVID-19 pneumonia admitted to the Infectious Diseases Clinic of the University Hospital of Modena, Italy from 21 February to 6 April 2020. COVID-19 was confirmed with PCR positive nasopharyngeal swabs while the presence of pneumonia was radiologically confirmed. Patients received standard of care according to national guidelines for clinical management of SARS-CoV-2 infection. The patients' full medical history, demographic and epidemiological features, clinical data, complete blood count, coagulation, inflammatory and biochemical markers were routinely collected and aggregated in a clinically-oriented logical framework in order to build different datasets. The dataset was used to train a learning framework relying on Microsoft LightGBM and leveraging a hybrid approach, where clinical expertise is applied alongside a data-driven analysis. Shapley Additive exPlanations (SHAP) values were used to quantify the positive or negative impact of each variable included in the model on the predicted outcome. The study outcome was the onset of moderate to severe respiratory failure defined as PaO2/FiO2 ratio < 150 mmHg (≥ 13.3 kPa) in at least one of two consecutive arterial blood gas analyses in the following 48 hours. Results A total of 198 patients contributed to generate 1068 valuable observations which allowed to build 3 prediction models based respectively on 31-variables signs and symptoms, 39-variables laboratory biomarkers and 91-variables as a composition of the two. A fourth boosted mixed model which included 20 variables was selected from the model 3, achieved the best predictive performance (AUC=0.84). Its clinical performance was applied in a narrative case report as an example. Conclusion This study developed a machine learning algorithm, with a 84% prediction accuracy, which is potentially able to assist clinicians in decision making process with therapeutic implications. [**note: from Italy a good analysis of one clinic's data set to identify markers and variables useful in assessing patients going forward.**]
https://www.medrxiv.org/content/10.1101/2020.05.30.20107888v1

- To determine the clinical outcomes of older COVID-19 patients who received DMB compared to those who did not. We hypothesized that fewer patients administered DMB would require oxygen therapy and/or intensive care support than those who did not. Methodology: Cohort observational study of all consecutive hospitalized COVID-19 patients aged 50 and above in a tertiary academic hospital who received DMB compared to a recent cohort who did not. Patients were administered oral vitamin D3 1000 IU OD, magnesium 150mg OD and vitamin B12 500mcg OD (DMB) upon admission if they did not require oxygen therapy. Primary outcome was deterioration post-DMB administration leading to any form of oxygen therapy and/or intensive care support. Results: Between 15 January and 15 April 2020, 43 consecutive COVID-19 patients aged ≥50 were identified. 17 patients received DMB and 26 patients did not. Baseline demographic characteristics between the two groups were similar. Significantly fewer DMB patients than controls required initiation of oxygen therapy subsequently throughout their hospitalization (17.6% vs 61.5%, P=0.006). DMB exposure was associated with odds ratios of 0.13 (95% CI: 0.03 − 0.59) and 0.15 (95% CI: 0.03 − 0.93) for oxygen therapy need and/or intensive care support on univariate and multivariate analyses respectively. Conclusions: DMB combination in older COVID-19 patients was associated with a significant reduction in proportion of patients with clinical deterioration requiring oxygen support and/or intensive care support. This study supports further larger randomized control trials to ascertain the full benefit of DMB in ameliorating COVID-19 severity. [**note: forget HCQ/zinc/azithromycin, Vitamins D &**

B12 coupled with magnesium is the way to go. Seriously, these observational studies on small patient populations, while interesting don't provide much of an answer. I'm sure these products will start selling well in pharmacies.]
https://www.medrxiv.org/content/10.1101/2020.06.01.20112334v1

- Both cellular and humoral immunities are critically important to control COVID19 infection but little is known about the kinetics of those responses and, in particular, in patients who will go on to develop a severe form of the disease over several weeks. We herein report the first set of data of our prospective cohort study of 90 hospitalized cases. Serological surveys were thoroughly performed over 2 month period by assessing IgG and IgM responses by immunofluorescence, immunoblot, Western blot and conventional ELISA using clinical RUN isolates of SARS-CoV-2 immobilized on 96 well plates. While the IgM and, unexpectedly, the IgG responses were readily detected early during the course of the disease (5-7 days post-first symptoms), our results (n=3-5 and over the full dilution set of the plasma 1/200 to 1/12800) demonstrated a significant decrease (over 2.5-fold) of IgG levels in severe (ICU) hospitalized patients (exemplified in patient 1) by WB and ELISA. In contrast, mild non-ICU patients had a steady and yet robust rise in their specific IgG levels against the virus. Interestingly, both responses (IgM and IgG) were initially against the nucleocapsid (50kDa band on the WB) and spreading to other major viral protein S and domains (S1 and S2. In conclusion, serological testing may be helpful for the diagnosis of patients with negative RT-PCR results and for the identification of asymptomatic cases. Moreover, medical care and protections should be maintained particularly for recovered patients (severe cases) who may remain at risk of relapsing or reinfection. Experiments to ascertain T cell responses but although their kinetics overtime are now highly warranted. All in all, these studies will help to delineate the best routes for vaccination. [**note: cool, we have a paper from researchers on Reunion island (looks like a great vacation destination when things get back to normal!) showing rapid antibody decrease in some patients.**] https://www.medrxiv.org/content/10.1101/2020.05.25.20112623v1
- The novel coronavirus SARS-Cov2 uses the angiotensin-converting enzyme 2 (ACE2) receptor as an entry point to the cell. Cardiovascular disease (CVD) is a risk factor for the novel coronavirus disease (Covid-19) with poor outcomes. We hypothesized that the rate of ACE inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) use is associated with the rate of Covid-19 confirmed cases and deaths. Methods: We conducted a geospatial study using publicly available county-level data. The Medicare ACEIs and ARBs prescription rate was exposure. The Covid-19 confirmed case and death rates were outcomes. Spatial autoregression models were adjusted for the percentage of Black residents, children, residents with at least some college degree, median household income, air quality index, CVD hospitalization rate in Medicare beneficiaries, and CVD death rate in a total county population. Results: The ACEI use had no effect on Covid-19 confirmed case rate. An average ACEIs use (compared to no-use) was associated with a higher Covid-19 death rate by 1.1 (95%CI 0.4-1.8)%. If the use of ACEIs increases by 0.5% for all counties, the Covid-19 death rate will drop by 0.4% to 0.7(95%CI 0.3-1.1)%; P<0.0001. An average ARBs use (compared to no-use) was associated with a higher Covid-19 confirmed case rate (by 4.2; 95%CI 4.1-4.3 %) and death rate (by 1.1; 95%CI 0.7-1.5 %). Each percent increase in ARBs use was associated with an increase in confirmed case rate by 0.2(0.03-0.4)% and death rate by 0.14(0.08-0.21)%. Conclusions: *ARBs, but not ACEIs use rate, is associated with Covid-19 confirmed case rate*. [**note: it is not clear how valuable this study is since it relies on some**

inferences that may not be truly linked. The best way to do this is directly though EHRs where there is confirmed infection and drug use. This has already been done in some papers and the OHDSI group will be doing this at a larger scale in multiple countries. Of course there are ongoing clinical trials with both classes of drugs.]
https://www.medrxiv.org/content/10.1101/2020.05.31.20118802v1

- Background New York City (NYC) has borne the greatest burden of COVID-19 in the United States, but information about characteristics and outcomes of racially/ethnically diverse individuals tested and hospitalized for COVID-19 remains limited. In this case series, we describe characteristics and outcomes of patients tested for and hospitalized with COVID-19 in New York City's public hospital system. Methods We reviewed the electronic health records of all patients who received a SARS-CoV-2 test between March 5 and April 9, 2020, with follow up through April 16, 2020. The primary outcomes were a positive test, hospitalization, and death. Demographics and comorbidities were also assessed. Results 22254 patients were tested for SARS-CoV-2. 13442 (61%) were positive; among those, the median age was 52.7 years (interquartile range [IQR] 39.5-64.5), 7481 (56%) were male, 3518 (26%) were Black, and 4593 (34%) were Hispanic. Nearly half (4669, 46%) had at least one chronic disease (27% diabetes, 30% hypertension, and 21% cardiovascular disease). Of those testing positive, 6248 (46%) were hospitalized. The median age was 61.6 years (IQR 49.7-72.9); 3851 (62%) were male, 1950 (31%) were Black, and 2102 (34%) were Hispanic. More than half (3269, 53%) had at least one chronic disease (33% diabetes, 37% hypertension, 24% cardiovascular disease, 11% chronic kidney disease). 1724 (28%) hospitalized patients died. The median age was 71.0 years (IQR 60.0, 80.9); 1087 (63%) were male, 506 (29%) were Black, and 528 (31%) were Hispanic. Chronic diseases were common (35% diabetes, 37% hypertension, 28% cardiovascular disease, 15% chronic kidney disease). Male sex, older age, diabetes, cardiac history, and chronic kidney disease were significantly associated with testing positive, hospitalization, and death. Racial/ethnic disparities were observed across all outcomes. Conclusions and Relevance This is the largest and most racially/ethnically diverse case series of patients tested and hospitalized for COVID-19 in the United States to date. Our findings highlight disparities in outcomes that can inform prevention and testing recommendations. [**note: large outcome study from the hard hit New York City public hospitals.**] https://www.medrxiv.org/content/10.1101/2020.05.29.20086645v1
- The outbreak of Coronavirus Disease 2019 (COVID-19) is threatening a surging number of populations worldwide, including women in breastfeeding period. Limited evidence is available concerning breastfeeding in women with COVID-19. Methods: Twenty-three pregnant women and puerperae were enrolled in the study. To evaluate the effect of breastfeeding on SARS-CoV-2 transmission, the presence of SARS-CoV-2, IgG and IgM in breast milk, maternal blood and infant blood were assessed. Feeding patterns were also recorded in follow-up. Results: No positive detection for SARS-CoV-2 of neonates was found. All breast milk samples were negative for the detection of SARS-CoV-2. The presence of IgM ofSARS-CoV-2 in breast milk was correlated with maternal blood. The results of IgG detection for SARS-CoV-2 were negative in all breast milk samples. All the infants were in healthy condition while six of them were fed with whole or partial breast milk. Eight infants received antibody test for SARS-CoV-2 in one month after birth and the results were all negative. Conclusion: Findings from this small number of cases suggest that there is currently no evidence for mother-to-child transmission via breast feeding in women with COVID-19 in the third trimester and puerperium. [**note: very small**

number of people here, but looks like no virus transmission via breast milk.]
https://www.medrxiv.org/content/10.1101/2020.05.30.20033407v1

- Smokers are generally more susceptible to infectious respiratory diseases and are at higher risk of developing severe complications from these infections. Conflicting reports exist regarding the impact of smoking on the risk of Coronavirus disease 2019 (COVID-19) infection. METHODS We carried out a population-based study among over 3,000,000 adult members of Clalit Health Services, the largest health provider in Israel. Since the beginning of the disease outbreak, 114,545 individuals underwent RT-PCR testing for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), and 4.0% had positive results. We performed a case-control study among patients who underwent SARS-CoV-2 testing, to assess the impact of smoking on infection incidence and severity. Individuals with positive tests were matched in a 1:5 ratio to individuals tested negative, of the same sex, age, and ethnicity/religion. Conditional logistic regressions were performed to evaluate odds ratios for current and previous smoking on the risk of testing positive. Multivariable logistic regressions were performed among patients infected with COVID-19 to estimate the association between smoking and fatal or severe disease requiring ventilation. Regressions were performed with and without adjustment for preexisting medical conditions. RESULTS In the matched cohort, current smokers (9.8%) were significantly less prevalent among members tested positive compared to the general population, and to matched members tested negative (19.4%, P<0.001). Current smoking was associated with significantly reduced odds ratio (OR) for testing positive OR=0.457 (95% confidence interval (CI) 0.407-0.514). Among patients tested positive, there was no evidence of significantly increased risk of developing severe or fatal disease. CONCLUSION The risk of infection by COVID-19 appears to be reduced by half among current smokers. This intriguing finding may reveal unique infection mechanisms present for COVID-19 which may be targeted to combat the disease and reduce its infection rate. [**note: another observational study on the effect of smoking and COVID-19, this time from Israel. This is similar to some other studies that have popped up but there have also been some that find the opposite. I DO NOT recommend smoking as a prophylaxis unless there is a presidential recommendation and even that might not be enough!**] https://www.medrxiv.org/content/10.1101/2020.06.01.20118877v1

DRUG DEVELOPMENT

- This study characterizes the first clinical application of CIGB-258 in COVID-19 patients. CIGB-258 is an immunoregulatory peptide, derived from the cellular heat shock protein 60 (HSP60). Sixteen patients with COVID-19 in serious (31%) or critical (69%) conditions were included in this study. All critically ill patients recovered from the respiratory distress condition. Two of these patients had a fatal outcome due to nosocomial infections. The five seriously ill patients considerably improved. C-reactive protein (CRP) and interleukin-6 (IL-6) levels significantly decreased during treatment. CIGB-258 seems to be an effective and safe treatment option in COVID-19 patients under cytokine storm. [**note: could it be that these Cuban researchers have found a good therapy? I don't know too much about this peptide. Wikipedia has an article on HSP60 from which this peptide is derived. Good to see the first Cuban paper! We are all in this together.**] https://www.medrxiv.org/content/10.1101/2020.05.27.20110601v1
- The precipitously increased death rates, its impact on livelihood and trembling economies warrant the urgent development of SARS-CoV-2 vaccine which would be safe, efficacious and

scalable. Owing to unavailability of the vaccine, *we propose a de novo synthesised avian orthoavulavirus 1 (AOaV-1)-based topical respiratory vaccine candidate against CoVID-19. Avirulent strain of Newcastle disease virus, proto-type virus of AOaV-1, was engineered to express full length spike (S) glycoprotein which is highly neutralizing and major protective antigen of the SARS-CoV-2.* Broad-scale in vitro characterization of recombinant vaccine candidate demonstrated efficient co-expression of the hemagglutinin-neuraminidase (HN) of AOaV-1 and S protein of SARS-CoV-2, and comparable replication kinetics were observed in cell culture model. The recombinant vaccine candidate virus actively replicated and spread within cells independently of exogenous trypsin. Interestingly, incorporation of S protein of SARS-CoV-2 into the recombinant AOaV-1 particles attributed the sensitivity to anti-SARS-CoV-2 antiserum and more prominently to anti-AOaV-1 antiserum. Finally, our results demonstrated that the recombinant vaccine vector stably expressed S protein after multiple propagation in chicken embryonated eggs, and this expression did not significantly impact the in vitro growth characteristics of the recombinant. Taken together, the presented respiratory vaccine candidate is highly attenuated in primates per se, safe and lacking pre-existing immunity in human, and carries the potential for accelerated vaccine development against CoVID-19 for clinical studies. [**note: and yet another approach to a COVID-19 vaccine!!! The downside to this approach is it *appears* to use the egg production system similar to that used for seasonal flu vaccine.**]
https://www.biorxiv.org/content/10.1101/2020.05.31.126524v1

- SARS-CoV-2 virus is the causative agent of COVID-19. Here we demonstrate that non-infectious SARS-CoV-2 virus like particles (VLPs) can be assembled by co-expressing the viral proteins S, M and E in mammalian cells. *The assembled SARS-CoV-2 VLPs display numerous S protein spikes ideal for vaccine development.* The particles have a spike to spike size of 103 ± 6 nm and a membrane diameter of 63 ± 5 nm. We further show that SARS-CoV-2 VLPs dried in ambient conditions can retain their structural integrity upon repeated scans with Atomic Force Microscopy up to a peak force of 1 nN. [**note: this is really a nice approach to creating a virus like particle. The conjecture about use as a vaccine is interesting but is the process scalable?**]
https://www.biorxiv.org/content/10.1101/2020.06.01.128058v1
- The shortage of effective sanitizing fluids, however, became a global challenge quickly after the coronavirus disease-19 (COVID-19) outbreak in December 2019. In this study, we present the effect of surfactants on coronavirus (SARS-CoV-2) virucidal efficiency in sanitizing fluids. Sodium dodecylbenzenesulfonate (SDBS), sodium laureth sulfate (SLS), and two commercial dish soap and liquid hand soap were studied with the goal of evaporation rate reduction in sanitizing liquids to maximize surface contact time. Twelve fluids with different recipes composed of ethanol, isopropanol, SDBS, SLS, glycerin, and water of standardized hardness (WSH) were tested for their evaporation time and virucidal efficiency. Evaporation time increased by 17-63% when surfactant agents were added to the liquid. In addition, surfactant incorporation enhanced the virucidal efficiency between 15-27% according to the 4-field test in the EN 16615:2015 European Standard method. Most importantly, however, we found that surfactant addition provides a synergistic effect with alcohols to inactivate the SARS-CoV-2 virus. This study provides a simple, yet effective solution to improve the virucidal efficiency of commonly used sanitizers. [**note: adding surfactants can improve**
https://www.biorxiv.org/content/10.1101/2020.05.29.124107v1

DIAGNOSTIC DEVELOPMENT

- Serological assays can detect anti-SARS-CoV-2 (SARS2) antibodies, but their sensitivity often comes at the expense of specificity. Here we used a Ternary Automated Blood Im-munoassay (TRABI) to assess the IgG response against SARS2 in 3,815 prepandemic plasma samples and 126 virologically and/or clinically confirmed COVID-19 samples. Posterior probabilities were calculated from 3x8 measurements of logarithmically diluted samples against the ectodomain and the receptor-binding domain of the spike protein and the nucleoprotein. We then performed 429,624 assays on 17,901 blood samples from patients of the University Hospital Zurich and from healthy blood donors. We found se-ropositivity in 44 of 8,591 patients and in 26 of 5,388 blood donors from December 2019 to May 2020. Western blotting confirmed seropositivity in COVID samples but in none of the prepandemic samples. Solution-equilibrium measurements revealed immunodominant antibodies with nanomolar affinity in COVID samples, whereas prepandemic plasma showed lower affinities despite similar titers for individual SARS2 antigens. Hence, TRABI identifies seropositive individuals in large unselected cohorts, discriminates be-tween SARS2 immunity and low-affinity crossreactivity, and is therefore suitable for large-scale nationwide screening campaigns. [**note: good work by this team to screen a huge number of samples and show how serology can be used to do large volume screening. We need to do this here in the US (to restate the obvious).**]
  https://www.medrxiv.org/content/10.1101/2020.05.31.20118554v1
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to more than 4 million confirmed infections worldwide and over 300,000 deaths. While Remdesivir has recently received FDA emergency use authorization for treatment of SARS-CoV-2 infection, convalescent plasma (CP) with high titers of SARS-CoV-2 neutralizing antibodies (NAbs) from recovered donors remains a promising and widely accessible method to mitigate severe disease symptoms. Here, we describe the development and validation of a cell-free neutralization PCR assay using SARS-CoV-2 spike protein S1 and human ACE2 receptor-DNA conjugates. By comparing with samples collected prior to the outbreak, we confirmed that NAbs were specifically detected in COVID-19 cases. Using our unique assay, the NAb signals are detectable as early as 10 days after onset of symptoms and continue to rise, plateauing after 18 days. Notably, we showed that the use of licensed pathogen reduction technology to inactivate potentially contaminating infectious pathogens in CP did not alter NAb signals, paving a path to safely administer effective CP therapies. The described neutralization PCR assay can serve as a qualification tool to easily identify suitable CP donors of a potentially lifesaving therapy. In addition, this assay tool is readily deployable in standard laboratories with biosafety level 2 capability, and can yield results within 2-3 hr. This advancement can facilitate research on factors driving diverse COVID-19 disease manifestations, and to evaluate the impact of various CP processing protocols on CP therapeutic efficacy. [**note: from a Swiss team, another method to help screen for antibodies from convalescent patients.**]
  https://www.medrxiv.org/content/10.1101/2020.05.28.20105692v1
- Rigorous testing is the way forward to fight the Covid-19 pandemic. Here we show that the currently used and most reliable RT-PCR based SARS-CoV-2 procedure can be further simplified to make it faster, safer and economical by bypassing the RNA isolation step. The modified method is not only fast and convenient but also at par with the traditional method in terms of accuracy, and therefore, can be used for mass screening. Our method takes about half the time and is cheaper by about 40% compared to current most widely used method. We also provide a

variant of the new method that increases the efficiency of detection by about 20% compared to the currently used method. Taken together, we demonstrate a more effective and reliable method of SARS-CoV-2 detection. [**note: another improvement to RT-PCR, this time from an Indian group.]** https://www.biorxiv.org/content/10.1101/2020.05.31.126342v1

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2020-06-03

How about Oakland's finest: The Pointer Sisters? Jump for My Love: https://www.youtube.com/watch?v=uyTVyCp7xrw I was attracted to this MTV video when it first came out. All the track and field athletes engaged in various jumping and hurdling activities and then ……… you can watch for yourself. **Time to run another contest to see if I can give away a prize!!! First one to correctly identify the player who has the flying slam dunk will be the winner! I can't think of an easier question to ask about this video (**I do know half the track and field athletes but those would be too obscure**).**

Is COVID-19 a blood vessel disease? This is a conjectural article on that topic that came over my news feed yesterday.

