2020-09-07

Welcome to Week 25 of the Ongoing Pandemic Newsletter

Sinatra, Baryshnikov, and Tharp. What do they all have in common? A great vocalist, a great dancer, and a great choreographer, that's what! Here they are in 'One for My Baby (and One More for the Road): https://www.youtube.com/watch?v=ifoOO5ZEC9g

This New York Times op-ed moans the loss of taste as a result of a COVID-19 infection. I hope she recovers it. To test or not to test; to test at home or not? Tough questions confronted in this article. If you are planning a road trip, these tips may help. In person choral singing is still risky but here is a London choir trying to make a go of it. HERE is the latest draft study of the musical aerosol research that is funded by a number of performing arts organizations.

The Washington Post reports on the rise in home schooling in Quebec as schools reopen during the pandemic. How much Formula 409 (a fine Clorox product) do you use during the normal day to keep ahead of stray SARS-CoV-2 viral particles? When the main form of transmission is aerosol, deep cleaning will make you feel good but likely not reduce your risk of COVID-19 that much. Still as a Clorox shareholder I invite you to purchase some of their very fine cleaning products!

STAT cover how coronavirus may damage the heart.

JAMA have a good article on the development of the leading mRNA COVID-19 vaccines.

How important are second COVID-19 infections? This article from Nature discusses some of the issues and HERE is an update of the vaccine landscape.

Medscape discuss how artificial intelligence (AI) is being used to identify potential COVID-19 therapeutics. While interesting, readers of this newsletter know all about my skepticism here. Many drug leads have been identified via a number of technologies but very few if any have entered clinical trials. Compounds demonstrating *in vitro* activity against SARS-CoV-2 infection may not be active *in vivo*.

MODELING

- Several outbreaks of COVID-19 were associated with seafood markets, raising concerns that fish-attached SARS-CoV-2 may exhibit prolonged survival in low-temperature environments. Here we showed that salmon-attached SARS-CoV-2 at 4oC could remain infectious for more than one week, suggesting that fish-attached SARS-CoV-2 may be a source of transmission. [**note: perhaps sushi is not a good menu choice these days. Cooked salmon should be just fine.**] https://www.biorxiv.org/content/10.1101/2020.09.06.284695v1