One of my readers pointed me to this interview with Erin Bromage. It's plain talk, no jargon, and it hits all the right points. Professor Bromage's blog is here and has some good stuff!!

Derek Lowe weighs in on the growing controversy over The Lancet large observational trial publication on HCQ which may be incorrect. There are some other papers from this company's data mining that now are suspect as well. This is really not good. Here is The Lancet expression of concern. STAT weighs in.

There is a lengthy story in The New York Times on what went wrong with the CDC. It is worth reading!

Researchers continue to pile on the preprints again today!!! The quality of some of the clinical results continues to be disappointing. Why bother doing small trials that are not controlled and who is going to publish these?

MODELING

- New York City (NYC) has emerged as one of the epicenters of the current SARS-CoV-2 pandemic. To identify the early transmission events underlying the rapid spread of the virus in the NYC metropolitan area, we sequenced the virus causing COVID-19 in patients seeking care at the Mount Sinai Health System. Phylogenetic analysis of 84 distinct SARS-CoV2 genomes indicates multiple, independent but isolated introductions mainly from Europe and other parts of the United States. Moreover, we find evidence for community transmission of SARS-CoV-2 as suggested by clusters of related viruses found in patients living in different neighborhoods of the city. [**note: I probably posed the preprint; here is the published paper from the Mt. Sinai group on the NYC spread.**] https://science.sciencemag.org/content/early/2020/05/28/science.abc1917

- We conducted immunologic testing of healthcare workers to determine the prevalence of SARS-CoV-2 IgG in this population. HCW were advised to wait at least two weeks from time of symptom onset or suspected exposure before undergoing testing. All participants were self-reported asymptomatic for at least three days at the time of testing. Results: Two hundred eighty-five samples were collected from March 24, 2020 to April 4, 2020. The average age of participants was 38 years (range 18-84), and 54% were male. Thirty-three percept tested IgG positive, 3% tested weakly positive, and 64% tested negative. Neither age nor sex was associated with antibody development. Conclusion: Thirty-six percent of HCW had IgG antibodies to SARS-CoV-2, reflecting the high exposure of inpatient and ambulatory frontline staff to this viral illness, most of whom had minimal symptoms and were working in the weeks preceding testing. While we continue to recommend standard protective precautions per CDC guidelines for all HCW, HCW with SARS-CoV-2 IgG may become our safest frontline providers as we learn if our IgG antibodies confer immunity. Knowing IgG antibody status may ease concerns regarding personal risk as this pandemic continues. [**note: this study was carried out on healthcare workers in the Mt. Sinai system. 35% of those tested had IgG to SARS-CoV-2 indicating infection.**] https://www.medrxiv.org/content/10.1101/2020.05.27.20090811v1
- The role of environmental transmission of SARS-CoV-2 remains unclear. Particularly the close contact of persons living together or cohabitating in domestic quarantine could result in high risk for exposure to the virus within the households. Therefore, the aim of this study was to investigate the whereabouts of the virus and whether useful precautions to prevent the dissemination can be given. 21 households under quarantine conditions were randomly selected for this study. All persons living in each household were recorded in terms of age, sex and time of household quarantine. Throat swabs for analysis were obtained from all adult individuals and most of the children. Air, wastewater samples and surface swabs (commodities) were obtained and analysed by RT-PCR. Positive swabs were cultivated to analyse for viral infectivity. 26 of all 43 tested adults (60.47 %) tested positive by RT-PCR. All 15 air samples were PCR-negative. 10 of 66 wastewater samples were positive for SARS-CoV-2 (15.15 %) as well as 4 of 119 object samples (3.36 *%). No statistically significant correlation between PCR-positive environmental samples and the extent of infection spread inside the household could be observed. No infectious virus could be isolated under cell culture conditions. As we cannot rule out transmission through surfaces, hygienic behavioural measures are important in the households of SARS-CoV-2 infected individuals to avoid potential transmission through surfaces.* The role of the domestic environment, in particular the wastewater load in washbasins and showers, in the transmission of SARS CoV-2 should be further clarified. [**note: just what I have waited for, a real world study of environmental surfaces and conditions!! Maybe surfaces are not that important, but still stand ready with your Clorox cleanser.**]
  https://www.medrxiv.org/content/10.1101/2020.05.28.20114041v1

NEWLY REGISTERED CLINICAL TRIALS

- The purpose of this study is to determine if administration of angiotensin-(1-7) / TXA127 prevents acute kidney injury and deterioration into multi-organ failure in patients with moderate to severe COVID-19. [**note: I don't know much about this one. It's been studied for a bunch of different things but approved for none. Maybe they will get lucky. Columbia University is the sponsor.**] NCT04401423

- The objectives of this study are to evaluate the safety, tolerability and efficacy of AT-527 in older subjects (ages 45-80 years) with moderate COVID-19 and risk factors for poor outcomes (such as obesity (BMI>30), hypertension, diabetes or asthma). Eligible subjects will be randomized to blinded AT-527 (nucleotide analog) tablets or matching placebo tablets to be administered orally for 10 days. Local supportive standard of care (SOC) will be allowed for all subjects. Efficacy and safety observations will be compared for treatment with active AT-527 tablets + SOC vs. placebo tablets + SOC. [**note: don't know the structure and the sponsor is Alea Pharmaceuticals.**] NCT04396106
- It has been found in intensive care patients hypoargininaemia, associated with the persistence of organ dysfunction (evaluated by the SOFA score), the occurrence of nosocomial infections and mortality. Also, it has been demonstrated that in these patients, the enteral administration of ARG was not deleterious and increased the synthesis of ornithine, suggesting a preferential use of ARG by the arginase route, without significant increase in argininaemia nor effect on immune functions. L-citrulline (CIT), an endogenous precursor of ARG, is an interesting alternative to increase the availability of ARG. Recent data demonstrate that the administration of CIT in intensive care is not deleterious and that it very significantly reduces mortality in an animal model of sepsis, corrects hypoargininemia, with convincing data on immunological parameters such as lymphopenia, which is associated with mortality, organ dysfunction and the occurrence of nosocomial infections. The availability of ARG directly impacts the mitochondrial metabolism of T lymphocytes and their function. The hypothesis is therefore that CIT supplementation is more effective than the administration of ARG to correct hypoargininaemia, decrease lymphocyte dysfunction, correct immunosuppression and organ dysfunction in septic patients admitted to intensive care. [**note: this is a French study of a common dietary supplement. It was first isolated from watermelon so eat up that melon this summer!**] NCT04404426
- 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. [**note: this is IL-7 by another name and the trial is sponsored by RevImmune.** NCT04407689
- Antioxidants, and particularly polyphenols, have shown protection in respiratory pathologies, which is related to the decrease in the severity of the clinical picture and suppression of inflammation. This suppression of inflammation may be related to the inhibition of NF-kB polyphenols, where its activation is related to the stimulation of 150 stimuli including cytokines (IL-1β, IL-6, THF-α, GM-CSF, MCP-1), TLRs, among others. There may be other additional mechanisms that can help control virus-induced respiratory pathologies, among which are the regulation of reactive oxygen species (ROS) associated with tissue destruction caused by the virus and a selective antiviral action can be reported. direct. The standardized P2Et extract obtained from C. spinosa, by the Immunobiology Group of the Pontificia Universidad Javeriana, is highly antioxidant, decreases lipid peroxidation and tissue damage and induces complete autophagy in stressed or tumor cells. The induction of a full autophagic flow could inhibit the replication of beta-coronaviruses like SARS-CoV-2. Furthermore, P2Et can decrease the factors involved in tissue damage by reducing IL-6 and decrease ILC2 cells of the lung in animals with lung metastases (unpublished data). [**note: another natural product trial this time from Columbia. I think this is the first time this country has made the newsletter.**] NCT04410510

CLINICAL TRIAL RESULTS

- Background. Respiratory failure is a key feature of severe Covid-19 and a critical driver of mortality, but for reasons poorly defined affects less than 10% of SARS-CoV-2 infected patients. Methods. We included 1,980 patients with Covid-19 respiratory failure at seven centers in the Italian and Spanish epicenters of the SARS-CoV-2 pandemic in Europe (Milan, Monza, Madrid, San Sebastian and Barcelona) for a genome-wide association analysis. After quality control and exclusion of population outliers, 835 patients and 1,255 population-derived controls from Italy, and 775 patients and 950 controls from Spain were included in the final analysis. In total we analyzed 8,582,968 single-nucleotide polymorphisms (SNPs) and conducted a meta-analysis of both case-control panels. Results. We detected cross-replicating associations with rs11385942 at chromosome 3p21.31 and rs657152 at 9q34, which were genome-wide significant (P<5x10-8) in the meta-analysis of both study panels, odds ratio [OR], 1.77; 95% confidence interval [CI], 1.48 to 2.11; P=1.14x10-10 and OR 1.32 (95% CI, 1.20 to 1.47; P=4.95x10-8), respectively. Among six genes at 3p21.31, SLC6A20 encodes a known interaction partner with angiotensin converting enzyme 2 (ACE2). The association signal at 9q34 was located at the ABO blood group locus and a blood-group-specific analysis showed higher risk for A-positive individuals (OR=1.45, 95% CI, 1.20 to 1.75, P=1.48x10-4) and a protective effect for blood group O (OR=0.65, 95% CI, 0.53 to 0.79, P=1.06x10-5). Conclusions. We herein report the first robust genetic susceptibility loci for the development of respiratory failure in Covid-19. Identified variants may help guide targeted exploration of severe Covid-19 pathophysiology. [**note: this paper has a huge number of co-authors and is the largest study that I've seen on blood group genetics. All you Type A folks need to take special care!**]
  https://www.medrxiv.org/content/10.1101/2020.05.31.20114991v1
- BACKGROUND: Non-steroidal anti-inflammatory drugs (NSAIDs) may exacerbate COVID-19 and worsen associated outcomes by upregulating the enzyme that SARS-CoV-2 binds to enter cells. However, to our knowledge, no study has examined the association between NSAID use and the risk of COVID-19-related outcomes among hospitalised patients. METHODS: We conducted a population-based cohort study using South Korea nationwide healthcare database, which contains data of all subjects who received a test for COVID-19 (n=69,793) as of April 8, 2020. We identified a cohort of adults hospitalised with COVID-19, where cohort entry was the date of hospitalisation. NSAIDs users were those prescribed NSAIDs while hospitalised and non-users were those not prescribed NSAIDs. Our primary outcome was a composite of death, intensive care unit admission, mechanical ventilation use, and sepsis; secondary outcome was cardiovascular or renal complications. We conducted logistic regression analysis to estimate adjusted odds ratio (aOR) with 95% confidence intervals (CI) for the risk of these outcomes associated with NSAIDs users versus non-users, using propensity score−inverse probability of treatment weighting to minimize potential confounding. In sensitivity analyses, we compared NSAIDs to paracetamol (acetaminophen) to minimize confounding by indication. FINDINGS: Of 1,824 adults hospitalised with COVID-19 (mean age 44.7 years; female 59%), 285 were NSAIDs users and 1,539 were non-users. Compared with non-users, NSAIDs users were associated with increased risks of the primary composite outcome (aOR 1.54, 95% CI 1.11−2.15) and cardiovascular or renal complications (aOR 2.64, 95% CI 1.67−4.16). The association with primary outcome remained consistent when comparing NSAIDs to paracetamol (aOR 1.31, 95% CI 0.89−1.95). INTERPRETATION: Use of NSAIDs, compared with non-use, is associated with worse outcomes among hospitalised COVID-19 patients. While awaiting the results of

confirmatory studies, we suggest NSAIDs be used with caution as the harms associated with their use may outweigh their benefits in this population. [**note: more confounding information on NSAIDs. There are some trials going on and maybe they will clear up this issue if they are ever completed.**] https://www.medrxiv.org/content/10.1101/2020.06.01.20119768v1

- OBJECTIVE To examine if baseline *soluble urokinase plasminogen activator receptor (suPAR)* can predict whether patients with COVID-19 symptoms will need mechanical ventilation during a 14-day follow-up. Furthermore, to examine differences in demographics, clinical signs, and biomarkers in patients tested either positive or negative for SARS-CoV-2. DESIGN Prospective cohort study including patients presenting with symptoms of COVID-19. SETTING Copenhagen University Hospital Amager and Hvidovre, Hvidovre, Denmark. PARTICIPANTS 407 patients presenting with symptoms of COVID-19 were included from the Emergency Department (ED). Patients were included from March 19 to April 3 and follow-up data was collected until April 17, 2020. MAIN OUTCOME MEASURES Primary outcomes were respiratory failure in patients presenting with symptoms of COVID-19 and in those with a positive SARS-CoV-2 RT-PCR test, respectively. Furthermore, we analysed differences between patients testing positive and negative for SARS-CoV-2, and disease severity outcomes in SARS-CoV-2 positive patients according to baseline suPAR. BACKGROUND Patients admitted to ED with clinical signs or symptoms of COVID-19 infection need a safe and quick triage, in order to determine if an in-hospital stay is necessary or if the patient can safely be isolated in their own home with relevant precautions. suPAR is a biomarker previously shown to be associated with adverse outcomes in acute medical patients. We aimed to examine if suPAR at baseline presentation is predictive of respiratory failure in patients presenting with symptoms of COVID-19. Furthermore, we examined demographic, clinical, and biochemical differences between SARS-CoV-2-positive and negative patients. RESULTS Among the 407 symptomatic patients, the median (interquartile range) age was 64 years (47-77), 58% were women, and median suPAR was 4.2 ng/ml (2.7-6.4). suPAR level below 4.75 ng/ml at admission ruled out respiratory failure during follow-up with an area under the curve (95% CI) of 0.89 (0.85-0.94) and a negative predictive value of 99.5%. Of the 407 symptomatic patients, 117 (28.8%) had a positive RT-PCR test for SARS-CoV-2 and presented with significant differences in vital signs, cell counts, and biomarkers compared to SARS-CoV-2 negative patients. In SARS-CoV-2 positive patients eligible for mechanical ventilation (N=87), 26 (30%) developed respiratory failure. Best baseline predictors of respiratory failure were suPAR with an area under the curve (95% CI) of 0.88 (0.80-0.95), EWS 0.84 (0.75-0.93), lactate dehydrogenase 0.82 (0.71-0.93), and C-reactive protein 0.80 (0.70-0.89). CONCLUSION SARS-CoV-2 affects several patient parameters underpinning the severe impact of the infection. A low suPAR level (<4.75 ng/ml) at baseline is a useful biomarker for aiding clinical decisions including discharge of patients presenting with symptoms of COVID-19. [**note: this may be a very useful biomarker to add to the list. Good work from Denmark.**] https://www.medrxiv.org/content/10.1101/2020.05.27.20114678v1
- The pandemic spread of the novel coronavirus SARS-CoV-2 is due, in part, to the immunological properties of the host-viral interaction. The clinical presentation varies greatly from individual to individual, with asymptomatic carriers, mild to moderate-presenting patients and severely affected patients. Variation in immune response to SARS-CoV-2 may underlie this clinical variation. Using a high dimensional systems immunology platform we have analysed the peripheral blood compartment of 6 healthy individuals, 23 mild-to-moderate COVID-19 patients

and 20 severe COVID-19 patients. We identify distinct immunological signatures in the peripheral blood of the mild-to-moderate and severe COVID-19 patients, including T cell lymphopenia, more consistent with peripheral hypo- than hyper-immune activation. Unique to the severe COVID-19 cases was a large increase in the proportion of IL-10-secreting regulatory T cells, a lineage known to possess anti-inflammatory properties in the lung. Annotated data is openly available (https://flowrepository.org/experiments/2713) with clinical correlates, as a systems immunology resource for the COVID-19 research community. [**note: from Belgium, more immune system signatures related to disease progression. Our understanding is increasing daily.**] https://www.medrxiv.org/content/10.1101/2020.05.31.20112979v1

- Background: We share our experience in COVID-19 pneumonia management at Saint George Hospital University Medical Center (SGHUMC) in Lebanon. In the absence of a standard of care, early diagnosis and opt-in therapy with Hydroxychloroquine and Azithromycin were offered. Methods: We reviewed records of COVID-19 pneumonia patients from March 16-April 26 2020. Based on NEWS score, we stratified patients as A: low B: medium, and C: high clinical severity and obtained pharmacotherapy data. Chest-CT-severity-score (CTSS) was used. We defined clinical cure as resolution of symptoms and biomarkers and virologic cure as a PCR above 35 cycles(Ct). Results: We recorded 21 COVID-19 pneumonia patients of whom 19 opted for treatment. Clinical symptoms and laboratory markers at presentation did not significantly correlate with severity. Lower initial viral load significantly correlated with lower levels of clinical and radiological severity (p=0.038). Virologic cure, Ct>35, by day 10, was only 33% in high severity significantly less than categories A and B. We observed 100% clinical cure at day 10 in Category-A, 67% in B, and 33% in C($p<0.05$). Patients with the lowest severity had the fastest virologic cure in a mean of 5.8 days from diagnosis, shortest hospitalization and earlier radiological improvement($p<0.005$). Ultimately, 18 patients were discharged home in good condition and one remains in the ICU. Conclusion: Viral dynamics matter in COVID-19 pneumonia. An early control of replication may be crucial in averting complications. Early administration of Hydroxychloroquine and Azithromycin potentially explains our 94.7% success rate in treating a fairly complex cohort of COVID-19 pneumonia. [**note: it looks like the HCQ/azithromycin fans will have more to cheer about with this small study. Two patients did not take the drug combination and since this paper is really quite messy, it was not apparent whether they recovered as well as the patients on treatment. The researchers also excluded a lot of patients from this trial. TIWWDCT!**]
https://www.medrxiv.org/content/10.1101/2020.05.28.20114835v1

DRUG DEVELOPMENT

- We report the isolation and characterization of an alpaca-derived, single domain antibody fragment (nanobody) that specifically targets the receptor binding domain (RBD) of the SARS-CoV-2 spike glycoprotein (spike) and potently neutralizes the virus. A cryo-electron microscopy structure of the bound complex at 2.9 Å resolution reveals that the nanobody (Ty1) binds to an epitope on the RBD accessible in both the "up" and "down" conformations and that Ty1 sterically hinders RBD-ACE2 binding. Mechanistic characterization confirms that Ty1 directly interferes with host cell receptor binding. This 12.8 kDa nanobody binds the SARS-CoV-2 spike with high specificity and affinity, and can be produced in high quantities recombinantly thereby offering potential as a potent and widely accessible SARS-CoV-2 antiviral agent. [**note: this is**

from Sweden and a different group from the one that did similar work using llama. They note in the paper that this 'nanobody Ty1' can be readily produced in bacteria at a very high yield (excess of 30 mg/L of culture) and shows high affinity. The authors even provide the amino acid sequence encouraging research. One question is whether it would be recognized as 'foreign' by the human immune system and cleared. Given the low cost of production, it is worth giving this one a trial! Good work to the Karolinska Institute team.]
https://www.biorxiv.org/content/10.1101/2020.06.02.130161v1

- Here we reported a humanized monoclonal antibody, H014, efficiently neutralizes SARS-CoV-2 and SARS-CoV pseudoviruses as well as authentic SARS-CoV-2 at nM level by engaging the S receptor binding domain (RBD). Importantly, H014 administration reduced SARS-CoV-2 replication and prevented pulmonary pathology in hACE2 mouse model. Cryo-EM characterization of the SARS-CoV-2 S trimer in complex with the H014 Fab fragment unveiled a novel conformational epitope, which is only accessible when the RBD is in open conformation. Biochemical, cellular, virological and structural studies demonstrated that H014 prevents attachment of SARS-CoV-2 to its host cell receptors. Epitope analysis of available neutralizing antibodies against SARS-CoV and SARS-CoV-2 uncover broad cross-protective epitopes. Our results highlight a key role for antibody-based therapeutic interventions in the treatment of COVID-19. [**note: more on the development of an mAb, this time from China.**]
https://www.biorxiv.org/content/10.1101/2020.06.02.129098v1
- Chloroquine/hydroxychloroquine have been proposed as potential treatments for COVID-19. These drugs have warning labels for use in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Analysis of whole-genome sequence data of 458 individuals from sub-Saharan Africa showed significant *G6PD* variation across the continent. We identified nine variants, of which four are potentially deleterious to G6PD function, and one (rs1050828) that is known to cause G6PD deficiency. We supplemented data for the rs1050828 variant with genotype array data from over 11,000 Africans. Although this variant is common in Africans overall, large allele frequency differences exist between sub-populations. African sub-populations in the same country can show significant differences in allele frequency (e.g. 16.0% in Tsonga vs 0.8% in Xhosa, both in South Africa, $\rho=2.4\times10^{-3}$). The high prevalence of variants in the *G6PD* gene found in this analysis suggests that it may be a significant interaction factor in clinical trials of chloroquine and hydrochloroquine for treatment of COVID-19 in Africans. [**note: this is a drug safety issue about HCQ that I was not aware of.**]
https://www.medrxiv.org/content/10.1101/2020.05.27.20114066v1
- OBJECTIVE To evaluate the safety and efficacy of leflunomide for the treatment of refractory COVID-19 in adult patients. DESIGN Open-label controlled study SETTING A designated hospital for patients with refractory COVID-19 in Wuhan, China. PARTICIPANTS 27 hospitalized adult patients (≥18 years of age) with radiologically confirmed pneumonia and SARS-CoV-2 positive for more than 28 days despite standard care were assigned to receive standard of care (SOC, grp I) or leflunomide + SOC (grp 2). After 2 weeks, grp 1 and grp 2 patients who continued to be SARS-CoV-2-positive received leflunomide for 14 days while continuing SOC. MAIN OUTCOME MEASURES The primary outcomes were the rate of and time to SARS-CoV-2 clearance and the 14-day and 30-day hospital discharge rate. RESULTS Twelve patients enrolled in grp 1 and 15 patients were in grp 2. The 14 days SARS-CoV-2 viral clearance rate was 80.0% (12/15) for grp 2 patients receiving leflunomide versus 16.7% for grp 1 patients (2/12) (P=0.002). By day 14, the

median time to SARS-CoV-2 clearance was 6.0 days (range 1-12; IQR 1-12) for grp 2 patients. In grp 1, two patients converted to viral negative on days 1 and 6 (P=0.002). The 14-day discharge rate was 73.3% (11/15) for the grp 2 versus 8.3% (1/12) for grp 1 (P=0.001). The 30-day discharge rate was 100% (15/15) for the grp 2 versus 66.7% (8/12) for grp 1. No severe adverse events or deaths were reported. CONCLUSION Leflunomide is effective in enhancing SARS-CoV-2 clearance and hospital discharge in refractory COVID-19 patients. The addition of leflunomide to SOC did not increase adverse events versus SOC. These preliminary observations underscore a need for a randomized clinical study of leflunomide in SARS-CoV-2 infection. [**note: this is from Wuhan and is an open label study on leflunomide. There is one trial I see registered at University of Chicago but it too is an open label study and only 20 patients will be studied. I don't know whether this drug is good, bad, or indifferent but it seems like a little more effort ought to be put in to see whether it is useful.**]
https://www.medrxiv.org/content/10.1101/2020.05.29.20114223v1

DIAGNOSTIC DEVELOPMENT

- The RT-PCR with virus DNA identification is still the benchmark Covid-19 diagnosis method. In this work we propose a new technique for representing DNA sequences: they are divided into smaller sequences with overlap in a pseudo-convolutional approach, and represented by co-occurrence matrices. This technique analyzes the DNA sequences obtained by the RT-PCR method, eliminating sequence alignment. Through the proposed method, it is possible to identify virus sequences from a large database: 347,363 virus DNA sequences from 24 virus families and SARSCov-2. Experiments with all 24 virus families and SARS-Cov-2 (multi-class scenario) resulted 0.822222 ± 0.05613 for sensitivity and 0.99974 ± 0.00001 for specificity using Random Forests with 100 trees and 30% overlap. When we compared SARS-Cov-2 with similar-symptoms virus families, we got 0.97059 ± 0.03387 for sensitivity, and 0.99187 ± 0.00046 for specificity with MLP classifier and 30% overlap. In the real test scenario, in which SARS-Cov-2 is compared to Coronaviridae and healthy human DNA sequences, we got 0.98824 ± 001198 for sensitivity and 0.99860 ± 0.00020 for specificity with MLP and 50% overlap. Therefore, the molecular diagnosis of Covid-19 can be optimized by combining RT-PCR and our pseudo-convolutional method to identify SARS-Cov-2 DNA sequences faster with higher specificity and sensitivity. [**note: yet another take on improving RT-PCR, this time from Brazil.**]
https://www.biorxiv.org/content/10.1101/2020.06.02.129775v1
- Here, we propose HiDRA-seq, a rapidly implementable, high throughput, and scalable solution that uses NGS lab infrastructure and reagents for population-scale SARS-CoV-2 testing. This method is based on the use of indexed oligo-dT primers to generate barcoded cDNA from a large number of patient samples. From this, highly multiplexed NGS libraries are prepared targeting SARS-CoV-2 specific regions and sequenced. The low amount of sequencing data required for diagnosis allows the combination of thousands of samples in a sequencing run, while reducing the cost to approximately 2 CHF/EUR/USD per RNA sample. Here, we describe in detail the first version of the protocol, which can be further improved in the future to increase its sensitivity and to identify other respiratory viruses or analyze individual genetic features associated with disease progression. [**note: the work to improve diagnostics never stops! This time it is a Swiss group coming up with a new approach.**]
https://www.biorxiv.org/content/10.1101/2020.06.02.130484v1

- We present here INSIGHT (Isothermal NASBA-Sequencing based hIGH-througput Test): a two-stage COVID-19 testing strategy, using a combination of an isothermal NASBA reaction and next generation sequencing. From commercially acquired human saliva with spiked-in viral RNA as input, the first stage employs isothermal amplification of viral RNA to give a rapid result in one to two hours, using either fluorescence detection or a dipstick readout, whilst simultaneously incorporating sample-specific barcodes into the amplification product. In the first stage, fluorescent viral RNA detection can be consistently achieved at 10-100 copies per 20 µl reaction. The second stage pools post-amplification barcoded products from multiple samples for scalable sequencing that could be centralised, to further improve the accuracy of the test in a massively parallel way. Our two-stage testing strategy is suitable for further development into a home-based or point-of-care assay, and is potentially scalable to population level. {**note: and another one, this time from a Cambridge, UK group. We are reaching a saturation point on new diagnostic approaches.**] https://www.biorxiv.org/content/10.1101/2020.06.01.127019v1 and here is another link from the UK on the SAMBA II rapid test that I have covered before. They do a larger validation study to show its usefulness: https://www.medrxiv.org/content/10.1101/2020.05.31.20114520v1
- Novel coronavirus SARS-CoV-2 outbreaks have rapidly spread to multiple countries, highlighting the urgent necessity for fast, sensitive, and specific diagnostic tools for virus surveillance. Here, the previously unknown collateral single-stranded DNA cleavage we observed with type I CRISPR-Cas3 highlights its potential for development as a Cas3-mediated rapid (within 40 min), low-cost, instrument-free detection method for SARS-CoV-2. This Cas3-based assay is comparable with Cas12- and real-time reverse-transcriptase PCR-based assays in its speed and sensitivity, but offers greater specificity for single-base-pair discrimination while negating the need for highly trained operators. These findings support the use of CRISPR diagnostics for point-of-care testing in patients with suspected SARS-CoV-2 infections. [**note: I've seen some other papers on the use of CRISPR. This one is from Japan.**] https://www.medrxiv.org/content/10.1101/2020.06.02.20119875v1
- Rationale Management of the COVID-19 pandemic is hampered by long delays associated with centralised laboratory PCR testing. In hospitals this leads to poor patient flow and nosocomial transmission and rapid, accurate diagnostic tests are urgently required. The FebriDx is a point-of-care test that detects an antiviral host response protein in finger prick blood within 10 minutes, but its accuracy for the detection of COVID-19 is unknown. Objectives To evaluate the diagnostic accuracy of FebriDx in hospitalised patients during the first wave of the pandemic. Methods Measures of diagnostic accuracy were calculated based on FebriDx results compared to the reference standard of PCR, and stratified by duration of symptoms. A multivariable predictive model was developed and underwent internal validation. Results FebriDx was performed on 251 patients and gave a valid result in 248. 118 of 248 (48%) were PCR positive for COVID-19. Sensitivity of FebriDx for the identification of COVID-19 was 93% (110/118; 95% CI 87 to 97%) and specificity was 86% (112/130; 95%CI 79 to 92%). Positive and negative likelihood ratios were 6.73 (95%CI 4.37 to 10.37) and 0.08 (95%CI 0.04 to 0.15) respectively. In the multivariate model diagnosis of COVID-19 was not significantly influenced by clinical symptoms and signs, and FebriDx accuracy was not improved by restricting testing to those with duration of symptoms of less than seven days. Conclusions During the first wave of the pandemic, FebriDx had high sensitivity for the identification of COVID-19 in hospitalised adults and could be

deployed as a front door triage tool. [**note: this is an interesting test that distinguishes between viral and bacterial infections. It does not measure SARS-CoV-2 directly but it's still an interesting finding.**] https://www.medrxiv.org/content/10.1101/2020.05.27.20114512v1

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2020-06-04

A while back I said NO Disco, NO way, NO how. Does singing a Disco classic at the Nobel Peace Prize Concert honoring President Obama count as Disco? NO way, NO how. Here is the estimable Donna Summer singing her great hit 'Last Dance:' https://www.youtube.com/watch?v=rFWbjz02OCQ

My goodness, none of my loyal YouTube music aficionados got the answer to yesterday's contest. I know for a fact that **one of my readers is a diehard NBA fan**; perhaps he doesn't like The Pointer Sisters. Of course, it was the great Julius 'Dr. J' Erving whose slam dunks for the Philadelphia 76ers were legendary. Prizes are still out there to be won!!!

Derek Lowe updates work on remdesivir and tocilizumab and there is not any exciting news at all.

Apparently the US government has chosen five vaccine candidates that will receive priority funding according to The New York Times. I wonder what criteria they used. There will be some unhappy campers who were left off the list. The J&J vaccine has not even entered trials and Merck is only on the list because of an acquisition of an Austrian company a couple of weeks ago (disclosure – I have equity holdings in both companies).

Some personal experiences from SARS-CoV-2 antibody testing from those living in New York City.

Here is a nice article on apps that can help with contact tracing. Privacy and location tracking issues probably will not lead to wide adoption. I do think the privacy concerns are overblown as there are ways to address those and I have cited some references in earlier posts.

Big news of the day is the HCQ prophylactic trial that showed no protection. Scroll down to the abstract and link to The New England Journal article.

MODELING

- The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, poses a tremendous threat to human health. There were more than 4 million confirmed cases and 300 000 reported deaths worldwide as of May 11, 2020. There have been warnings of a major coronavirus pandemic since the SARS outbreak in 2002–04, and in 2020 that threat has been realised. Now we need to consider the challenges ahead; the current prevalence, the incidence of asymptomatic infections, and the true mortality remains unclear. We also need to ascertain the probable effectiveness of current control measures so future

strategies can be prioritised accordingly. At the end of 2019, the COVID-19 outbreak was first reported in the city of Wuhan, China, and has since spread worldwide. To contain the spread of the disease, a cordon sanitaire was imposed on Wuhan city on Jan 23, 2020, and travel restrictions were subsequently imposed on other cities across Hubei province the next day. After 61 days of lockdown in Hubei province, the province reopened again on March 25, 2020, and after 76 days of lockdown in Wuhan, the city reopened again on April 8, 2020. The screening of individuals in these areas provides essential information on how immunity, and potentially herd immunity, is shaped in the community that has so far had the longest chain of community transmission but also some of the strongest physical distancing measures. During early-2020, many studies attempted to estimate the reporting rate (or ascertainment rate) of COVID-19 in Wuhan. Seroprevalence surveys of the general population are key to understanding reporting rates, the underlying number of infections, the build-up of immunity, and to reconstruct chains of transmission of SARS-CoV-2. In *The Lancet Microbe*, Kelvin To and colleagues present the results of a seroepidemiological survey of SARS-CoV-2 in the general population in Hong Kong and returnees evacuated from Hubei, China. The investigators enrolled 1938 individuals before and after the COVID-19 pandemic between 2018 and 2020, and 452 asymptomatic Hubei returnees in March, 2020. The study showed that as an emergent virus, SARS-CoV-2 had a seroprevalence of 3·8% among Hubei returnees (17 of 452 returnees); very far from any plausible level of herd immunity. Although the study assessed a small sample—452 of approximately 60 million people in Hubei province—it provides an essential baseline for public health authorities when evaluating the effect of current and future interventions. Judging by patterns of circulation of endemic coronaviruses (HCoV-NL63, HCoV-HKU1, HCoV-229E, and HCoV-OC4), it is probable that broader immunity might build up in the years ahead. However, To and colleagues' study shows that the current level of immunity is far below the herd immunity threshold and will not appreciably slow future spread, so testing, screening, and contact tracing from symptomatic and asymptomatic infections are still key to stopping further infections. [**note: this is an article well worth looking at as there are some good papers that are referenced.]** https://www.thelancet.com/journals/lanmic/article/PIIS2666-5247(20)30055-0/fulltext

- With anecdotal reports of viral shedding from COVID-19 patients for several weeks, there is a need to quantify the prevalence of long-term SARS-CoV-2 shedding. Here, we characterize the temporal distribution of diagnostic SARS-CoV-2 PCR outcomes from nasopharyngeal swabs and associated EHR-derived features over two months for 874 COVID-19 patients with longitudinal data. Among a cohort of 379 COVID-19 patients with at least one positive follow-up SARS-CoV-2 PCR test, 53 patients remain SARS-CoV-2-positive after four weeks of initial diagnosis. Surprisingly, a majority of COVID-19 patients with long-term viral shedding are not hospitalized (40 of 53 patients), and have no enrichments among symptoms, demographics, or medical history. In a cohort of 370 COVID-19 patients that transition to a confirmed negative status, the upper bound of viral shedding duration has a mean of 21.2 days with standard deviation of 9.3 days. Of the 81 PCR-confirmed COVIDpos patients who have undergone serologic testing, 68 patients have developed anti-SARS-CoV-2 IgG to date, with a mean upper bound of time to seroconversion of 38.1 days (95% C.I. = 35.2-41.1 days). *Given that SARS-CoV-2 PCR testing may detect replication incompetent virus and that serologic tests do not imply neutralizing immunity, we suggest that the development of novel assays for measuring infectious viral load in non-*

hospitalized long-term shedders may help mitigate community transmission. This study motivates a platform that can link longitudinal diagnostic and serologic testing with real-time epidemiological data, towards proactively identifying and managing emerging hotspots of COVID-19. [**note: these Mayo Clinic researchers highlight an important issue related to long term viral shedding of clinically recovered patients. More work needs to be done to fully diagnose recovered patients.**]
https://www.medrxiv.org/content/10.1101/2020.06.02.20120774v1

- To investigate the relationship between viral load and secondary transmission in novel coronavirus disease 2019 (COVID−19), we reviewed epidemiological and clinical data obtained from immunocompetent laboratory-confirmed patients with COVID−19 at Toyama University Hospital. In total, 28 patients were included in the analysis. Median viral load at the initial sample collection was significantly higher in adults than in children and in symptomatic than in asymptomatic patients. Among symptomatic patients, non−linear regression models showed that the estimated viral load at onset was higher in the index (patients who transmitted the disease to at least one other patient) than in the non−index patients (patients who were not the cause of secondary transmission; median [95% confidence interval]: 6.6 [5.2−8.2] vs. 3.1 [1.5−4.8] log copies/μL, respectively). High nasopharyngeal viral loads around onset may contribute to secondary transmission of COVID−19. [**note: good study from Japan on viral load in infected patients. Lower levels in children.**]
https://www.medrxiv.org/content/10.1101/2020.06.02.20120014v1
- There are concerns that both the experience of adversities during the COVID-19 pandemic and worries about experiencing adversities will have substantial and lasting effects on physical and mental health. One pathway through which both experience of and worries about adversity may impact health is through effects on sleep. Psychosocial stress can reduce sleep length and increase sleep disturbance, which can in turn reduce individuals ability to cope and respond to stressors, and worsen health outcomes. Therefore this study explored whether either worries about adversities during the pandemic or the experience of adversities were associated with impaired sleep. We used data from 45,109 adults in the COVID-19 Social Study assessed weekly from 01/04/2020-11/05/2020 in the UK during the pandemic. *We studied six categories of adversity including both worries and experiences of: illness with COVID-19, financial difficulty, loss of paid work, difficulties acquiring medication, difficulties accessing food, and threats to personal safety. We used random-effect within-between models that automatically account for all time-invariant confounders. Both the total number of adversity experiences and total number of adversity worries were associated with lower quality sleep. Each additional experience was associated with a 1.17 (95% CI = 1.11, 1.24) times higher odds of poor quality sleep while each additional worry was associated with a 1.20 (95% CI = 1.17, 1.23) times higher odds of poor quality sleep. When considering specific experiences and worries, all worries and experiences were significantly related to poorer quality sleep except experiences relating to employment and finances. Having a larger social network offered some buffering effects on associations but there was limited further evidence of moderation by social or psychiatric factors. Results suggest that poor sleep may be a mechanism by which adversities are affecting mental health and highlight the importance of interventions that seek to reassure individuals and support adaptive coping strategies during the pandemic*. [**note: anything to do with sleep quality deserves a mention in my newsletter.**] https://www.medrxiv.org/content/10.1101/2020.06.02.20120311v1