NEWLY REGISTERED CLINICAL TRIALS

- Provide updates yesterday

CLINICAL TRIAL RESULTS

- Objectives: The World Health Organisation (WHO) and National Institute for Health and Care Excellence (NICE) recommend various triage tools to assist decision-making for patients with suspected COVID-19. We aimed to estimate the accuracy of triage tools for predicting severe illness in adults presenting to the emergency department (ED) with suspected COVID-19 infection. Methods: We undertook a mixed prospective and retrospective observational cohort study in 70 EDs across the United Kingdom (UK). We collected data from people attending with suspected COVID-19 between 26 March 2020 and 28 May 2020, and used presenting data to determine the results of assessment with the following triage tools: the WHO algorithm, NEWS2, CURB-65, CRB-65, PMEWS and the swine flu adult hospital pathway (SFAHP). We used 30-day outcome data (death or receipt of respiratory, cardiovascular or renal support) to determine prognostic accuracy for adverse outcome. Results: We analysed data from 20892 adults, of whom 4672 (22.4%) died or received organ support (primary outcome), with 2058 (9.9%) receiving organ support and 2614 (12.5%) dying without organ support (secondary outcomes). C-statistics for the primary outcome were: CURB-65 0.75; CRB-65 0.70; PMEWS 0.77; NEWS2 (score) 0.77; NEWS2 (rule) 0.69; SFAHP (6-point) 0.70; SFAHP (7-point) 0.68; WHO algorithm 0.61. All triage tools showed worse prediction for receipt of organ support and better prediction for death without organ support. At the recommended threshold, PMEWS and the WHO criteria showed good sensitivity (0.96 and 0.95 respectively), at the expense of specificity (0.31 and 0.27 respectively). NEWS2 showed similar sensitivity (0.96) and specificity (0.28) when a lower threshold than recommended was used. Conclusion: *CURB-65, PMEWS and NEWS2 provide good but not excellent prediction for adverse outcome in suspected COVID-19, and predicted death without organ support better than receipt of organ support. PMEWS, the WHO criteria and NEWS2 (using a lower threshold than usually recommended) provide good sensitivity at the expense of specificity*. [**note: this is from the UK and looks at several triage tools for clinicians treating COVID-19 patients.]**
  https://www.medrxiv.org/content/10.1101/2020.09.02.20185892v1
- At the beginning of the COVID-19 pandemic, there was high mortality and a lack of effective treatment for critically ill patients. Build on the experience in argentine hemorrhagic fever with convalescent plasma, we incorporated 90 patients into a multicenter study, and 87 were evaluable. We collected 397 donations from 278 convalescent donors. Patients received plasma with an IgG concentration of 0.7-0.8 (measured by Abbott chemiluminescence) for every 10 kg of body weight. Survival during the first 28 days was the primary objective. 77% were male, age 54 (+/-15.6 y/o (range 27-85); body mass index 29.7 +/-; 4,4; hypertension 39% and diabetes 20%; 19.5% had an immunosuppression condition; 23% were healthcare workers. Plasma was administered to 55 patients (63%) on spontaneous breathing with oxygen supplementation (mainly oxygen mask with reservoir bag in 80%), and 32 patients (37%) were infused on mechanical ventilation. The 28-day survival rate was 80%, with 91% in patients infused on spontaneous breathing and 63% in those infused on mechanical ventilation (p = 0.0002). There was a significant improvement in the WHO pneumonia clinical scale at 7 and 14 days, and in PaO2 / FiO2, ferritin and LDH, in the week post-infusion. We observed an episode of circulatory volume overload and a febrile reaction, both mild. *Convalescent plasma infusions are feasible, safe, and potentially effective, especially before requiring mechanical ventilation, and are an attractive clinical option for treating severe forms of COVID-19 until other effective therapies become available*. [**note: this is from an open label trial of convalescent plasma in Buenos**

Aires. 28 day survival was the primary endpoint.]
https://www.medrxiv.org/content/10.1101/2020.09.01.20184390v1

- BACKGROUND Recent studies suggest a link between vitamin D deficiency and Covid-19 infection. In our population we observe major differences in Covid-19 incidence in ethnic groups and genders in each group. METHODS We carried out a population-based study among 4.6 million members of Clalit Health Services (CHS). We collected results from vitamin D tests performed between 2010 and 2019 and used weighted linear regression to assess the relationship between prevalence of vitamin D deficiency and Covid-19 incidence in 200 localities. Additionally, we matched 52,405 infected patients with 524,050 control individuals of the same sex, age, geographical region and used conditional logistic regression to assess the relationship between baseline vitamin D levels, acquisition of vitamin D supplements in the last 4 months, and positive Covid-19. RESULTS *We observe a highly significant correlation between prevalence of vitamin D deficiency and Covid-19 incidence, and between female-to-male ratio for severe vitamin D deficiency and female-to-male ratio for Covid-19 incidence in localities (P<0.001). In the matched cohort, we found a significant association between low vitamin D levels and the risk of Covid-19, with the highest risk observed for severe vitamin D deficiency. A significant protective effect was observed for members who acquired liquid vitamin D formulations (drops) in the last 4 months.* CONCLUSION In this large observational population study, we show a strong association between vitamin D deficiency and Covid-19 occurrence. After adjustment for baseline characteristics and prior vitamin D levels, acquisition of liquid vitamin D formulations is associated with decreased risk for Covid-19 infection. [**note: get your Vitamin D levels up!! That is the conclusion of this a large Israeli cohort study}**
https://www.medrxiv.org/content/10.1101/2020.09.04.20188268v1

DRUG DEVELOPMENT

- There is an urgent need to develop efficacious vaccines against SARS-CoV-2 that also address the issues of deployment, equitable access, and vaccine acceptance. Ideally, the vaccine would prevent virus infection and transmission as well as preventing COVID-19 disease. We previously developed an oral adenovirus-based vaccine technology that induces both mucosal and systemic immunity in humans. Here we investigate the immunogenicity of a range of candidate adenovirus-based vaccines, expressing full or partial sequences of the spike and nucleocapsid proteins, in mice. We demonstrate that, compared to expression of the S1 domain or a stabilized spike antigen, the full length, wild-type spike antigen induces significantly higher neutralizing antibodies in the periphery and in the lungs, when the vaccine is administered mucosally. Antigen-specific CD4+ and CD8+ T cells were induced by this leading vaccine candidate at low and high doses. This full-length spike antigen plus nucleocapsid adenovirus construct has been prioritized for further clinical development. **[note: this is preclinical data from Vaxart, a California biotech company that is developing oral vaccines. This uses an adenovirus vector. Clinical trials are planned.]**
https://www.biorxiv.org/content/10.1101/2020.09.04.283853v1
- Background: The SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is a positive-sense single-stranded RNA coronavirus responsible for the ongoing 2019-2020 COVID-19 outbreak. The highly contagious COVID-19 disease has spread to 216 countries in less than six months. Though several vaccine candidates are being claimed, an effective vaccine is yet to

come. In present study we have designed and theoretically validated novel Multi-Patch Vaccines against SARS-CoV-2. Methodology: A novel reverse epitomics approach, overlapping-epitope-clusters-to-patches method is utilized to identify multiple antigenic regions from the SARS-CoV-2 proteome. These antigenic regions are here termed as Ag-Patch or Ag-Patches, for Antigenic Patch or Patches. The identification of Ag-Patches is based on clusters of overlapping epitopes rising from a particular region of SARS-CoV-2 protein. Further, we have utilized the identified Ag-Patches to design Multi-Patch Vaccines (MPVs), proposing a novel methodology for vaccine design and development. The designed MPVs were analyzed for immunologically crucial parameters, physiochemical properties and cDNA constructs. Results: We identified 73 CTL (Cytotoxic T-Lymphocyte), 49 HTL (Helper T-Lymphocyte) novel Ag-Patches from the proteome of SARS-CoV-2. The identified Ag-Patches utilized to design MPVs cover 768 (518 CTL and 250 HTL) overlapping epitopes targeting different HLA alleles. Such large number of epitope coverage is not possible for multi-epitope vaccines. The large number of epitopes covered implies large number of HLA alleles targeted, and hence large ethnically distributed human population coverage. The MPVs:Toll-Like Receptor ectodomain complex shows stable nature with numerous hydrogen bond formation and acceptable root mean square deviation and fluctuation. Further, the cDNA analysis favors high expression of the MPVs constructs in human cell line. Conclusion: Highly immunogenic novel Ag-Patches are identified from the entire proteome of SARS CoV-2 by a novel reverse epitomics approach. We conclude that the novel Multi-Patch Vaccines could be a highly potential novel approach to combat SARS-CoV-2, with greater effectiveness, high specificity and large human population coverage worldwide. [**note: this is an interesting theoretical vaccine design paper. I'm unsure of how it will translate to actual R&D**] https://www.biorxiv.org/content/10.1101/2020.09.06.284992v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Nothing Today

DIAGNOSTIC DEVELOPMENT

- Nothing Today

>>

2020-09-08

Let's listen to some opera! Here is the great Peruvian tenor, Juan Diego Flores with the Austrian coloratura Daniela Fally in Bellini's La Sonnambula. No sleepwalking going on here, just some great bel canto singing: https://www.youtube.com/watch?v=5I8SNXbpNZo there was nothing in the notes to indicate when and where this performance took place but a Google search indicates Vienna in 2017.

The Washington Post notes that New York's infection rate has been below one percent for 30 days. Let us hope this trend continues. Nine things that have changed since the pandemic; I don't think I have used cash for anything other than to pay out my Scrabble losses to Ms. Goldhammer. Speaking of changes, look no further than on-campus dinning choices. Here is more on the pledge by the companies who are developing COVID-19 vaccines. Pediatricians are facing new problems with COVID-19.

The Guardian reports on an infectious disease specialist who is also president of a major university. Some of the faculty don't have faith in his decision-making.

STAT cover Operation Warp Speed. Yes, it is fast but will we get the 'best' vaccine from this research effort (if there is one)? Here is their take on the vaccine pledge letter.