- Half a year after the emergence of COVID-19, research is still going on to gain insight in the importance of different SARS-CoV-2 transmission routes and their impact on the clinical picture of COVID-19. Our findings suggest that coughing is not as important for transmission as initially anticipated and we discuss the potentially important role for loud conversation as a driver for transmission. [**note: interesting conjecture from these Belgian researchers. However, a study that relies on recollection is not entirely robust.**]
https://www.medrxiv.org/content/10.1101/2020.06.03.20121004v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- We conducted a randomized, double-blind, placebo-controlled trial across the United States and parts of Canada testing hydroxychloroquine as postexposure prophylaxis. We enrolled adults who had household or occupational exposure to someone with confirmed Covid-19 at a distance of less than 6 ft for more than 10 minutes while wearing neither a face mask nor an eye shield (high-risk exposure) or while wearing a face mask but no eye shield (moderate-risk exposure). Within 4 days after exposure, we randomly assigned participants to receive either placebo or hydroxychloroquine (800 mg once, followed by 600 mg in 6 to 8 hours, then 600 mg daily for 4 additional days). The primary outcome was the incidence of either laboratory-confirmed Covid-19 or illness compatible with Covid-19 within 14 days. We enrolled 821 asymptomatic participants. Overall, 87.6% of the participants (719 of 821) reported a high-risk exposure to a confirmed Covid-19 contact. The incidence of new illness compatible with Covid-19 did not differ significantly between participants receiving hydroxychloroquine (49 of 414 [11.8%]) and those receiving placebo (58 of 407 [14.3%]); the absolute difference was −2.4 percentage points (95% confidence interval, −7.0 to 2.2; P=0.35). Side effects were more common with hydroxychloroquine than with placebo (40.1% vs. 16.8%), but no serious adverse reactions were reported. *After high-risk or moderate-risk exposure to Covid-19, hydroxychloroquine did not prevent illness compatible with Covid-19 or confirmed infection when used as postexposure prophylaxis within 4 days after exposure*. [**note: this will be disappointing to the HCQ fans. The authors are aware of the limitations of the study and you can all read the paper. It's free from The New England Journal of Medicine.]**
https://www.nejm.org/doi/full/10.1056/NEJMoa2016638 & an accompanying editorial:
https://www.nejm.org/doi/full/10.1056/NEJMe2020388
- Background. Emerging evidence indicates a potential role for monocyte in COVID-19 immunopathology. We investigated two soluble markers of monocyte activation, sCD14 and sCD163, in covid19 patients with the aim of characterizing their potential role in monocyte-macrophage disease immunopathology. To the best of our knowledge, this is the first study of its kind. Methods. Fifty-nine SARS-Cov-2 positive hospitalized patients, classified according to ICU or non-ICU admission requirement, were prospectively recruited and analyzed by ELISA for levels of sCD14 and sCD163, along with other laboratory parameters, and compared to a healthy control group. Results. sCD14 and sCD163 levels were significantly higher among COVID-19 patients, independently of ICU admission requirement, compared to the control group. We found a significant correlation between sCD14 levels and other inflammatory markers,

particularly Interleukin-6, in the non-ICU patients group. sCD163 showed a moderate positive correlation with the time at sampling from admission, increasing its value over time, independently of severity group. Conclusions. Monocyte-macrophage activation markers are increased and correlate with other inflammatory markers in SARS-Cov-2 infection, in association to hospital admission. These data suggest a potentially preponderant role for monocyte-macrophage activation in the development of immunopathology of covid19 patients. [**note: some good work from Spain on the immunopathology of COVID-19 patients.**]
https://www.medrxiv.org/content/10.1101/2020.06.02.20120295v1

- Renalase (RNLS), a novel secreted plasma flavoprotein, has anti-inflammatory effects in a variety of disease processes. Severe COVID-19 disease is associated with disordered inflammatory responses. We hypothesized that reduced plasma RNLS levels could be a marker of COVID-19 disease severity. Methods: Plasma was collected from 51 hospitalized COVID-19 patients and 15 uninfected non-hospitalized controls. Plasma RNLS and cytokine levels were measured and sociodemographic and clinical data were collected from chart review. Data were analyzed using nonparametric analyses and Kaplan Meir curve log rank analysis. Results: Plasma RNLs levels were negatively correlated with inflammatory markers, including IL-1b, IL-6, and TNFa ($p = 0.04$, $p = 0.03$, $p = 0.01$, respectively). Patients with COVID-19 disease had lower levels of RNLS than controls. Lower levels of RNLS were associated with more severe disease among COVID-19 patients. Low RNLS was also associated with worse survival among COVID-19 patients (HR = 4.54; 95% CI: 1.06-19.43; $p = 0.005$). Conclusion: Low plasma RNLS levels are associated with severe COVID-19 disease and may be a useful additional biomarker when identifying patients with severe COVID-19 disease. Given RNLS anti-inflammatory properties and negative correlation with inflammatory markers, these findings also suggest evidence of a potential pathophysiological mechanism for severe COVID-19 disease. [**note: another biomarker for severe COVID-19 from a Yale group.**]
https://www.medrxiv.org/content/10.1101/2020.06.02.20120865v1
- We used a new strategy to screen cytokines associated with SARS-CoV-2 infection. Cytokines that can classify populations in different states of SARS-CoV-2 infection were first screened in cross-sectional serum samples from 184 subjects by 2 statistical analyses. The resultant cytokines were then analyzed for their interrelationships and fluctuating features in sequential samples from 38 COVID-19 patients. Three cytokines, M-CSF, IL-8 and SCF, which were clustered into 3 different correlation groups and had relatively small fluctuations during SARS-CoV-2 infection, were selected for the construction of a multiclass classification model. This model discriminated healthy individuals and asymptomatic and nonsevere patients with accuracy of 77.4% but was not successful in classifying severe patients. Further searching led to a single cytokine, hepatocyte growth factor (HGF), which classified severe from nonsevere COVID-19 patients with a sensitivity of 84.6% and a specificity of 97.9% under a cutoff value of 1128 pg/ml. The level of this cytokine did not increase in nonsevere patients but was significantly elevated in severe patients. Considering its potent antiinflammatory function, we suggest that HGF might be a new candidate therapy for critical COVID-19. In addition, our new strategy provides not only a rational and effective way to focus on certain cytokine biomarkers for infectious diseases but also a new opportunity to probe the modulation of cytokines in the immune response. [**note: and another biomarker from China. I assume that someone is aggregating all of these!**]
https://www.medrxiv.org/content/10.1101/2020.05.31.20118315v1

- The SARS-CoV-2 spike (S) protein, the viral mediator for binding and entry into the host cell, has sparked great interest as a target for vaccine development and treatments with neutralizing antibodies. Initial data suggest that the virus has low mutation rates, but its large genome could facilitate recombination, insertions, and deletions, as has been described in other coronaviruses. Here, we deep-sequenced the complete SARS-CoV-2 S gene from 18 patients (10 with mild and 8 with severe COVID-19), and found that the virus accumulates deletions upstream and very close to the S1/S2 cleavage site, generating a frameshift with appearance of a stop codon. These deletions were found in a small percentage of the viral quasispecies (2.2%) in samples from all the mild and only half the severe COVID-19 patients. Our results suggest that the virus may generate free S1 protein released to the circulation. We propose that natural selection has favored a Do not burn down the house strategy, in which free S1 protein may compete with viral particles for the ACE2 receptor, thus reducing the severity of the infection and tissue damage without losing transmission capability. [**note: this is a really fascinating paper from a group of Spanish researchers. It points to a mutation(s) that leads to mild infection and also points out that the virus may weaken over time as a result. The mutation leads to free S1 protein that can bind to the ACE2 receptor reducing anchoring of the virus. We will need to see more data from other regions and investigators to see if this conjecture holds up.**]
  https://www.biorxiv.org/content/10.1101/2020.06.03.129585v1
- Objective: To evaluate differences in morbidity and mortality among mechanically ventilated patients with COVID-19 treated with therapeutic versus prophylactic anticoagulation. Methods: We performed a retrospective review of 245 COVID-19 positive patients admitted to the ICU requiring mechanical ventilation from March 1, 2020 through April 11, 2020 at Mount Sinai Hospital. Patients either received therapeutic anticoagulation for a minimum of 5 days or prophylactic dose anticoagulation. Morbidity and mortality data were analyzed. Results: Propensity score (PS) weighted Kaplan-Meier plot demonstrated a survival advantage (57% vs. 25%) at 35 days from admission to the ICU in patients who received therapeutic anticoagulation for a minimum of 5 days compared to those who received prophylactic anticoagulation during their hospital course. A multivariate Cox proportional hazard regression model with PS weights to adjust for baseline differences found a 79% reduction in death in patients who were therapeutically anticoagulated HR 0.209, [95% CI (0.10, 0.46), p <0.001]. Bleeding complications were similar between both groups. A 26.7% [95% CI (1.16, 1.39), p<0.001] excess mortality was found for each 1 mg/dL rise in serum creatinine over a 21-day period. Conclusions: Therapeutic anticoagulation is associated with a survival advantage among patients with COVID-19 who require mechanical ventilation in the ICU. [**note: it looks like use of therapeutic anticoagulants for seriously ill COVID-19 patients should be the standard of care.**]
  https://www.medrxiv.org/content/10.1101/2020.05.30.20117929v1

DRUG DEVELOPMENT

- The recent spillover of SARS-CoV-2 in the human population resulted in the ongoing COVID-19 pandemic which has already caused 4.9 million infections and more than 326,000 fatalities. To initiate infection the SARS-CoV-2 spike (S) glycoprotein promotes attachment to the host cell surface, determining host and tissue tropism, and fusion of the viral and host membranes. Although SARS-CoV-2 S is the main target of neutralizing antibodies and the focus of vaccine design, its stability and conformational dynamics are limiting factors for developing

countermeasures against this virus. We report here the design of a prefusion SARS-CoV-2 S ectodomain trimer construct covalently stabilized in the closed conformation. Structural and antigenicity analysis showed we successfully shut S in the closed state without otherwise altering its architecture. Finally, we show that this engineering strategy is applicable to other beta-coronavirus S glycoproteins and might become an important tool for vaccine design, structural biology, serology and immunology studies. [**note: good work from some University of Washington researchers. I still think this kind of approach can be useful in vaccine validation.**] https://www.biorxiv.org/content/10.1101/2020.06.03.129817v1

- Replication of the SARS-CoV-2 genome is a fundamental step in the virus life cycle and inhibiting the SARS-CoV2 replicase machinery has been proven recently as a promising approach in combating the virus. Despite this recent success, there are still several aspects related to the structure, function and dynamics of the CoV-2 polymerase that still need to be addressed. This includes understanding the dynamicity of the various polymerase subdomains, analyzing the hydrogen bond networks at the active site and at the template entry in the presence of water, studying the binding modes of the nucleotides at the active site, highlighting positions for acceptable nucleotides substitutions that can be tolerated at different positions within the nascent RNA strand, identifying possible allosteric sites within the polymerase structure and studying their correlated dynamics relative to the catalytic site. Here, we combined various cutting-edge modelling tools with the recently resolved SARS-CoV-2 cryo-EM polymerase structures to fill this gap in knowledge. Our findings provide a detailed analysis of the hydrogen bond networks at various parts of the polymerase structure and suggest possible nucleotides substitutions that can be tolerated by the polymerase complex. We also report here three druggable allosteric sites within the nsp12 RdRp that can be targeted by small molecule inhibitors. Our correlated motion analysis shows that the dynamics within one of the newly identified sites are linked to the active site, indicating that targeting this site can significantly impact the catalytic activity of the SARS-CoV-2 polymerase. [**note: another possible target approach to the polymerase.**] https://www.biorxiv.org/content/10.1101/2020.06.02.130849v1

DIAGNOSTIC DEVELOPMENT

- To assess the current coronavirus pandemic, there is a pressing need to determine the exposure and seroconversion to SARS-CoV-2 on a local and global level. Here, we demonstrate a sensitive and specific S-protein based assay that is well suited for detection of weak SARS- CoV-2-directed IgG responses, and that could identify exposed individuals with asymptomatic infection without the requirement of PCR diagnostics. Our results raise the possibility that on- going population-based studies using less sensitive state-of-the-art serological assays may significantly underestimate the frequency of exposure and seroconversion to SARS-CoV-2. [**note: another diagnostic tool for the arsenal. It also highlights the need to identify those who are infected but asymptomatic.**] https://www.medrxiv.org/content/10.1101/2020.06.02.20120477v1
- There is a clear requirement for an accurate SARS−CoV−2 antibody test, both as a complement to existing diagnostic capabilities and for determining community seroprevalence. We therefore evaluated the performance of a variety of antibody testing technologies and their potential as diagnostic tools. A highly specific in−house ELISA was developed for the detection of anti−spike (S), −receptor binding domain (RBD) and −nucleocapsid (N) antibodies and used for the cross−comparison of ten commercial serological assays − a chemiluminescence−based platform,

two ELISAs and seven colloidal gold lateral flow immunoassays (LFIAs) − on an identical panel of 110 SARS−CoV−2−positive samples and 50 pre−pandemic negatives. There was a wide variation in the performance of the different platforms, with specificity ranging from 82% to 100%, and overall sensitivity from 60.9% to 87.3%. However, the head to head comparison of multiple serodiagnostic assays on identical sample sets revealed that performance is highly dependent on the time of sampling, with sensitivities of over 95% seen in several tests when assessing samples from more than 20 days post onset of symptoms. Furthermore, these analyses identified clear outlying samples that were negative in all tests, but were later shown to be from individuals with mildest disease presentation. Rigorous comparison of antibody testing platforms will inform the deployment of point of care technologies in healthcare settings and their use in the monitoring of SARS−CoV−2 [**note: from the UK, an extensive study of serology testing platforms. They note that quick Lateral Flow tests require an experienced user to assure optimal performance, particularly when scoring borderline cases and this may require two readers. There will also be a need to evaluate alternative sources of blood collection particularly pin-prick collection and even samples such as saliva.**]
https://www.medrxiv.org/content/10.1101/2020.06.02.20120345v1

- SARS-CoV-2 causes ongoing infections worldwide, and identifying people with immunity is becoming increasingly important. Available point-of-care diagnostic systems as lateral flow assays have high potential for fast and easy on-site antibody testing but are lacking specificity, sensitivity or possibility for quantitative measurements. Here, a new point-of-care approach for SARS CoV 2 specific antibody detection in human serum based on magnetic immuno-detection is described and compared to standard ELISA. For magnetic immuno-detection, immunofiltration columns were coated with a SARS-CoV-2 spike protein peptide. SARS CoV 2 peptide reactive antibodies, spiked at different concentrations into PBS and human serum, were rinsed through immunofiltration columns. Specific antibodies were retained within the IFC and labelled with an isotype specific biotinylated antibody. Streptavidin-functionalized magnetic nanoparticles were applied to label the secondary antibodies. Enriched magnetic nanoparticles were then detected by means of frequency magnetic mixing detection technology, using a portable magnetic read-out device. Measuring signals corresponded to the amount of SARS CoV 2 specific antibodies in the sample. Our preliminary magnetic immuno-detection setup resulted in a higher sensitivity and broader detection range and was four times faster than ELISA. Further optimizations could reduce assay times to that of a typical lateral flow assay, enabling a fast and easy approach, well suited for point-of-care measurements without expensive lab equipment. [**note: another novel approach to serology testing from a German group.]**
https://www.biorxiv.org/content/10.1101/2020.06.02.131102v1
- The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has presented significant challenges for laboratories including supply chain limitations with restricted access to reagents and sample collection materials (i.e. swabs, viral transport media (VTM)) for patients testing. Therefore, saliva has been evaluated as an alternative specimen for COVID-19 diagnosis. comparable performance between dry nasal swabs (NS) and nasopharyngeal swabs (NPS) collected in VTM has been observed with the ID NOW for SARS-CoV-2; the majority of false-negative results occur with higher cycle number (CN) or cycle threshold (Ct) values suggesting low viral load in these specimens. We performed clinical validation of saliva specimens on the ID NOW molecular platform to detect SARS-CoV-2. Saliva was compared to nasopharyngeal swabs

tested on the ID NOW and the Cepheid molecular assay. We also performed stability studies of saliva samples over 5 days. A total of 96 saliva samples and 64 paired NPS were analyzed. We observed 78% (18/23) positive percent agreement (PPA) and 100% (44/44) negative percent agreement (NPA) between matched saliva and nasopharyngeal specimens performed on ID NOW. We found 83% (19/23) PPA and 100% NPA (25/25) between saliva run on the ID NOW and Cepheid assay. Six saliva specimens positive for SARS-CoV-2 were continuously positive for five days when stored at room temperature. Therefore, we propose further investigation of saliva as an alternative sample type for testing symptomatic patients with ID NOW as a promising method to address COVID-19 testing. [**note: maybe White House visitors won't have to have invasive nasal swabs! This group from UT Southwestern Med School look at the performance of the Abbott quick test on saliva samples. More validation work needs to be done but maybe 'spit and test' will work.**] https://www.medrxiv.org/content/10.1101/2020.06.01.20119198v1

- The outbreak of the SARS-CoV-2 in early 2020 found health authorities worldwide unprepared to control the pandemic. The adoption of accurate, rapid and inexpensive methods to identify infected subjects in the general population is of paramount relevance for the control of the disease. We evaluated one of the available serological tests, the Prima Lab Covid-19 IgG/IgM Rapid Tests, on 739 volunteers. We first assessed the test's reproducibility by administering it twice on the same day on 104 subjects obtaining and overall score of 93 percent. Since the intensity of the color in the test line regions varies depending on the concentration of Covid-19 antibodies in each sample and that the determination of the positivity depends strictly on the subjective assessment by the reader, after excluding the subjects whose color intensity was too tenuous to be deemed unquestionably positive by the reader the reproducibility increased to 96%. The test would not perform properly for 6 subjects for a very limited overall technical failure of 0.83%. For 138 subjects information was available regarding a previous Real Time PCR nasopharyngeal swab test performed elsewhere. The correspondence of positive results between the two tests was 90.58% (125/138). In spite of some limitation owing especially to the choice of a self selected population sample, we conclude that Prima Lab Covid-19 IgG/IgM Rapid Test represents a low-cost, easily applicable and reproducible tool in detecting SARS-Cov-2 diffusion in the general population. [**note: analysis of another quick LFA serology test from Italy. It's probably good enough for community surveillance.**]
  https://www.medrxiv.org/content/10.1101/2020.05.30.20117424v1

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2020-06-05

Back on April 16, I provided a music selection from the Joan Chamorro Quintet. Chamorro is a phenomenal music teacher and his Sant Andreu Jazz Band is one phenomenal orchestra made up of kids between ages of 7 and 20. You can queue up any one of the groups performances or play lists and just smile the time away. The vocalists are always female, much as they were in the big band era here in the US. Here is a fine performance of 'Lover Come Back to Me' with drummer Abril Sauri singing: https://www.youtube.com/watch?v=T9_x09c-Xd8 Enjoy!