MODELING

- The timing of SARS-CoV-2 transmission is a critical factor to understand the epidemic trajectory and the impact of isolation, contact tracing and other non- pharmaceutical interventions on the spread of COVID-19 epidemics. We examined the distribution of transmission events with respect to exposure and onset of symptoms. We show that for symptomatic individuals, the timing of transmission of SARS-CoV-2 is more strongly linked to the onset of clinical symptoms of COVID-19 than to the time since infection. We found that it was approximately centered and symmetric around the onset of symptoms, with three quarters of events occurring in the window from 2-3 days before to 2-3 days after. However, we caution against overinterpretation of the right tail of the distribution, due to its dependence on behavioural factors and interventions. We also found that the pre-symptomatic infectious period extended further back in time for individuals with longer incubation periods. This strongly suggests that information about when a case was infected should be collected where possible, in order to assess how far into the past their contacts should be traced. Overall, the fraction of transmission from strictly pre-symptomatic infections was high (41%; 95%CI 31-50%), which limits the efficacy of symptom-based interventions, and the large fraction of transmissions (35%; 95%CI 26-45%) that occur on the same day or the day after onset of symptoms underlines the critical importance of individuals distancing themselves from others as soon as they notice any symptoms, even if they are mild. Rapid or at-home testing and contextual risk information would greatly facilitate efficient early isolation. [**note: this is an interesting paper that looks at the distribution of transmission events. They hypothesize that the peak of infectiousness depends on the onset of symptoms rather than time of infection. This is a good paper to read thoroughly.**] https://www.medrxiv.org/content/10.1101/2020.09.04.20188516v1
- A well-known characteristic of pandemics such as COVID-19 is the high level of transmission heterogeneity in the infection spread: not all infected individuals spread the disease at the same rate and some individuals (superspreaders) are responsible for most of the infections. To quantify this phenomenon requires the analysis of the effect of the variance and higher moments of the infection distribution. Working in the framework of stochastic branching processes, we derive an approximate analytical formula for the probability of an outbreak in the high variance regime of the infection distribution, verify it numerically and analyze its regime of validity in various examples.We show that it is possible for an outbreak not to occur in the high variance regime even when the basic reproduction number R0 is larger than one and discuss the implications of our results for COVID-19 and other pandemics. [**note: here is another useful model looking variants of infection distribution. It's good to see new approaches to disease modeling that move beyond the traditional SIR model. This is an interesting paper to read.**] https://www.medrxiv.org/content/10.1101/2020.09.06.20189258v1
- The SARS-CoV-2 coronavirus has proven difficult to control not only because of its high transmissibility, but because those who are infected readily spread the virus before symptoms appear, and because some infected individuals, though contagious, never exhibit symptoms.

Proactive testing of asymptomatic individuals is therefore a powerful, and probably necessary, tool for preventing widespread infection in many settings. This paper explores the effectiveness of alternative testing regimes, in which the frequency, the accuracy, and the delay between testing and results determine the time path of infection. *For a simple model of disease transmission, we present analytic formulas that determine the effect of testing on the expected number of days of during which an infectious individual is exposed to the population at large. This allows us to estimate the frequency of testing that would be required to prevent uncontrolled outbreaks, and to explore the trade-offs between frequency, accuracy, and delay in achieving this objective. We conclude by discussing applications to outbreak control on college and university campuses*. [**note: this is a paper modeling the frequency and accuracy of proactive testing for COVID-19 Although it is a little too late, there is a good discussion for university administrators regarding amount and frequency of testing.**] https://www.medrxiv.org/content/10.1101/2020.09.05.20188839v1