King Arthur flour have an isolation baking show. Why was I not paying attention? Here is the 10th episode and it is a good one. Jeffery Hamelman, the bread baker, is also the author of the most authoritative book on bread baking and one I constantly refer to. Here is the King Arthur YouTube Channel. Happy pandemic baking!!!

I read one piece of sad news this morning. Brooks Brothers are looking to close their last remaining US manufacturing facilities. Among them is the Southwick suit factory in Massachusetts that Brooks Brothers purchased just over a decade ago. Southwick have been making fine suits since 1929. For the fashionistas reading this newsletter, Southwick were my exclusive suit maker over the years. Here is an amusing anecdote. In early spring 1990, I went to my clothier to pick out a Southwick fabric for a new light weight suit. I was waited on by Arthur Adler, owner of the venerable DC haberdashery of the same name, and he complemented me on the choice of fabric noting that then Vice President Dan Quayle had selected the exact same one the day before!!!

More on long term symptoms of COVID-19 from Ed Yong in The Atlantic.

The co-authors of the large observational HCQ analysis published last week in The Lancet have retracted the paper. The could not do an audit of the database that was maintained by Surgisphere Corporation. This one is pretty ugly.

The Lancet also has a good overview of vaccine development.

One of the downsides of the early days of the SARS-CoV-2 pandemic was the over prescribing of antibiotics for what was suspected to be bacterial pneumonia. Perhaps this will be a teaching moment. A group of researchers at the NIH Clinical Center that includes one of my good friends has issued a needs assessment for novel gram-negative antibiotics.

Here is a good New York Times article on the genetic vulnerability of some people to severe COVID-19. I've been discussing this as preprints have appeared defining some of the genetic linkages.

Can the US and the world avoid screwing up if a resurgence appears (or better yet when the next pandemic arrives. That's the topic of this STAT piece. I especially liked the suggestions *avoid magical thinking* and *communicate better*!

Derek Lowe weighs in on the HCQ prophylactic study reported out yesterday in the NEJM. There are still a lot of HCQ fans writing in the comment section. I'm constantly amazed by the number of people who have no concept of how trials are run and analyzed.

I'm putting this preprint above the fold because of its good advice to clinicians. "How Should Clinicians Interpret Imprecise Trials Assessing Drugs for COVID-19 Patients?" ABSTRCT: As the COVID-19 pandemic progresses, researchers are reporting findings of randomized trials comparing standard care with care augmented by experimental drugs. The trials have small sample sizes, so estimates of treatment effects are imprecise. Seeing imprecision, clinicians reading research articles may find it difficult to decide when to treat patients with experimental drugs. Whatever decision criterion one uses, there is always some probability that random variation in trial outcomes will lead to prescribing sub-optimal treatments. A conventional practice when comparing standard care and an innovation is to choose the innovation only if the estimated treatment effect is positive and statistically significant. This practice defers to standard care as the status quo. We argue that clinicians should ignore conventional

measures of statistical significance. They should choose treatments that work best in trials, taking side effects into account and recognizing that treatment effects may vary with patient risk factors. To evaluate treatments, we use the concept of near optimality, which jointly considers the probability and magnitude of decision errors. An appealing decision criterion from this perspective is the empirical success rule, which chooses the treatment with the highest observed average patient outcome in the trial. Considering the design of COVID-19 trials, we show that the empirical success rule yields treatment results that are much closer to optimal than those generated by prevailing decision criteria based on hypothesis tests. [**note: do read the whole paper!**]

MODELING

- Extraordinary finding!!! No New Models Today!!!

NEWLY REGISTERED CLINICAL TRIALS

- The purpose of this study is to determine if therapeutic dose anticoagulation (experimental group) improves 30-day mortality in participants with COVID-19 compared to those patients receiving the intermediate dose prophylaxis (control group). Following screening, subjects will be randomized 1:1 to intermediate dose prophylaxis or therapeutic dose anticoagulation treatment arms.Treatment will continue for 28 days, followed by a 6 month follow-up period. [**note: this is from Cornell Medical school. Anti-coagulants are looking to be the standard of care for seriously ill patients.**] NCT04406389
- Patients with moderate to severe COVID-19 present a very high risk of thromboembolic disease.This multicenter, prospective, randomized, event-driven study evaluates rivaroxaban compared with standard of care including low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH) at prophylactic doses if applicable in the prevention of the composite of venous thromboembolism (deep vein thrombosis and/or fatal or non-fatal pulmonary embolism), arterial thromboembolism, new myocardial infarction, non-hemorrhagic stroke, all-cause mortality or progression to intubation and invasive ventilation 35 days post randomization in patients with moderate to severe COVID-19. [**note: another anti-coagulation study, this time from Germany.]** NCT04416048
- A randomized, double-blind, placebo-controlled Phase 2 Study of LAU-7b against confirmed COVID-19 Disease in hospitalized patients at a higher risk of complications. [**note: I have no idea about this drug, it is from a Canadian company Laurent Pharmaceuticals. The drug has been studied for cystic fibrosis and Is a form of the retinoid fenretidine.**] NCT04417257
- The purpose of this study is to determine if prophylaxis with RTB101 decreases the severity of laboratory-confirmed COVID-19 among adults ≥ 65 years who reside in a nursing homes in which one or more residents or staff have laboratory-confirmed COVID-19. [**note: another trial from a small company, resTORbio who are engaged in anti-aging research (this is where I'm putting my money!). Here is the press release; I'm curious about the structure of this one.**] NCT04409327
- The aim of this study is to test the hypothesis that prophylaxis of severe COVID-19 patients with treatment dose LMWH leads to better thromboembolic-free outcomes and associated complications during hospitalization than prophylaxis with institutional standard of care with

prophylactic to intermediate-doses of UFH or LMWH [**note: and another anti-coagulant study.]** NCT04401293

CLINICAL TRIAL RESULTS

- Here is a really small trial in a subgroup of patients with COVID-19 using canakinumab, a monoclonal against IL-1β. A positive clinical effect was noted. The rapid improvement in serum inflammatory biomarkers after the administration of canakinumab therefore implicates the IL-1β inflammasome pathway in the pathophysiology of COVID-19. \[**note: this is study from Italy.]** **https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(20)30167-3/fulltext**
- The newly identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes COVID-19, a pandemic respiratory disease presenting with fever, cough, and often pneumonia. Moreover, thromboembolic events throughout the body including the central nervous system (CNS) have been described. Given first indication for viral RNA presence in the brain and cerebrospinal fluid and in light of neurological symptoms in a large majority of COVID-19 patients, SARS-CoV-2-penetrance of the CNS is likely. By precisely investigating and anatomically mapping oro- and pharyngeal regions and brains of 32 patients dying from COVID-19, we not only describe CNS infarction due to cerebral thromboembolism, but also demonstrate SARS-CoV-2 neurotropism. SARS-CoV-2 enters the nervous system via trespassing the neuro-mucosal interface in the olfactory mucosa by exploiting the close vicinity of olfactory mucosal and nervous tissue including delicate olfactory and sensitive nerve endings. Subsequently, SARS-CoV-2 follows defined neuroanatomical structures, penetrating defined neuroanatomical areas, including the primary respiratory and cardiovascular control center in the medulla oblongata. [**note: this is probably another great reason to mask up! Clearly not every COVID-19 patient suffer severe symptoms but I wonder if those who suffer longer term effects might have neurological involvement.**] https://www.biorxiv.org/content/10.1101/2020.06.04.135012v1

DRUG DEVELOPMENT

- Here, we compare induced pluripotent stem cell (iPSC)-derived alveolar and airway epithelial cells to primary lung epithelial cell controls, focusing on expression levels of genes relevant for COVID-19 disease modeling. iPSC-derived alveolar epithelial type II-like cells (iAT2s) and iPSC-derived airway epithelial lineages express key transcripts associated with lung identity in the majority of cells produced in culture. They express ACE2 and TMPRSS2, transcripts encoding essential host factors required for SARS-CoV-2 infection, in a minor subset of each cell sub-lineage, similar to frequencies observed in primary cells. In order to prepare human culture systems that are amenable to modeling viral infection of both the proximal and distal lung epithelium, we adapt iPSC-derived alveolar and airway epithelial cells to two-dimensional air-liquid interface cultures. These engineered human lung cell systems represent sharable, physiologically relevant platforms for SARS-CoV-2 infection modeling and may therefore expedite the development of an effective pharmacologic intervention for COVID-19. [**note: this may be a good tissue model for studying potential drug therapies.**] https://www.biorxiv.org/content/10.1101/2020.06.03.132639v1
- Recent evidence shows that the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is highly sensitive to interferons (IFNs). However, the underlying antiviral effectors remain to be defined. Here, we show that Zinc finger antiviral protein (ZAP) that specifically targets CpG

dinucleotides in viral RNA sequences restricts SARS-CoV-2. We demonstrate that ZAP and its cofactors KHNYN and TRIM25 are expressed in human lung cells. Type I, II and III IFNs all strongly inhibited SARS-CoV-2 and further induced ZAP expression. Strikingly, SARS-CoV-2 and its closest relatives from bats show the strongest CpG suppression among all known human and bat coronaviruses, respectively. Nevertheless, knock-down of ZAP significantly increased SARS-CoV-2 production in lung cells, particularly upon treatment with IFN-alpha or IFN-gamma. Thus, our results identify ZAP as an effector of the IFN response against SARS-CoV-2, although this pandemic pathogen may be preadapted to the low CpG environment in humans. [**note: Here is the definition of zinc finger, which is a structural motif that binds one or more zinc ions. The structure of the ZAP protein with RNA is here. For all the zinc dietary supplement fans who read my newsletter, there is NO evidence that I have found that taking zinc supplements regulates production of this protein. Anyway this current paper is intriguing in terms of viral pathogenicity.**] https://www.biorxiv.org/content/10.1101/2020.06.04.134379v1

- There is an urgent need for a vaccine with efficacy against SARS-CoV-2. We hypothesize that peptide vaccines containing epitope regions optimized for concurrent B cell, CD4+ T cell, and CD8+ T cell stimulation would drive both humoral and cellular immunity with high specificity, potentially avoiding undesired effects such as antibody-dependent enhancement (ADE). Additionally, such vaccines can be rapidly manufactured in a distributed manner. In this study, we combine computational prediction of T cell epitopes, recently published B cell epitope mapping studies, and epitope accessibility to select candidate peptide vaccines for SARS-CoV-2. We begin with an exploration of the space of possible T cell epitopes in SARS-CoV-2 with interrogation of predicted HLA-I and HLA-II ligands, overlap between predicted ligands, protein source, as well as concurrent human/murine coverage. Beyond MHC affinity, T cell vaccine candidates were further refined by predicted immunogenicity, viral source protein abundance, sequence conservation, coverage of high frequency HLA alleles and co-localization of CD4+ and CD8+ T cell epitopes. B cell epitope regions were chosen from linear epitope mapping studies of convalescent patient serum, followed by filtering to select regions with surface accessibility, high sequence conservation, spatial localization near functional domains of the spike glycoprotein, and avoidance of glycosylation sites. From 58 initial candidates, three B cell epitope regions were identified. By combining these B cell and T cell analyses, as well as a manufacturability heuristic, we propose a set of SARS-CoV-2 vaccine peptides for use in subsequent murine studies and clinical trials. [**note: good work that needs to be explored much further. I am afraid the vaccine train in the US has left the station with the selection of candidate vaccines that will receive funding. There is no question in my mind that much more work needs to be done in epitope selection as well as using targeted antibody analysis for proof of clinical efficacy. We have the tools now to make this achievable.**] https://www.biorxiv.org/content/10.1101/2020.06.04.135004v1

DIAGNOSTIC DEVELOPMENT

- Resolving the COVID-19 pandemic requires diagnostic testing to determine which individuals are infected and which are not. The current gold standard is to perform RT-PCR on nasopharyngeal samples. Best-in-class assays demonstrate a limit of detection (LoD) of ~100 copies of viral RNA per milliliter of transport media. However, LoDs of currently approved assays vary over 10,000-fold. Assays with higher LoDs will miss more infected patients, resulting in more false negatives.

However, the false-negative rate for a given LoD remains unknown. Here we address this question using over 27,500 test results for patients from across our healthcare network tested using the Abbott RealTime SARS-CoV-2 EUA. These results suggest that each 10-fold increase in LoD is expected to increase the false negative rate by 13%, missing an additional one in eight infected patients. *The highest LoDs on the market will miss a majority of infected patients, with false negative rates as high as 70%. These results suggest that choice of assay has meaningful clinical and epidemiological consequences. The limit of detection matters*. [**note: as we all should know by now, diagnostic test kits vary considerably by limits of detection. All test kits must be independently validated!**]
https://www.biorxiv.org/content/10.1101/2020.06.02.131144v1

- To combat the COVID-19 pandemic, millions of PCR tests are performed worldwide. Any deviation of the diagnostic sensitivity and specificity will reduce the predictive values of the test. Here, we report the occurrence of contaminations of commercial primers/probe sets with the SARS-CoV-2 target sequence of the RT-qPCR as an example for pitfalls during PCR diagnostics affecting diagnostic specificity. In several purchased in-house primers/probe sets, quantification cycle values as low as 17 were measured for negative control samples. However, there were also primers/probe sets that displayed very low-level contaminations, which were detected only during thorough internal validation. Hence, it appears imperative to pre-test each batch of reagents extensively before use in routine diagnosis, to avoid false-positive results and low positive predictive value in low-prevalence situations. As such contaminations may have happened more widely, COVID-19 diagnostic results should be re-assessed retrospectively to validate the epidemiological basis for control measures. [**note: a cautionary note from Germany about the contamination of commercial PCR reagents highlighting the need to reassess validation at intervals as part of SOP.**]
https://www.biorxiv.org/content/10.1101/2020.06.03.132357v1

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2020-06-06

Why haven't we had any comedy songs? One of my alert readers posed this exact question yesterday and I will remedy this oversight right now. Tom Lehrer is still with us at age 92, though he retired from performing many years ago. He also claims to have invented the Jell-O Shot while in the army. Here is 'We Will all Go Together When We Go' that refers back to the Cold War Days when we all had to do 'duck and cover' drills in school for fear of the nuclear apocalypse which, although I cannot be 100% certain, has not yet occurred: https://www.youtube.com/watch?v=frAEmhqdLFs

I see that the data from the UK COVID-19 RECOVERY Trial on HCQ are in. I'll spare you all the waiting while you hurriedly scroll down to the Clinical Trial Results section. The DSMB unblinded the results as they deemed it important to communicate that ……..wait for it……. "There was no significant difference in the primary endpoint of 28-day mortality (25.7% hydroxychloroquine vs. 23.5% usual care; hazard ratio 1.11 [95% confidence interval 0.98-1.26]; p=0.10). There was also no evidence of beneficial effects on hospital stay duration or other outcomes." Derek Lowe weighs in on this as well but this still does not satisfy all the HCQ fan boys and girls judging from the comments section.

The Lancet commentary on what the WHO should look like.

Some interesting perspectives from The New England Journal of Medicine (all should be free): False Negative Tests for SARS-CoV-2 Infection-Challenges and Implications, Waiting for Certainty on COVID-19 Antibody Tests – At What Cost?, and Putting the Public Back in Public Health – Surveying Symptoms of COVID-19.

Oh dear, this is really dispiriting. Here is a story from STAT about Americans misusing cleaning products in response to the SARS-CoV-2 pandemic. I covered this matter a while back in response to a certain political official's comment (referred to in the article) about the use of such products.

As there was a shortage of interesting papers yesterday, I started thinking about scaling the newsletter back to every other day. This morning's plethora of interesting papers disabused me of this! I think I will start to reduce the posting of new diagnostic approaches as I'm seeing too many similar approaches from various groups. I'll try to highlight only novel (in my estimation) approaches going forward.

MODELING

- The purpose of this study was to explore the factors underlying variability in compliance with CDC guidelines in response to the novel coronavirus, or COVID-19. To do this, we examined the frequency of once ordinary, but newly risky behavior (as deemed by CDC guidelines) in a sample of 482 MTurkers. We ran analyses probing the situational and dispositional variables that predicted variance in risky behavior using data-driven and hypothesis-generated approaches. We found situational and dispositional variables contributed unique variance to risky behavior, controlling for variability accounted for by demographic factors. More frequent report of risky activity was associated with higher extraversion, need for cognitive closure, behavior activation, and perceived resource scarcity; in contrast, more frequent report of risky activity was associated with less empathy and living space access, as well as younger age. To break down these findings, we used a cluster analysis to profile individuals, using only situational and dispositional variables belonging to seven clusters. Combined with testing differences in risk taking by cluster identity, we suggest this profile approach might allow consideration of multi-faceted attributes that influence adherence with public health guidance in the context of health emergencies like the COVID-19 pandemic. [**note: I don't think the US will go through another lock down regardless of the trending cases of SARS-CoV-2 infection. This paper is interesting in it attempts to look at why risky behavior continues to be prevalent. The absence of mask wearing in crowded public spaces continues to puzzle me. I understand some peoples reflex against social distancing but there is no question regarding the public health value of wearing a mask at this point in time.**]
  https://www.medrxiv.org/content/10.1101/2020.06.04.20122754v1
- The ongoing COVID-19 pandemic caused by SARs-CoV-2 was considered to be transmitted person to person via droplet infections and fecal-oral transmission. To determine this, we used the existing polio environment surveillance network in Pakistan to investigate presence of SARs-CoV-2 using three commercially available kits and E-Gene detection published assay for surety and confirmatory of positivity. A Two-phase separation method is used for sample clarification and concentration. Before proceeding directly for RNA extraction, an additional high-speed centrifugation (14000Xg for 30 min) step was introduced to increase viral RNA yield resulting

decrease in Cq value. A total of 78 wastewater samples collected from 38 districts across Pakistan, 74 wastewater samples from existing polio environment surveillance sites, 3 from drains of COVID-19 infected areas and 1 from COVID 19 quarantine center drainage, were tested for presence of SARs-CoV-2. 21 wastewater samples (27%) from 13 districts turned to be positive on RT-qPCR. SARs-COV-2 RNA positive samples from areas with COVID patients and COVID 19 patient quarantine center drainage strengthen the findings and use of wastewater surveillance in future. Furthermore, sequence data of partial ORF 1a generated from COVID 19 patient quarantine center drainage sample also reinforce our findings that SARs-CoV-2 can be detected in wastewater. This study finding indicates that SARs-CoV-2 detection through wastewater surveillance has an epidemiologic potential that can be used as early warning system to monitor viral tracking and circulation in cities with lower COVID-19 disease burden or heavily populated areas where door-to-door tracing may not be possible. However, attention needed on virus concentration and detection assay to increase the sensitivity. Development of highly sensitive assay will be an indicator for virus monitoring and to provide early warning signs. [**note: good work from Pakistan showing the utility of waste water monitoring!**] https://www.medrxiv.org/content/10.1101/2020.06.03.20121426v1

- One of the significant unanswered questions about COVID-19 epidemiology relates to the role of children in transmission. In this study we estimate susceptibility and infectivity of children compared to those of adults. Understanding the age-structured transmission dynamics of the outbreak provides precious and timely information to guide epidemic modelling and public health policy. Data were collected from households in the city of Bnei Brak, Israel, in which all household members were tested for COVID-19 using PCR. To estimate relative transmission parameters in the absence of data on who infected whom, we developed an estimation method based on a discrete stochastic dynamic model of the spread of the epidemic within a household. The model describes the propagation of the disease between household members allowing for susceptibility and infectivity parameters to vary among two age groups. The parameter estimates are obtained by a maximum likelihood method, where the likelihood function is computed based on the stochastic model via simulations. Inspection of the data reveals that children are less likely to become infected compared to adults (25% of children infected over all households, 44% of adults infected over all households, excluding index cases), and the chances of becoming infected increases with age. An interesting exception is that infants up to age one year are more likely to be infected than children between one and four. Using our modelling approach, we estimate that the susceptibility of children (under 20 years old) is 45% [40%, 55%] of the susceptibility of adults. The infectivity of children was estimated to be 85% [65%,110%] relative to that of adults. It is widely observed that the percentage of children within confirmed cases is low. A common explanation is that children who are infected are less likely to develop symptoms than adults, and thus are less likely to be tested. We estimate that children are less susceptible to infection, which is an additional factor explaining their relatively low rate of occurrence within confirmed cases. Moreover, our results indicate that children, when infected, are somewhat less prone to infect others compared to adults; however, this result is not statistically significant. The resulting estimates of susceptibility and infectivity of children compared to adults are crucial for deciding on appropriate interventions, and for controlling the epidemic outbreak and its progress. These estimates can guide age-dependent public health policy such as school closure and opening. However, while our estimates of children's

susceptibility and infectivity are lower than those of adults within a household, it is important to bear in mind that their role in the spread of COVID-19 outside the household, is also affected by different contact patterns and hygiene habits. [**note: good work from Israel in looking at infectivity in children and the role they might play in transmission of SARS-CoV-2. This will be important as schools begin to reopen in the US.**]
https://www.medrxiv.org/content/10.1101/2020.06.03.20121145v1

- The genome of SARS-CoV-2 (SARS2) encodes for two viral proteases (NSP3/ papain-like protease and NSP5/ 3C-like protease or major protease) that are responsible for cleaving viral polyproteins for successful replication. NSP3 and NSP5 of SARS-CoV (SARS1) are known interferon antagonists. Here, we examined whether the protease function of SARS2 NSP3 and NSP5 target proteins involved in the host innate immune response. We designed a fluorescent based cleavage assay to rapidly screen the protease activity of NSP3 and NSP5 on a library of 71 human innate immune proteins (HIIPs), covering most pathways involved in human innate immunity. By expressing each of these HIIPs with a genetically encoded fluorophore in a cell-free system and titrating in the recombinant protease domain of NSP3 or NSP5, we could readily detect cleavage of cognate HIIPs on SDS-page gels. We identified 3 proteins that were specifically and selectively cleaved by NSP3 or NSP5: IRF-3, and NLRP12 and TAB1, respectively. Direct cleavage of IRF3 by NSP3 could explain the blunted Type-I IFN response seen during SARS-CoV-2 infections while NSP5 mediated cleavage of NLRP12 and TAB1 point to a molecular mechanism for enhanced production of IL-6 and inflammatory response observed in COVID-19 patients. Surprisingly, both NLRP12 and TAB1 have each two distinct cleavage sites. We demonstrate that in mice, the second cleavage site of NLRP12 is absent. We pushed this comparative alignment of IRF-3 and NLRP12 homologs and show that the lack or presence of cognate cleavage motifs in IRF-3 and NLRP12 could contribute to the presentation of disease in cats and tigers, for example. Our findings provide an explanatory framework for in-depth studies into the pathophysiology of COVID-19 and should facilitate the search or development of more effective animal models for severe COVID-19. Finally, we discovered that one particular species of bats, Davids Myotis, possesses the five cleavage sites found in humans for NLRP12, TAB1 and IRF3. These bats are endemic from the Hubei province in China and we discuss its potential role as reservoir for the evolution of SARS1 and SASR2. [**note: I don't know how to classify interesting papers such as this one, hence it goes in the modelling section. This is an interesting observation in that the proteases from SARS-CoV-2 may blunt the immune response in some manner by cleaving important human proteins. The ecological observation about bats in Hubei province is intriguing as this is where SARS-like viruses intermix.**]
https://www.biorxiv.org/content/10.1101/2020.06.05.135699v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow!

CLINICAL TRIAL RESULTS

- Objective We aimed to describe the influence of tocilizumab on the need of transfer to ICU or death in non-critically ill patients. Methods A retrospective study of 171 patients with SARS-CoV-2 infection that did not qualify as requiring transfer to ICU during the first 24h after admission to a conventional ward, were included. The criteria to receive tocilizumab was radiological

impairment, oxygen demand or an increasing of inflammatory parameters, however, the ultimate decision was left to the attending physician judgement. The primary outcome was the need of ICU admission or death whichever came first. Results 77 patients received tocilizumab and 94 did not. The tocilizumab group had less ICU admissions (10.3% vs. 27.6%, P= 0.005) and need of invasive ventilation (0 vs 13.8%, P=0.001). In the multivariable analysis, tocilizumab remained as a protective variable (OR: 0.03, CI 95%: 0.007-0·1, P=0.0001) of ICU admission or death. Conclusions Tocilizumab in the early stages of the inflammatory flare, could avoid an important number of ICU admissions and mechanical ventilation use. The mortality rate of 10.3% among patients receiving tocilizumab appears to be lower than other reports. [**note: more early data on the usefulness of tocilizumab from Spain. It will be important to see the clinical trial data since this now seems to point to a decent treatment option. I wonder if any of the DSMBs associated with those trials will take that step if the data looks good enough.**[https://www.medrxiv.org/content/10.1101/2020.06.05.20113738v1

- Background: Tocilizumab blocks pro-inflammatory activity of interleukin-6 (IL-6) which might be important in the pathogenesis of interstitial pneumonia. Objective: to evaluate efficacy of tocilizumab in COVID-19 pneumonia patients. Design: multicenter single-arm phase 2 trial, powered to detect 10% absolute lethality rate reduction at 14 and 30-days, with 20% and 35% expected rates. A consecutive prospective validation cohort was also evaluated. Setting: 185 Italian public hospitals, during coronavirus breakout. Patients: 1221 patients hospitalized with pneumonia, from March 19th to 24th, 2020. Intervention: tocilizumab 8 mg/kg, intravenously, one or two administrations with 12 hours interval. Measurements: lethality rates at 14 and 30-days; safety according to CTCAE. Results: 301 and 920 cases were available for intention-to-treat (ITT) analysis in phase 2 and validation cohorts. Due to delayed drug availability, 60% of patients received tocilizumab, and with some delays. In phase 2, 67 patients died; lethality rates were 18.4% (97.5%CI: 13.6-24.0, P=0.52) and 22.4% (97.5%CI: 17.2-28.3, P<0.001) at 14 and 30-days. Lower rates (15.6% and 20.0%) were reported in the modified ITT including only treated patients (mITT). Lethality rates in the validation cohort were smaller than in phase 2, at 14 and 30 days and in ITT and mITT populations. Multivariable logistic regression model suggests tocilizumab be more effective in patients not requiring mechanical respiratory support at baseline. No relevant signal of specific drug toxicity was reported, many severe adverse events being disease-related. Limitations: single-arm design. In addition, delayed availability of the drug induced indication bias and immortal time bias. Conclusion: Tocilizumab reduced lethality rate at 30 but not at 14-days, compared with the expectations, without significant toxicity. Efficacy was more evident among patients not requiring mechanical respiratory support. [**note: more data on tocilizumab also pointing to early use.**] https://www.medrxiv.org/content/10.1101/2020.06.01.20119149v1
- Background: Absolute numbers of COVID-19 cases and deaths reported to date in the sub-Saharan Africa (SSA) region have been significantly lower than those across the Americas, Asia, and Europe. As a result, there has been limited information about the demographic and clinical characteristics of deceased cases in the region, as well as the impacts of different case management strategies. Methods: Data from deceased cases reported across SSA through May 10, 2020 and from hospitalized cases in Burkina Faso through April 15, 2020 were analyzed. Demographic, epidemiological, and clinical information on deceased cases in SSA was derived through a line-list of publicly available information and, for cases in Burkina Faso, from aggregate records at the Center Hospitalier Universitaire de Tengandogo in Ouagadougou. A

synthetic case population was derived probabilistically using distributions of age, sex, and underlying conditions from populations of West African countries to assess individual risk factors and treatment effect sizes. Logistic regression analysis was conducted to evaluate the adjusted odds of survival for patients receiving oxygen therapy or convalescent plasma, based on therapeutic effectiveness observed for other respiratory illnesses. Results: Across SSA, deceased cases for which demographic data are available have been predominantly male (63/103, 61.2%) and over 50 years of age (59/75, 78.7%). In Burkina Faso, specifically, the majority of deceased cases either did not seek care at all or were hospitalized for a single day (59.4%, 19/32); hypertension and diabetes were often reported as underlying conditions. After adjustment for sex, age, and underlying conditions in the synthetic case population, the odds of mortality for cases not receiving oxygen therapy was significantly higher than those receiving oxygen, such as due to disruptions to standard care (OR: 2.07; 95% CI: 1.56-2.75). Cases receiving convalescent plasma had 50% reduced odds of mortality than those who did not (95% CI: 0.24-0.93). Conclusion: Investment in sustainable production and maintenance of supplies for oxygen therapy, along with messaging around early and appropriate use for healthcare providers, caregivers, and patients could reduce COVID-19 deaths in SSA. Further investigation into convalescent plasma is warranted, as data on its effectiveness specifically in treating COVID-19 becomes available. The success of supportive or curative clinical interventions will depend on earlier treatment seeking, such that community engagement and risk communication will be critical components of the response. [**note: we have to remember that we are all in this together. It is good to give a shout out to researchers from Burkina Faso for this preprint.**] https://www.medrxiv.org/content/10.1101/2020.06.04.20119784v1

- Aims: Severity of outcome in COVID-19 is disproportionately higher among the obese, males, smokers, those suffering from hypertension, kidney disease, coronary heart disease (CHD) and/or type 2 diabetes (T2D). We examined if serum levels of ACE2, the cellular entry point for the coronavirus SARS-CoV-2, were altered in these high-risk groups. Methods: Associations of serum ACE2 levels to hypertension, T2D, obesity, CHD, smokers and males in a single center population-based study of 5457 Icelanders from the Age, Gene/Environment Susceptibility Reykjavik Study (AGES-RS) of the elderly (mean age 75+/-6 years). Results: Smokers, males, and individuals with T2D or obesity have altered serum levels of ACE2 that may influence productive infection of SARS-CoV-2 in these high-risk groups. Conclusion: ACE2 levels are upregulated in some patient groups with comorbidities linked to COVID-19 and as such may have an emerging role as a circulating biomarker for severity of outcome in COVID-19. [**note: this is from Icelandic data and points to another biomarker for infection. That smokers are in this group is interesting in that smoking has been thought to be mildly protective from several preprints.**] https://www.medrxiv.org/content/10.1101/2020.06.04.20122044v1
- We estimated the association between patients characteristics and COVID-19 diagnosis, hospitalization and adverse outcome in Mexico. **Methods:** This retrospective case series used a publicly available nation-level dataset released on May 31, 2020 by the Mexican Ministry of Health, with patients classified as suspected cases of viral respiratory disease. Patients with COVID-19 were laboratory-confirmed. Their profile was stratified by COVID-19 diagnosis or not. Differences among COVID-19 patients based on two separate clinical endpoints, hospitalization and adverse outcome, were examined. Multivariate logistic regressions examined the associations between patient characteristics and hospitalization and adverse outcome. **Results:**

*Overall, 236,439 patients were included, with **89,756** (38.0%) being diagnosed with COVID-19.* COVID-19 patients were disproportionately older, males and with increased prevalence of one or more comorbidities, particularly diabetes, obesity, and hypertension. Age, male gender, diabetes, obesity and having one or more comorbidities were independently associated with laboratory-confirmed COVID-19. Current smokers were 23% less likely to be diagnosed with COVID-19 compared to non-smokers. Of all COVID-19 patients, 34.8% were hospitalized and 13.0% experienced an adverse outcome. Male gender, older age, having one or more comorbidities, and chronic renal disease, diabetes, obesity, COPD, immunosuppression and hypertension were associated with hospitalization and adverse outcome. *Current smoking was not associated with adverse outcome*. **Conclusion:** This largest ever case series of COVID-19 patients identified risk factors for COVID-19 diagnosis, hospitalization and adverse outcome. The findings could provide insight for the priorities the need to be set, especially by LMICs, to tackle the pandemic. [**note: here is a very large population analysis from Mexico. As with some other studies, current smoking was not associated with adverse outcome. Why this is so in light of the Icelandic data above, confounds me (but there is a lot about this virus that puzzles me).**] https://www.medrxiv.org/content/10.1101/2020.06.04.20122481v1

- We analyzed 184 patients hospitalized for Covid-19 in Livingston, New Jersey for clinical characteristics associated with severe disease. Results: The majority of Covid-19 patients had diabetes mellitus (DM) (62.0%), Pre-DM (23.9%) with elevated FBG, or a BMI > 30 with normal HbA1C (4.3%). SARS-CoV-2 infection was associated with new and persistent hyperglycemia in 29 patients, including several with normal HbA1C levels. Forty-four patients required intubation, which occurred significantly more often in patients with DM as compared to non-diabetics. Conclusions: Severe Covid-19 occurs in the presence of impaired glucose metabolism in patients with SARS-CoV-2 infection. The association of dysregulated glucose metabolism and severe Covid-19 suggests a previously unrecognized manifestation of primary SARS-CoV-2 infection. Exploration of pathways by which SARS-CoV-2 impacts glucose metabolism is critical for understanding disease pathogenesis and developing therapies. [**note: a further sub-analysis of diabetic associated severe COVID-19.N]**
  https://www.medrxiv.org/content/10.1101/2020.06.04.20122507v1
- To date, there have been reports of neurologic manifestations in Covid-19 patients including ischemic strokes, Guillain-Barre Syndrome and anosmia. In this case report, we report a patient who presented with dysosmia, dysgeusia along with monocular peripheral vision loss after being diagnosed with Covid-19. [**note: it is only a one patient case report but may be worrying if this begins to show up more frequently.**]
  https://www.medrxiv.org/content/10.1101/2020.06.03.20112540v1
- Background A high proportion of COVID-19 patients develop acute liver dysfunction. Early research has suggested that pre-existing fatty liver disease may be a significant risk factor for hospitalisation. Liver fat, in particular, is a modifiable parameter and can be a target for public health policy and individual patient plans. In this study we aimed to assess pre-existing liver disease as a risk factor for developing symptomatic COVID-19. Methods From 502,506 participants from the UK Biobank, 42,146 underwent MRI (aged 45-82), and had measures of liver fat, liver fibroinflammatory disease and liver iron. Patients were censored on May 28th to determine how many had tested for COVID-19 with symptomatic disease. UK testing was restricted to those with symptoms in hospital. COVID-19 symptoms included fever, dry cough,

sore throat, diarrhoea and fatigue. Univariate analysis was performed on liver phenotypic biomarkers to determine if these variables increased risk of symptomatic COVID-19, and compared to previously described risk factors associated with severe COVID-19, including to age, ethnicity, gender and obesity, Findings Increased liver fat was associated with a higher risk for symptomatic confirmed COVID-19 in this population in univariate analysis(OR:1.85, p=0.03). In obese participants, only those with concomitant fatty liver(≥10%) were at increased risk(OR:2.96, p=0.02), with those having normal liver fat (<5%) showing no increased risk(OR:0.36, p=0.09). Conclusions UK Biobank data demonstrated an association between pre-existing liver disease and obesity with severe COVID-19, with higher proportions of liver fat in obese individuals a likely risk factor for symptomatic disease and severity. Public policy measures to protect patients with liver disease who may have almost double the risk of the general population should be considered, especially as dietary and pharmacological strategies to reduce body weight and liver fat already exist. [**note: in light of the Surgisphere data base associated paper retractions, it is good to know that there are large databases that can be usefully mined for important information!! The UK Biobank is one such data set.**]
https://www.medrxiv.org/content/10.1101/2020.06.04.20122457v1

- An outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been widely spread. We aim to investigate the therapeutic effect of arbidol and moxifloxacin in patients infected with SARS-CoV-2. Methods We collected and analyzed data on 94 patients with COVID-19 including 27 severe patients at the Intensive Care Unit (ICU) and 74 ordinary patients at general isolation ward in Wuhan Xiehe Hospital, from February 15, 2020 to March 15, 2020. All patients were treated with arbidol (100mg each time, three times a day for 14 days) and moxifloxacin (0.4g each time, once a day for 7-14 days). Other data was also collected including demographic data, symptoms, laboratory findings, treatments and clinical outcomes. Results In basic characteristics, compared with the ordinary patients, the severe patients were older (median age was 63.0 years V.S 57.0 years, p=0.03), had higher proportion of hypertension (30% V.S 9%, p=0.03), higher possibility of getting fatigue and/or myalgia (26% V.S 6%, p=0.03), and had more obvious dyspnea symptom (26% V.S 3%, p=0.006). In regarding to laboratory results, we found the severe patients have higher white blood cell counts (p=0.003), neutrophil counts (p=0.007), higher levels of D-dimer (p<0.001), ALT (p<0.001) and AST (p=0.013) than the ordinary patients. After treatment of arbidol and moxifloxacin for one week, the rates of SARS-CoV-2 nucleic acid turning negative were 69.2% in the severe group and 77.8% in the ordinary group. A peculiar phenomenon was that IL-6 stands out among the cytokines in both groups, and higher in severe group than the ordinary one (p=0.011). After treating with arbidol and moxifloxacin for one week, IL-6 decreased significantly in severe group (p=0.023). Conclusion In summary, we proved the treatment of arbidol and moxifloxacin could be helpful in reducing viral load and inflammation during SARS-CoV2 infection, especially for negatively regulating fatal inflammation in severe COVID-19 patients. However, more evidence awaits further clinical verification. [**note: this is the first result paper I've seen on umifenovir (arbidol) and I've not seen any mention of moxifloxacin before. There are a small number of ex-US trials on umifenovir but they all appear to be underpowered so I don't know if any meaningful data will come out.**]
https://www.medrxiv.org/content/10.1101/2020.05.30.20117598v1

- A subset of patients, however, progresses to severe disease and respiratory failure with acute respiratory distress syndrome (ARDS). Severe COVID-19 has been associated with increased neutrophil counts and dysregulated immune responses. The mechanisms of protective immunity in mild forms and the pathogenesis of dysregulated inflammation in severe courses of COVID-19 remain largely unclear. Here, we combined two single-cell RNA-sequencing technologies and single-cell proteomics in whole blood and peripheral blood mononuclear cells (PBMC) to determine changes in immune cell composition and activation in two independent dual-center patient cohorts (n=46 + n=54 COVID-19 samples), each with mild and severe cases of COVID-19. We observed a specific increase of HLA-DR high CD11c high inflammatory monocytes that displayed a strong interferon (IFN)-stimulated gene signature in patients with mild COVID-19, which was absent in severe disease. Instead, we found evidence of emergency myelopoiesis, marked by the occurrence of immunosuppressive pre-neutrophils and immature neutrophils and populations of dysfunctional and suppressive mature neutrophils, as well as suppressive HLA-DR low monocytes in severe COVID-19. Our study provides detailed insights into systemic immune response to SARS-CoV-2 infection and it reveals profound alterations in the peripheral myeloid cell compartment associated with severe courses of COVID-19. [**note; more information on changes to the immune system.**]
  https://www.medrxiv.org/content/10.1101/2020.06.03.20119818v1