NEWLY REGISTERED CLINICAL TRIALS

- The purpose of this study is to assess the efficacy of Bacille Calmette-Guérin (BCG) vaccination compared to placebo in reducing severe Covid-19 disease among elderly residents of skilled nursing facilities. The investigators hypothesize that BCG vaccination can reduce severity of Covid-19 disease. Patients who are residents of participating long-term care facilities (LTCFs), with the ability to understand and cooperate with study procedures, who agree to participate in the study will be randomly assigned to receive BCG vaccination or a placebo. Participants will be followed for up to twelve months to assess severity of Covid-19 outcomes. [**note: there are several BCG trials registered but this one singles out residents of nursing home facilities who are at risk. Harvard Med School is the sponsor.]** NCT04534803 **and here is a Dutch trial registration:** NCT04537663
- Clinical trial to compare sublingual low dose thimerosal in adults that have symptoms of SARS-CoV-2 Infection against placebo to show a difference in physical characteristics and viral levels. [**note: this is for people >60 years of age. I really want to know the rationale for this drug study!**] NCT04522830
- The primary purpose of this research is to determine whether Valproate alone, and in combination with Quetiapine, lowers confusion and agitation in persons with severe Corona Virus Disease (COVID)19 pneumonia during weaning from the breathing machine (ventilator). Though Valproate and Quetiapine are often given to persons with severe confusion with agitation, the purpose of this small research study is specifically for: a) persons infected with COVID 2019 on a ventilator whose agitation is not responding to the usual medications (like dexmedetomidine), and b) to reduce the time persons are treated with dexmedetomidine, which requires continuous close monitoring in an ICU. [**note: this is a trial to look at those with severe COVID-19 who require ventilation**] NCT04513314
- In this randomized double blind Phase 3 clinical trial we will study the efficacy and safety of oral polio vaccine with and without NA-831 versus placebo. [**note: sponsor is NeuroActivia Inc. and the experimental drug is a neuroprotective and neorgenesis drug that is in trials for early onset Alzheimer's disease. Interesting rationale for the study provided by the company.**] NCT04540185

CLINICAL TRIAL RESULTS

- To determine whether severe acute respiratory syndrome coronavirus 2 (SARS CoV 2, the cause of COVID 19 disease) exposure in pregnancy, compared to non exposure, is associated with infection related obstetric morbidity. Design and setting: Throughout Spain, 45 hospitals took part in the universal screening of pregnant women going into labour using polymerase chain reaction (PCR) for COVID 19 since late March 2020. Methods: The cohort of exposed and unexposed pregnancies was followed up until 6 weeks postpartum. Multivariate logistic regression analysis, adjusting for known confounding variables, determined the adjusted odds ratio (aOR) with 95% confidence intervals (95% CI) of the association of COVID 19 exposure, compared to non exposure, with infection related obstetric outcomes. Main outcome measures: Preterm delivery (primary), premature rupture of membranes and neonatal intensive care unit admissions. Results: In the cohort of 1,009 screened pregnancies, 246 were COVID 19 positive. Compared to non exposure, COVID 19 exposure increased the odds of preterm birth (34 vs 51, 13.8% vs 6.7%, aOR 2.12, 95% CI 1.32 3.36, p=0.002), premature rupture of membranes at term (39 vs 75, % vs 9.8%, aOR 1.70, 95% CI 1.11 2.57, p=0.013) and neonatal intensive care unit admissions (23 vs 18, 9.3% vs 2.4%, aOR 4.62, 95% CI 2.43 8.94, p<0.001). Conclusion: *This first prospective cohort study demonstrated that pregnant women infected with SARS CoV 2 have more infection related obstetric morbidity. This hypothesis merits evaluation of a causal association in further research*. **[note: this is the first large observational study I've seen that looks at COVID-19 positive obstetrics cases.**]
  https://www.medrxiv.org/content/10.1101/2020.09.05.20188458v1
- Rationale: Activins are inflammatory and tissue-repair-related members of the TGFβ-superfamily that have been implicated in the pathogenesis of several immuno-inflammatory disorders including sepsis/acute respiratory distress syndrome (ARDS). We hypothesized that they might be of particular relevance to COVID-19 pathophysiology. Objectives: To assess the involvement of the Activin-Follistatin-axis in COVID-19 pathophysiology. Methods: Levels of Activins -A, -B and their physiological inhibitor Follistatin, were retrospectively analyzed in 314 serum samples from 117 COVID-19 patients derived from two independent centers and compared with common demographic, clinical and laboratory parameters. Optimal-scaling with ridge-regression was used to screen variables and establish a prediction model. Main Results: The Activin/Follistatin-axis was significantly deregulated during the course of COVID-19 and was independently associated with severity and in-hospital mortality. FACT-CLINYCoD, a novel disease scoring system, adding one point for each of Follistatin >6235 pg/ml, Activin-A >591 pg/ml, Activin-B >249 pg/ml, CRP >10.3 mg/dL, LDH >427 U/L, Intensive Care Unit (ICU) admission, Neutrophil/Lymphocyte-Ratio >5.6, Years of Age >61, Comorbidities >1 and D-dimers >1097 ng/ml, efficiently predicted and monitored fatal outcome independently of multiplicity and timing of sampling (AUC: 0.951±0.032, p<10-6). Validation in 35 samples derived from a third hospital indicated comparable AUC (0.958±0.086, p=0.032). Conclusion: This study unravels the link between Activin/Folistatin-axis and COVID-19 mortality and introduces FACT-CLINYCoD, a novel pathophysiology-based tool that copes with the dynamic and heterogeneous nature of COCVID-19, predicts disease outcome and supports clinical decision making. Prospective large-scale validation of this calculator, as well as investigation of the mechanisms linking Activin/Folistatin-axis to COVID-19 pathogenesis is warranted. [**note: from Greece another possible predictor of disease outcome.]**
  https://www.medrxiv.org/content/10.1101/2020.09.05.20184655v1

- Coronavirus disease-2019 (COVID-19) has poorer clinical outcomes in males compared to females, and immune responses underlie these sex-related differences in disease trajectory. As immune responses are in part regulated by metabolites, we examined whether the serum metabolome has sex-specificity for immune responses in COVID-19. In males with COVID- 19, kynurenic acid (KA) and a high KA to kynurenine (K) ratio was positively correlated with age, inflammatory cytokines, and chemokines and was negatively correlated with T cell responses, revealing that KA production is linked to immune responses in males. Males that clinically deteriorated had a higher KA:K ratio than those that stabilized. In females with COVID-19, this ratio positively correlated with T cell responses and did not correlate with age or clinical severity. KA is known to inhibit glutamate release, and we observed that serum glutamate is lower in patients that deteriorate from COVID-19 compared to those that stabilize, and correlates with immune responses. Analysis of Genotype-Tissue Expression (GTEx) data revealed that expression of kynurenine aminotransferase, which regulates KA production, correlates most strongly with cytokine levels and aryl hydrocarbon receptor activation in older males. This study reveals that KA has a sex-specific link to immune responses and clinical outcomes, in COVID-19 infection. [**note: from Yale, possible linkage of kynurenic acid to sex differential response and outcome of COVID-19**]
  https://www.medrxiv.org/content/10.1101/2020.09.06.20189159v1

DRUG DEVELOPMENT

- After the SARS-CoV outbreak in 2003, a second zoonotic coronavirus named SARS-CoV-2, emerged late 2019 in China and rapidly caused the COVID-19 pandemic leading to a public health crisis of an unprecedented scale. Despite the fact that SARS-CoV-2 uses the same receptor as SARS-CoV, transmission and pathogenesis of both viruses seem to be quite distinct. A remarkable feature of the SARS-CoV-2 spike is the presence of a multibasic cleavage site, which is absent in the SARS-CoV spike. The viral spike protein not only attaches to the entry receptor, but also mediates fusion after cleavage by host proteases. Here, we report that the SARS-CoV-2 spike multibasic cleavage site increases infectivity on differentiated organoid-derived human airway cells. Compared with SARS-CoV, SARS-CoV-2 entered faster into the lung cell line Calu-3, and more frequently formed syncytial cells in differentiated organoid-derived human airway cells. Moreover, the multibasic cleavage site increased entry speed and plasma membrane serine protease usage relative to endosomal entry using cathepsins. Blocking serine protease activity using the clinically approved drug camostat mesylate effectively inhibited SARS-CoV-2 entry and replication in differentiated organoid-derived human airway cells. Our findings provide novel information on how SARS-CoV-2 enters relevant airway cells and highlight serine proteases as an attractive antiviral target. [**note: from Erasmus Univ in Rotterdam, more on the cleavage site and serine protease usage. There are some clinical trials of camostat but I've not seen any results.**] https://www.biorxiv.org/content/10.1101/2020.09.07.286120v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The adaptive immunity that protects patients from coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is not well characterized. In particular, the asymptomatic patients have been found to induce weak and transient SARS-CoV-2 antibody responses, but the underlying mechanisms remain unknown; meanwhile, the