DRUG DEVELOPMENT

- Importance Aside from supportive management, there remains no specific treatment for Coronavirus Disease 2019 (COVID-19). Objective: Determine whether ozonated autohemotherapy is associated with a shorter time to clinical improvement in patients with severe COVID-19 pneumonia. Design: Single-center proof-of-concept prospective cohort study. Setting: Internal Medicine ward at Policlinica Ibiza Hospital, Spain. Participants: Eighteen consecutive patients with laboratory confirmed COVID-19 infection and severe pneumonia who were admitted to hospital between 20th March and 19th April 2020. Exposures: Patients in the ozonated autohemotherapy arm received hemotherapy twice daily starting on the day of admission for a median of 4 days. Each treatment involved administration of 200 mL autologous whole blood enriched with 200 mL of oxygen-ozone mixture with a 40 µg/mL ozone concentration. Patients in the control arm received supportive care. Assignment to hemotherapy versus usual care was determined based on the admitting physician on the day of admission, with only one of the three possible physicians prescribing ozonated autohemotherapy Main Outcomes: The primary outcome was time from hospital admission to clinical improvement, which was defined as either hospital discharge or a two-point improvement in clinical status measured on a six-point ordinal scale. Secondary outcomes were clinical improvement measured on the 7th, 14th and 28th day after admission, as well as time to a 2-fold reduction in concentrations of C-protein reactive, ferritin, D-dimer and lactate dehydrogenase. Results: The mean age of the cohort was 68 y and 72% (n=13) were male. Nine patients (50%) received ozonated autohemotherapy beginning on the day of admission. In unadjusted comparisons, ozonated autohemotherapy was associated with significantly shorter time to clinical improvement (median [IQR]), 7 days [6-10] vs 28 days [8-31], p=0.04) and significantly higher proportion of patients achieving 14-day clinical improvement (88.8% vs 33.3%, p=0.01). In risk-adjusted analyses, ozonated autohemotherapy was associated with a

shorter mean time to clinical improvement (-11.3 days, p=0.04, 95% CI -22.25 to -0.42). Conclusions and Relevance: Ozonated autohemotherapy was associated with a significantly shorter time to clinical improvement in this prospective cohort study. Given the small sample size and single-center study design, these observations require evaluation in larger randomized controlled trials. [**note: through out this whole newsletter enterprise, I am often reminded of the famous Hunter S. Thompson quote, "When the going gets weird, the weird turn pro." Why not try something like this when there are no really good therapies. There are some who swear by inhaled hydrogen peroxide and you can DIY (THIS IS BY NO MEANS A MEDICAL RECOMMENDATION – IT IS ONLY A PUBLIC SERVICE TO SHOW THAT PATENT MEDICINE IS STILL ALIVE IN THE 21st CENTURY). I don't know of any peroxide clinical trial but given the weirdness of some recently registered trials, I won't be surprised if someone dose one. Isn't it amazing what knowledge is imparted through these newsletters?**]
https://www.medrxiv.org/content/10.1101/2020.06.03.20117994v1

- G-quadruplex, one of the non-canonical secondary structures, has shown potential antiviral values. However, little is known about G-quadruplexes on the emerging SARS-CoV-2. Herein, we characterized the potential G-quadruplexes both in the positive and negative-sense viral stands. The identified potential G-quadruplexes exhibits similar features to the G-quadruplexes detected in the human transcriptome. Within some bat and pangolin related beta coronaviruses, the G-quartets rather than the loops are under heightened selective constraints. We also found that the SUD-like sequence is retained in the SARS-CoV-2 genome, while some other coronaviruses that can infect humans are depleted. Further analysis revealed that the SARS-CoV-2 SUD-like sequence is almost conserved among 16,466 SARS-CoV-2 samples. And the SARS-CoV-2 SUDcore-like dimer displayed similar electrostatic potential pattern to the SUD dimer. Considering the potential value of G-quadruplexes to serve as targets in antiviral strategy, we hope our fundamental research could provide new insights for the SARS-CoV-2 drug discovery. [n**ote: a new target for drug development.**]
https://www.biorxiv.org/content/10.1101/2020.06.05.135749v1
- There are no known cures or vaccines for COVID-19, the defining pandemic of this era. Animal models are essential to fast track new interventions and nonhuman primate (NHP) models of other infectious diseases have proven extremely valuable. Here we compare SARS-CoV-2 infection in three species of experimentally infected NHPs (rhesus macaques, baboons, and marmosets). During the first 3 days, macaques developed clinical signatures of viral infection and systemic inflammation, coupled with early evidence of viral replication and mild-to-moderate interstitial and alveolar pneumonitis, as well as extra-pulmonary pathologies. Cone-beam CT scans showed evidence of moderate pneumonia, which progressed over 3 days. Longitudinal studies showed that while both young and old macaques developed early signs of COVID-19, both groups recovered within a two-week period. Recovery was characterized by low-levels of viral persistence in the lung, suggesting mechanisms by which individuals with compromised immune systems may be susceptible to prolonged and progressive COVID-19. The lung compartment contained a complex early inflammatory milieu with an influx of innate and adaptive immune cells, particularly interstitial macrophages, neutrophils and plasmacytoid dendritic cells, and a prominent Type I-interferon response. While macaques developed moderate disease, baboons exhibited prolonged shedding of virus and extensive pathology following infection; and marmosets demonstrated a milder form of infection. These results

showcase in critical detail, the robust early cellular immune responses to SARS-CoV-2 infection, which are not sterilizing and likely impact development of antibody responses. Thus, various NHP genera recapitulate heterogeneous progression of COVID-19. Rhesus macaques and baboons develop different, quantifiable disease attributes making them immediately available essential models to test new vaccines and therapies. [**note: another paper that the folks at PETA won't like; more work on animal models for SARS-CoV-2 infection.**] https://www.biorxiv.org/content/10.1101/2020.06.05.136481v1

- COVID-19 is a severe acute respiratory disease caused by SARS-CoV-2, a novel betacoronavirus discovered in December 2019 and closely related to the SARS coronavirus (CoV). Both viruses use the human ACE2 receptor for cell entry, recognizing it with the Receptor Binding Domain (RBD) of the S1 subunit of the viral spike (S) protein. The S2 domain mediates viral fusion with the host cell membrane. Experience with SARS and MERS coronavirus has shown that potent monoclonal neutralizing antibodies against the RBD can inhibit the interaction with the virus cellular receptor (ACE2 for SARS) and block the virus cell entry. Assuming that a similar strategy would be successful against SARS-CoV-2, we used phage display to select from the human naive universal antibody gene libraries HAL9/10 anti SARS2 spike antibodies capable of inhibiting interaction with ACE2. 309 unique fully human antibodies against S1 were identified. 17 showed more than 75% inhibition of spike binding to cells expressing ACE2, assessed by flow cytometry and several antibodies showed even an 50% inhibition at a molar ratio of the antibody to spike protein or RBD of 1:1. Furthermore, these antibodies neutralized active SARS-Cov-2 virus infection of VeroE6 cells. All 17 were all able to bind the isolated RBD, four of them with sub-nanomolar EC50. Epitope analysis of the antibodies revealed that six bind at the RBD-ACE2 interface and two on the opposite side of the domain. Universal libraries from healthy donors offer the advantage that antibodies can be generated quickly and independent from the availability of material from recovered patients in a pandemic situation. [**note: good work from these German researchers on another way to identify neutralizing antibodies to SARS-CoV-2.**] https://www.biorxiv.org/content/10.1101/2020.06.05.135921v1
- The National Center for Advancing Translational Sciences (NCATS) has developed an online open science data portal for its COVID-19 drug repurposing campaign, named OpenData, with the goal of making data across a range of SARS-CoV-2 related assays available in real-time. The assays developed cover a wide spectrum of the SARS-CoV-2 life cycle, including both viral and human (host) targets. In total, over 10,000 compounds are being tested in full concentration-response ranges from across multiple annotated small molecule libraries, including approved drug, repurposing candidates and experimental therapeutics designed to modulate a wide range of cellular targets. The goal is to support research scientists, clinical investigators and public health officials through open data sharing and analysis tools to expedite the development of SARS-CoV-2 interventions, and to prioritize promising compounds and repurposed drugs for further development in treating COVID-19. [**note: not to be critical (but I will!), this is a good effort and should have been up and running two months ago. One has to wonder whether it will serve any utility right now. It certainly can go down in the "lessons-learned" category as something to keep up and running to aid drug development collaboration for the future.**] https://www.biorxiv.org/content/10.1101/2020.06.04.135046v1
- To identify drugs that are potentially used for the treatment of COVID-19, the potency of 1403 FDA-approved drugs were evaluated using a robust pseudovirus assay and the candidates were

further confirmed by authentic SARS-CoV-2 assay. Four compounds, Clomiphene (citrate), Vortioxetine, Vortioxetine (hydrobromide) and Asenapine (hydrochloride), showed potent inhibitory effects in both pseudovirus and authentic virus assay. The combination of Clomiphene (citrate), Vortioxetine and Asenapine (hydrochloride) is much more potent than used alone, with IC50 of 0.34 μM. [**note: what a strange set of drugs, a fertility agents, an antidepressant and an anti-psychotic. While the mixture of the three has a great IC50, one wonders what the side effect profile of the cocktail might be. Any bets on when a clinical trial will be started?**] https://www.biorxiv.org/content/10.1101/2020.06.05.135996v1

DIAGNOSTIC DEVELOPMENT

- The aim of this study is to evaluate if the sweat produced by COVID-19 persons (SARS-CoV-2 PCR positive) has a different odour for trained detection dogs than the sweat produced by non COVID-19 persons. The study was conducted on 3 sites, following the same protocol procedures, and involved a total of 18 dogs. A total of 198 armpits sweat samples were obtained from different hospitals. For each involved dog, the acquisition of the specific odour of COVID-19 sweat samples required from one to four hours, with an amount of positive samples sniffing ranging from four to ten. For this proof of concept, we kept 8 dogs of the initial group (explosive detection dogs and colon cancer detection dogs), who performed a total of 368 trials, and will include the other dogs in our future studies as their adaptation to samples scenting takes more time. The percentages of success of the dogs to find the positive sample in a line containing several other negative samples or mocks (2 to 6) were 100p100 for 4 dogs, and respectively 83p100, 84p100, 90p100 and 94p100 for the others, all significantly different from the percentage of success that would be obtained by chance alone. We conclude that there is a very high evidence that the armpits sweat odour of COVID-19+ persons is different, and that dogs can detect a person infected by the SARS-CoV-2 virus. [**note: you have to hand it to the French for out of the box thinking. Perhaps we have all be 'barking' up the wrong tree in looking for quick diagnostics. Let's speed up the training of dogs to do the sentinel research. Hey, the White House can do away with the nasal swabs and have a good pet at that same time!**] https://www.biorxiv.org/content/10.1101/2020.06.03.132134v1
- Antibodies testing in the coronavirus era is frequently promoted, but the underlying statistics behind their validation has come under more scrutiny in recent weeks. We provide calculations, interpretations, and plots of positive and negative predictive values under a variety of scenarios. Prevalence, sensitivity, and specificity are estimated within ranges of values from researchers and antibodies manufacturers. Illustrative examples are highlighted, and interactive plots are provided in the Supplementary Material. Implications are discussed for society overall and across diverse locations with different levels of disease burden. Specifically, the proportion of positive serology tests that are false can differ drastically from up to 3% to 88% for people from different places with different proportions of infected people in the populations while the false negative rate is typically under 10%. [**note: unsurprising and related to one of the commentaries from the NEJM that I posted above. A 'perfect' test is not required for field epidemiology work, as long as the false positive statistics are known and disclosed.**] https://www.medrxiv.org/content/10.1101/2020.06.04.20122358v1
- The advent of the COVID-19 pandemic in the United States created a unique situation where multiple molecular diagnostic assays with various indications for use in the detection of SARS-

CoV-2 rapidly received Emergency Use Authorization by the FDA, were validated by laboratories and utilized clinically, all within a period of a few weeks. We compared the performance of four of these assays that were being evaluated for use at our institution: Abbott RealTime m2000 SARS-CoV-2 Assay, DiaSorin Simplexa COVID-19 Direct, Cepheid Xpert Xpress SARS-CoV-2 and Abbott ID NOW COVID-19. Nasopharyngeal and nasal specimens were collected from 88 ED and hospital-admitted patients and tested by the four methods in parallel to compare performance. ID NOW performance stood out as significantly worse than the other three assays despite demonstrating comparable analytic sensitivity. Further study determined that the use of a foam nasal swab compared to a nylon flocked nasopharyngeal swab, as well as use in a population chronically vs. acutely positive for SARS-CoV-2, were significant factors in the poor comparable performance. [**note: a further comparison of RT-PCR assays shows the Abbott quick test does not perform as well and that the type of nasal swab impacts test performance.**] https://www.biorxiv.org/content/10.1101/2020.06.04.135616v1 and more on the Abbott test here: https://www.medrxiv.org/content/10.1101/2020.06.03.20116327v1 similar data showing the need for independent validation and using a second test as necessary.

- The recently emerged coronavirus disease COVID-19 has now evolved into a global pandemic. Early detection is crucial for its effective control. Nucleic acid testing for viral pathogen and serological testing for host antibodies are playing important roles in current COVID-19 diagnosis. However, while nucleic acid testing is complicated, facility-restricted and time-consuming, antibody testing may result in high rates of false-negative diagnoses, especially during the early stages of viral infection. Thus, a more rapid and reliable test for both early COVID-19 diagnosis and whole-population screening is urgently needed. Here, we developed a novel nanozyme-based chemiluminescence paper assay for rapid and high-sensitive testing of SARS-CoV-2 spike antigen. Our paper test uses a newly established peroxidase-mimic Co-Fe@hemin nanozyme instead of natural HRP that catalytically amplifies the chemiluminescent signal, allowing for target concentrations to be as low as 0.1 ng/ml. Furthermore, our nanozyme-based chemiluminescence test exhibits a linear range that is 32-fold wider compared to ELISA tests. Importantly, testing is completed in less than 16 min, compared to 1-2 h required for ELISA or nucleic acid tests. Critically, signal detection is feasible using a smartphone camera. Ingredients for our test are simple and readily available, rendering overall cost considerably lower than those used in current diagnoses. In conclusion, our novel test provides a high-sensitive, rapid and convenient approach for SARS-CoV-2 antigen detection, which should greatly increase current early screening capacities for suspected infections, and considerably lower demand for national healthcare resources. [**note: way cool new diagnostic test from China. I have a smart phone!!! Now that I have rebuilt my website, I have time for app development. Send me some test strips and I'll do some neighborhood field studies.**] https://www.biorxiv.org/content/10.1101/2020.06.05.131748v1
- To date, there are limited data available on the analytical performance of emerging commercially available assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and integration of these assays into laboratory workflows. Here, we performed a prospective validation study of a commercially available assay, the AusDiagnostics Coronavirus Typing (8-well) assay. Respiratory tract samples for SARS-CoV-2 testing were collected between 1st March and 25th March 2020. All positive samples and a random subset of negative samples were sent to a reference laboratory for confirmation. In total, 2,673 samples were analyzed using the

Coronavirus Typing assay. The predominant sample type was a combined nasopharyngeal/throat swab (2,640/2,673; 98.8%). Fifty-four patients were positive for SARS-CoV-2 (0.02%) using the Coronavirus Typing assay; 53/54 (98.1%) positive results and 621/621 (100%) negative results were concordant with the reference laboratory. Compared to the reference standard, sensitivity of the Coronavirus Typing assay for SARS-CoV-2 was 100% [95% CI 93.2%-100%], specificity 99.8% [95% CI 99.1%-100%], positive predictive value 98.1% (95% CI 90.2%-99.7%] and negative predictive value 100% [95% CI 99.4%-100%]. In many countries, standard regulatory requirements for the introduction of new assays have been replaced by emergency authorizations and it is critical that laboratories share their post-market validation experiences, as the consequences of widespread introduction of a sub-optimal assay for SARS-CoV-2 are profound. *Here, we share our in-field experience, and encourage other laboratories to follow suit.* [**note: you will get no argument from me about the need to share validation experiences!!!**] https://www.medrxiv.org/content/10.1101/2020.06.03.20117267v1

- Detection platforms that are efficient, rapid, accurate, specific, sensitive, and user friendly are urgently needed to manage and control the spread of SARS-CoV-2. RT-qPCR based methods are the gold standard for SARS-CoV-2 detection. However, these methods require trained personnel, sophisticated infrastructure, and a long turnaround time, thereby limiting their usefulness. Reverse transcription-loop-mediated isothermal amplification (RT-LAMP), a one-step nucleic acid amplification method conducted at a single temperature, has been used for colorimetric virus detection. CRISPR-Cas12 and CRISPR-Cas13 systems, which possess collateral activity against ssDNA and RNA, respectively, have also been harnessed for virus detection. Here, we built an efficient, rapid, specific, sensitive, user-friendly SARS-CoV-2 detection module that combines the robust virus amplification of RT-LAMP with the specific detection ability of SARS-CoV-2 by CRISPR-Cas12. Furthermore, we combined the RT-LAMP-CRISPR-Cas12 module with lateral flow cells to enable highly efficient point-of-care SARS-CoV-2 detection. Our iSCAN SARS-CoV-2 detection module, which exhibits the critical features of a robust molecular diagnostic device, should facilitate the effective management and control of COVID-19. [**note: good stuff from this Saudi group!]**
  https://www.medrxiv.org/content/10.1101/2020.06.02.20117739v1

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2020-06-07

For reflection Sunday I will turn again to Mahler for what is one of my favorite symphonic pieces 'Das Lied von der Erde.' I always find something new in this piece and have maybe 10 recordings. Perhaps the best interpretation is Bruno Walter's 1952 recording with Kathleen Ferrier and Julius Patzak. I don't think anyone has sung the final song as well as Ferrier who died of cancer at the young age of 41 about one year after the recording was made. Walter conducted the premier of the piece in 1909. For a video performance, here is the Toronto Symphony under the direction of Peter Oundijian with soloists, Michael Schade and Susan Platts: https://www.youtube.com/watch?v=R81yPON9IHI

One of my loyal readers points to this Guardian article on monoclonal antibodies. There is no question that mAbs represent the best approach to therapy and results coming out on the use of sera from recovered individuals that is enriched for neutralizing antibodies substantiates this. I have covered this issue in the past and Derek Lowe has also written a couple of blogs on it. Lilly has an mAb in trials right now and we need more of this scale up going on.

Here is an interesting piece by Eduardo Porter in The New York Times about the use of targeted closings and reopenings. This accords with my thinking *as long as there is a public health infrastructure to respond quickly to new outbreaks.*

Masking up is a generally accepted practice in Japan and it works! For those of us living in the US and seeing the reopening of many shops, it is important to remember the 4Cs, *close contact, confined spaces, crowds and choices* as we begin to start getting back to a sense of normalcy.