protective immunity that guide the recovery of these asymptomatic patients is also not well studied. Here, we characterized SARS-CoV-2-specific B-cell and T-cell responses in 10 asymptomatic patients and 49 patients with other disease severity (mild, n=10, moderate, n=32, severe, n=7) and found that asymptomatic or mild symptomatic patients failed to mount virus-specific germinal center (GC) B cell responses that result in robust and long-term humoral immunity, assessed by GC response indicators including follicular helper T (TFH) cell and memory B cell responses as well as serum CXCL13 levels. Alternatively, these patients mounted potent virus-specific TH1 and CD8+ T cell responses. In sharp contrast, patients of moderate or severe disease induced vigorous virus-specific GC B cell responses and associated TFH responses; however, the virus-specific TH1 and CD8+ T cells were minimally induced in these patients. *These results therefore uncovered the protective immunity in asymptomatic patients and revealed the strikingly dichotomous and unbalanced humoral and cellular immune responses in COVID-19 patients with different disease severity, providing important insights into rational design of COVID-19 vaccines*. [**note: this is from China and shows the difference in immune response between various COVID-19 severities. I'm not sure how it relates to vaccine design.**] https://www.medrxiv.org/content/10.1101/2020.09.05.20187435v1

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the pandemic respiratory infectious disease COVID-19. However, clinical manifestations and outcomes differ significantly among COVID-19 patients, ranging from asymptomatic to extremely severe, and it remains unclear what drives these disparities. Here, we studied 159 hospitalized Italian patients with pneumonia from the NIAID-NCI COVID-19 Consortium using a phage-display method to characterize circulating antibodies binding to 93,904 viral peptides encoded by 1,276 strains of human viruses. SARS-CoV-2 infection was associated with a marked increase in individual's immune memory antibody repertoires linked to trajectories of disease severity from the longitudinal analysis also including anti-spike protein antibodies. By applying a machine-learning-based strategy, we developed a viral exposure signature predictive of COVID-19-related disease severity linked to patient survival. These results provide a basis for understanding the roles of memory B-cell repertoires in COVID-19-related symptoms as well as a predictive tool for monitoring its clinical severity. [**note: this is another look at differential immune response based on disease severity from NIH reserachers.**] https://www.medrxiv.org/content/10.1101/2020.09.04.20187088v1

DIAGNOSTIC DEVELOPMENT

- Not a thing today.

>>

2020-09-09

What better name for a daughter who you just know will be an opera singer when she grows up. Aida Garifullina was born in Kazan and has been enjoying a very nice career as a lyric soprano. Of course she is unlikely to sing Norma on stage but this is about as nice a 'Casta Diva' in concert that I've seen: https://www.youtube.com/watch?v=rK6GsRUl4WI I don't think the eyelashes are real.

The New York Times covers seven ways food shopping has changed. Maybe masks let in just enough SARS-CoV-2 virus to provoke an immune response. Someone needs to do a randomized controlled trial here!!! Commercial real estate in Manhattan is suffering because of COVID-19.

The Washington Post notes that the hard-hit Italian city of Bergamo is doing surveillance of recovered COVID-19 patients to gauge long term health effects. Let's hope they get some good outcomes data from this effort. Florida continues to be a COVID-19 trendsetter in a not so good way. Not disclosing health data is never a good thing. Winning an Olympic Gold Medal and graduating from Stanford is not a guarantee of having common sense when it comes to public health protection. The July motorcycle rally in Sturgis SD may have led to over 250K new COVID-19 cases. Cases of COVID-19 are leveling off in the US but still remain high in some areas.

Here is the New England Journal of Medicine commentary on mask controlled variolation as mentioned in the NY Times article. The Journal also has a perspective on when will we have a vaccine?