Finally, although she doesn't read the newsletters, I would like to wish a happy birthday to my wife!!! As we say in our family, "…it's her happy day…!"

MODELING

- Current models for flu-like epidemics insufficiently explain multi-cycle seasonality. Meteorological factors alone do not predict seasonality, given substantial climate differences between countries that are subject to flu-like epidemics or COVID-19. Pollen is documented to be antiviral and allergenic, play a role in immuno-activation, and seems to create a bio-aerosol lowering the reproduction number of flu-like viruses. Therefore, we hypothesize that pollen may explain the seasonality of flu-like epidemics including COVID-19. We tested the Pollen-Flu Seasonality Theory for 2016-2020 flu-like seasons, including COVID-19, in The Netherlands with its 17 million inhabitants. We combined changes in flu-like incidence per 100K/Dutch citizens (code: ILI) with weekly pollen counts and meteorological data for the same period. Finally, a discrete, predictive model is tested using pollen and meteorological threshold values displaying inhibitory effects on flu-like incidence. We found a highly significant inverse association of $r(224)= -.38$ between pollen and changes in flu-like incidence corrected for incubation period, confirming our expectations for the 2019/2020 COVID-19 season. We found that our predictive model has the highest inverse correlation with changes in flu-like incidence of $r(222) = -.48$ ($p < .001$) when pollen thresholds of 610 total pollen grains/m3 per week, 120 allergenic pollen grains/m3 per week, and a solar radiation threshold of 510 J/cm2 are passed. The passing of at least the pollen thresholds, preludes the beginning and end of flu-like seasons. Solar radiation is a supportive factor, temperature makes no difference, and relative humidity associates even with flu-like incidence increases. We conclude that pollen is a predictor for the inverse seasonality of flu-like epidemics including COVID-19, and solar radiation is a co-inhibitor. The observed seasonality of COVID-19 during Spring, suggests that COVID-19 may revive in The Netherlands after week 33, the start being preceded by the relative absence of pollen, and follows standard pollen-flu seasonality patterns. [**note: great news for all you seasonal allergy sufferers!! According to this Dutch model, pollen allergies may be protective against SARS-**

CoV-2 infection. Had I known this, I would have been collecting oak pollen all spring long and taken a quick sniff every day (is another patent application in the offing???). I still think high production of mucopolysaccharides may be protective and this would of course explain the pollen protection. I still want someone to do *in vitro* testing of my nasal secretions. Maybe I am the cure!] https://www.medrxiv.org/content/10.1101/2020.06.05.20123133v1

- Wastewater-based epidemiology can be a powerful tool to understand the actual incidence of coronavirus disease 2019 (COVID-19) in a community because severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19, can be shed in the feces of infected individuals regardless of their symptoms. The present study aimed to assess the presence of SARS-CoV-2 RNA in wastewater and river water in Yamanashi Prefecture, Japan, using four quantitative and two nested PCR assays. Influent and secondary-treated (before chlorination) wastewater samples and river water samples were collected five times from a wastewater treatment plant and three times from a river, respectively, between March 17 and May 7, 2020. The wastewater and river water samples (200-5,000 mL) were processed by using two different methods: the electronegative membrane-vortex (EMV) method and the membrane adsorption-direct RNA extraction method. Based on the observed concentrations of indigenous pepper mild mottle virus RNA, the EMV method was found superior to the membrane adsorption-direct RNA extraction method. SARS-CoV-2 RNA was successfully detected in one of five secondary-treated wastewater samples with a concentration of 2.4×10^3 copies/L by N_Sarbeco qPCR assay following the EMV method, whereas all the influent samples were tested negative for SARS-CoV-2 RNA. This result could be attributed to higher limit of detection for influent (4.0×10^3-8.2×10^4 copies/L) with a lower filtration volume (200 mL) compared to that for secondary-treated wastewater (1.4×10^2-2.5×10^3 copies/L) with a higher filtration volume of 5,000 mL. None of the river water samples tested positive for SARS-CoV-2 RNA. Comparison with the reported COVID-19 cases in Yamanashi Prefecture showed that SARS-CoV-2 RNA was detected in the secondary-treated wastewater sample when the cases peaked in the community. This is the first study reporting the detection of SARS-CoV-2 RNA in wastewater in Japan. [**note: first report on wastewater screening from Japan. They compared wastewater to river water and unsurprisingly did not find see any positive viral assays from the latter. It was good to do that control!**]
  https://www.medrxiv.org/content/10.1101/2020.06.04.20122747v1
- Background: Covid-19 seroprevalence rates and serological tests are important tools in understanding the epidemiology of the disease and help in the fight against it. Seroprevalence rates vary according to the population studied and the test employed and they range from 0.133 to 25.7%. The purpose of this study is to assess the seroprevalence rate in a population of healthy blood donors living under strict lockdown measures in Jordan which has in total 71 confirmed cases per million population. Methods: Left over sera and plasma samples from 746 healthy blood donors were tested using a commercially available FDA approved kit having a specificity of 100%. External positive controls were used for validation. Results: More than 80% of the donors were men 18-63 year old and residing in the capital city of Jordan, Amman. All tested specimens were negative yielding a zero seroprevalence rate in this healthy blood donor population. Conclusion: Strict lockdown measures effectively limit intracommunity spread of the infection, however at the cost of lack of any acquired community immunity. Additionally the use of highly specific test is recommended in low prevalence setting. [**note: a predictable study**

result from Jordan (this may be that country's first citation in the newsletter!) on negative serology findings in lockdowned blood donors. It is still good to do these studies for confirmation purposes.] https://www.medrxiv.org/content/10.1101/2020.06.06.20123919v1

- Background: Contact tracing and lockdown are health policies being used worldwide to combat the coronavirus (COVID-19). While easing lockdown, the UK National Health Service (NHS) launched its Track and Trace Service at the end of May 2020, and aims by end of June 2020 also to include app-based notification and advice to self-isolate for those who have been in contact with a person known to have COVID-19. To be successful, such an app will require high uptake, the determinants and willingness for which are unclear but essential to understand for effective public health benefit. Objectives: To measure the determinants of willingness to participate in an NHS app-based contact tracing programme using a questionnaire within the Care Information Exchange (CIE) - the largest patient-facing electronic health record in the NHS. Methods: Observational study of 47,708 registered NHS users of the CIE, 27% of whom completed a novel questionnaire asking about willingness to participate in app-based contact tracing, understanding of government advice, mental and physical wellbeing and their healthcare utilisation -- related or not to COVID-19. Descriptive statistics are reported alongside univariate and multivariable logistic regression models, with positive or negative responses to a question on app-based contact tracing as the dependent variable. Results: 26.1% of all CIE participants were included in the analysis (N = 12,434, 43.0% male, mean age 55.2). 60.3% of respondents were willing to participate in app-based contact tracing. Out of those who responded "no", 67.2% stated that this was due to privacy concerns. In univariate analysis, worsening mood, fear and anxiety in relation to changes in government rules around lockdown were associated with lower willingness to participate. Multivariable analysis showed that difficulty understanding government rules was associated with a decreased inclination to download the app, with those scoring 1-2 and 3-4 in their understanding of the new government rules being 45% and 27% less inclined to download the contact tracing app, respectively; when compared to those who rated their understanding as 5-6/10 (OR for 1-2/10 = 0.57 [CI 0.48 - 0.67]; OR for 3-4/10 = 0.744 [CI 0.64 - 0.87]), whereas scores of 7-8 and 9-10 showed a 43% and 31% respective increase. Those reporting an unconfirmed belief of having previously had and recovered from COVID-19 were 27% less likely to be willing to download the app; belief of previous recovery from COVID-19 infection OR 0.727 [0.585 - 0.908]). Conclusions: In this large UK-wide questionnaire of wellbeing in lockdown, a willingness for app-based contact tracing is 60% - close to the estimated 56% population uptake, and substantially less than the smartphone-user uptake considered necessary for an app-based contact-tracing to be an effective intervention to help suppress an epidemic. Given this marginal level of support over an appropriate age range, the impacts of difficulty comprehending government advice and a policy of not testing to confirm self-reported COVID-19 infection during lockdown indicate that uncertainty in communication and diagnosis in adopted public health policies will negatively impact the effectiveness of a government contact tracing app. [**note: I find this very disappointing. We need to employ technology to help further public health. If apps are rejected, it means that more people will have to do the grunt work of surveillance. I have noted before that the privacy issues can be satisfactorily addressed but It may be that there is an increasing lack of communitarian belief that we are all in this together.**] https://www.medrxiv.org/content/10.1101/2020.06.03.20120337v1

NEWLY REGISTERED CLINICAL TRIALS

- Opaganib, a sphingosine kinase-2 (SphK2) inhibitor, has been broadly tested in Phase I/II studies. Extensive nonclinical data indicates both anti-viral and anti-inflammatory activity via selective SphK2 inhibition which may prove beneficial for treating COVID-19 infection and resulting pneumonia. This proof of concept study will take place in the US and will enroll about 40 hospitalized patients diagnosed with COVID-19 infection who have developed pneumonia and require supplemental oxygen. Half of the patients, i.e. 20 patients, will receive opaganib in addition to standard of care for 14 days. The other 20 will receive matching placebo (capsules that do not contain the medication) in addition to standard of care. Study drug will be administered every day for 14 days, twice each day, unless the patient has been discharged from the hospital without requiring supplemental oxygen, in which case study drug will only be administered for 10 days. All participants will be followed up for 4 weeks after their last dose of study drug. [**note: interesting molecular structure but is just a developmental drug for several types of cancer. Trial is sponsored by RedHill Biopharma, a company new to me. Some of you may remember June Almenoff from GSK; she was on the PhRMA Drug Safety committee and is the chief scientific officer.]** NCT04414618

CLINICAL TRIAL RESULTS

- Objective: To measure heart rate variability metrics in critically ill COVID-19 patients with comparison to all-cause critically ill sepsis patients. Design and patients: Retrospective analysis of COVID-19 patients admitted to an ICU for at least 24h at any of Emory Healthcare ICUs between March and April 2020. The comparison group was a cohort of all-cause sepsis patients prior to COVID-19 pandemic. Interventions: none. Measurements: Continuous waveforms were captured from the patient monitor. The EKG was then analyzed for each patient over a 300 second (s) observational window, that was shifted by 30s in each iteration from admission till discharge. A total of 23 HRV metrics were extracted in each iteration. We use the Kruskal-Wallis and Steel-Dwass tests ($p < 0.05$) for statistical analysis and interpretations of HRV multiple measures. Results: A total of 141 critically-ill COVID-19 patients met inclusion criteria, who were compared to 208 patients with all-cause sepsis. Demographic parameters were similar apart from a high proportion of African-Americans in the COVID-19 cohort. Three non-linear markers, including SD1:SD2, sample entropy, approximate entropy and four linear features mode of Beat-to-Beat interval (NN), Acceleration Capacity (AC), Deceleration Capacity (DC), and pNN50, were statistical significance between more than one binary combinations of the sub-groups (comparing survivors and non-survivors in both the COVID-19 and sepsis cohorts). The three nonlinear features and AC, DC, and NN (mode) were statistically significant across all four combinations. Temporal analysis of the main markers showed low variability across the 5 days of analysis, compared with sepsis patients. Conclusions: Heart rate variability is broadly implicated across patients infected with SARS-CoV-2, and admitted to the ICU for critical illness. Comparing these metrics to patients with all-cause sepsis suggests a unique set of expressions that differentiate this viral phenotype. This finding could be investigated further as a potential biomarker to predict poor outcome in this patient population, and could also be a starting point to measure potential autonomic dysfunction in COVID-19. [**note: from Emory University, heart rate variability metrics in critically ill COVID-19 patients. Patients were compared to those with all-cause sepsis and showed a different set of expressions.**]
https://www.medrxiv.org/content/10.1101/2020.06.05.20123752v1

- Objective: To describe the symptom course in outpatients with coronavirus disease 2019 (COVID-19). Design: Retrospective chart review of standardized symptom checklist for patients followed at home by telephone calls during their acute COVID-19 illness. Compile results by day of illness into a single heatmap representation of symptoms. Setting: COVID-19 Virtual Outpatient Management Clinic (VOMC) in Atlanta, Georgia; a practice that follows patients with mild COVID-19 at home. Participants: 272 patients with confirmed COVID-19 by nasopharyngeal PCR, who presented to the VOMC within 10 days of symptom onset and within 5 days of screening PCR test. Main outcome measure: Each symptom is recorded as yes/no for each patient on each day. The total number of yes replies is divided by the total number of patients in VOMC to generate a result for each cell in the heatmap. Patients admitted to the hospital are censored from the denominator. Results: The mean duration of follow-up was 20.2 days. The most commonly reported symptoms in the course of illness were cough (83%), headache (73%) loss of smell or taste (71%), sinus congestion (71%), and body ache (67%). Symptoms remained common at 3 weeks, including cough (41%), shortness of breath on exertion (24%), loss of smell or taste (23%), sinus congestion (23%), and headache (20%). Conclusions: Symptoms of acute COVID-19 frequently last longer than the minimum duration of isolation and patients and healthcare providers should be aware that symptom resolution may be gradual. [**note: more good work from Emory. I've seen very little focus on outpatient COVID-19 resolution and this paper highlights some important items. Most of the viral clearance data we have is from hospital patients and it would be good to get similar data from the community. Perhaps saliva collection and testing is the way to go as I doubt nasal swabbing can be done by non-trained people in the community.**]
  https://www.medrxiv.org/content/10.1101/2020.06.05.20123471v1
- Background. During the COVID-19 outbreak, reports have surfaced of children who present with features of a multisystem inflammatory syndrome with overlapping features of Kawasaki disease and toxic shock syndrome - Paediatric Inflammatory Multisystem Syndrome- temporally associated with SARS-CoV-2 pandemic (PIMS-TS). Initial reports find that many of the children are PCR-negative for SARS-CoV-2, so it is difficult to confirm whether this syndrome is a late complication of viral infection in an age group largely spared the worst consequences of this infection, or if this syndrome reflects enhanced surveillance. Methods. Children hospitalised for symptoms consistent with PIMS-TS between 28 April and 8 May 2020, and who were PCR-negative for SARS-CoV-2, were tested for antibodies to viral spike glycoprotein using an ELISA test. Results. Eight patients (age range 7-14 years, 63% male) fulfilled case-definition for PIMS-TS during the study period. Six of the eight patients required admission to intensive care. All patients exhibited significant IgG and IgA responses to viral spike glycoprotein. Further assessment showed that the IgG isotypes detected in children with PIMS-TS were of the IgG1 and IgG3 subclasses, a distribution similar to that observed in samples from hospitalised adult COVID-19 patients. In contrast, IgG2 and IgG4 were not detected in children or adults. IgM was not detected in children, which contrasts with adult hospitalised adult COVID-19 patients of whom all had positive IgM responses. Conclusions. Strong IgG antibody responses can be detected in PCR-negative children with PIMS-TS. The low detection rate of IgM in these patients is consistent with infection having occurred weeks previously and that the syndrome onset occurs well after the control of SARS-CoV-2 viral load. This implies that the disease is largely immune-mediated. Lastly, this indicates that serology can be an appropriate diagnostic tool in

select patient groups. [**note: this is a fascinating clinical result as the children come down with COVID-19 inflammatory syndrome well after the virus has been cleared by the immune system. Unfortunately, it points to some long lasting impacts on the immune system that are not fully understood and one should not sanguinely assume that the young are resilient in terms of their immune systems.**]
https://www.medrxiv.org/content/10.1101/2020.06.05.20123117v1

DRUG DEVELOPMENT

- Coronaviruses (CoV) are a large family of enveloped, RNA viruses that circulate in mammals and birds but have crossed the species barrier to infect humans seven times. Of these, three pathogenic strains have caused zoonotic infections in humans that result in severe respiratory syndromes including the Middle East Respiratory Syndrome (MERS-CoV), severe acute respiratory syndrome (SARS-CoV), and now SARS-CoV-2 coronaviruses, the latter of which is the cause of the ongoing pandemic of coronavirus disease 2019 (COVID-19). Here, we describe a panel of synthetic monoclonal antibodies, built on a human framework, that bind SARS-CoV-2 spike protein, compete for binding with ACE2, and potently inhibit infection by SARS-CoV-2. These antibodies were found to have a range of neutralization potencies against live virus infection in Vero E6 cells, potently inhibiting authentic SARS-CoV-2 virus at sub-nanomolar concentrations. These antibodies represent strong immunotherapeutic candidates for treatment of COVID-19. [**note: more mAb development, this time from Toronto**]
https://www.biorxiv.org/content/10.1101/2020.06.05.137349v1
- The Spike (S) protein of the virus forms projections on the virion surface responsible for host cell attachment and penetration. This viral glycoprotein is synthesized as a precursor in infected cells and, to be active, must be cleaved to two associated polypeptides: S1 and S2. For SARS-CoV-2 the cleavage is catalysed by furin, a host cell protease, which cleaves the S protein precursor at a specific sequence motif that generates a polybasic Arg-Arg-Ala-Arg (RRAR) C-terminal sequence on S1. This sequence motif conforms to the C-end rule (CendR), which means that the C-terminal sequence may allow the protein to associate with cell surface neuropilin-1 (NRP1) and neuropilin-2 (NRP2) receptors. Here we demonstrate using immunoprecipitation, site-specific mutagenesis, structural modelling, and antibody blockade that, in addition to engaging the known receptor ACE2, S1 can bind to NRP1 through the canonical CendR mechanism. This interaction enhances infection by SARS-CoV-2 in cell culture. NRP1 thus serves as a host factor for SARS-CoV-2 infection, and provides a therapeutic target for COVID-19. [**note: another therapeutic target.**] https://www.biorxiv.org/content/10.1101/2020.06.05.134114v1

DIAGNOSTIC DEVELOPMENT

- Constituting important correlates of protection, determination of virus-neutralizing antibodies (NAbs) is indispensable for convalescent plasma selection, vaccine candidate evaluation, and immunity certificates. In contrast to standard serology ELISAs, plaque reduction neutralization tests (PRNTs) are laborious, time-consuming, expensive, and restricted to specialized laboratories. To replace microscopic counting-based SARS-CoV-2 PRNTs by a novel assay exempt from genetically modified viruses, which are inapplicable in most diagnostics departments, we established a simple, rapid, and automated SARS-CoV-2 neutralization assay employing an in-cell ELISA (icELISA) approach. After optimization of various parameters such as virus-specific

antibodies, cell lines, virus doses, and duration of infection, SARS-CoV-2-infected cells became amenable as direct antigen source for quantitative icELISA. Using commercially available nucleocapsid protein-specific antibodies, viral infection could easily be quantified in human and highly permissive Vero E6 cells by icELISA. Antiviral agents such as human sera containing NAbs or antiviral interferons dose-dependently reduced the SARS-CoV-2-specific signal. Applying increased infectious doses, the icNT was superior to PRNT in discriminating convalescent sera with high from those with intermediate neutralizing capacities. The SARS-CoV-2 icELISA test allows rapid (<48h in total, read-out in seconds) and automated quantification of virus infection in cell culture to evaluate the efficacy of NAbs as well as antiviral drugs, using reagents and equipment present in most routine diagnostics departments. We propose the icELISA and the icNT for COVID-19 research and diagnostics. [**note: from Germany, another improved approach to testing.**] https://www.biorxiv.org/content/10.1101/2020.06.05.135806v1