STAT reports that the AZ/Oxford adenovirus based COVID-19 vaccine trial has been put on hold because of suspected serious adverse reaction. We will have to see how this affects other trials. The New York Times also has a story on this safety issue. STAT also has a good piece on how nature impacts pandemic releases of microorganisms.

There is an interesting paper from India with results on a trial of convalescent plasma that does not show positive results. This is why we do clinical trials!

MODELING

- Bats are the suggested natural hosts for severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2, the latter of which caused the coronavirus disease 2019 (COVID-19) pandemic. The interaction of viral Spike proteins with their host receptor angiotensin-converting enzyme 2 (ACE2) is a critical determinant of potential hosts and cross-species transmission. Here we use virus-host receptor binding and infection assays to show that ACE2 orthologs from 24, 21, and 16 of 46 phylogenetically diverse bat species, including those in close and distant contact with humans, do not support entry of SARS-CoV, SARS-CoV-2, and both of these coronaviruses, respectively. Furthermore, we used genetic and functional analyses to identify genetic changes in bat ACE2 receptors associated with viral entry restrictions. Our study demonstrates that many, if not most, bat species are not potential hosts of SARS-CoV and SARS-CoV-2, and provides important insights into pandemic control and wildlife conservation. [**note: from Wuhan evidence that a lot of bat species do not support entry of SARS-CoV-2 and are not potential hosts.**] https://www.biorxiv.org/content/10.1101/2020.09.08.284737v1

NEWLY REGISTERED CLINICAL TRIALS

- I checked yesterday

CLINICAL TRIAL RESULTS

- Objectives: Convalescent plasma (CP) as a passive source of neutralizing antibodies and immunomodulators is a century-old therapeutic option used for the management of viral diseases. We investigated its effectiveness for the treatment of COVID-19. Design: Open-label, parallel-arm, phase II, multicentre, randomized controlled trial. Setting: Thirty-nine public and

private hospitals across India. Participants: Hospitalized, moderately ill confirmed COVID-19 patients (PaO2/FiO2: 200-300 or respiratory rate > 24/min and SpO2 ≤ 93% on room air). Intervention: Participants were randomized to either control (best standard of care (BSC)) or intervention (CP + BSC) arm. Two doses of 200 mL CP was transfused 24 hours apart in the intervention arm. Main Outcome Measure: Composite of progression to severe disease (PaO2/FiO2<100) or all-cause mortality at 28 days post-enrolment. Results: Between 22 nd April to 14 th July 2020, 464 participants were enrolled; 235 and 229 in intervention and control arm, respectively. Composite primary outcome was achieved in 44 (18.7%) participants in the intervention arm and 41 (17.9%) in the control arm [aOR: 1.09; 95% CI: 0.67, 1.77]. Mortality was documented in 34 (13.6%) and 31 (14.6%) participants in intervention and control arm, respectively [aOR) 1.06 95% CI: -0.61 to 1.83]. Interpretation: *CP was not associated with reduction in mortality or progression to severe COVID-19. This trial has high generalizability and approximates real-life setting of CP therapy in settings with limited laboratory capacity. A priori measurement of neutralizing antibody titres in donors and participants may further clarify the role of CP in management of COVID-19*. [**note: there are a large number of co-authors on the clinical trial of convalescent plasma from India. Does not look like it helped out at all in terms of mortality and disease progression. However, it was also a modest patient population.**] https://www.medrxiv.org/content/10.1101/2020.09.03.20187252v1

DRUG DEVELOPMENT

- SARS-CoV-2 infection is the cause of a worldwide pandemic, currently with limited therapeutic options. It is characterised by being highly contagious and nasal mucosa appears to be the primary site with subsequent spread to the lungs and elsewhere. BromAc (Bromelain & Acetylcysteine) has been described to disrupt glycoproteins by the synchronous breakage of glycosidic linkages and disulphide bonds. The spike protein of SARS-CoV-2 is an attractive target as it is essential for binding to the ACE2 receptor in host cells and is formed of glycoprotein and disulphide bridges for stabilisation. Hence, we sought to determine whether BromAc has activity on the spike and envelope protein specific to SARS-CoV-2 virus. Design: Gel electrophoresis analysis was carried out on recombinant spike and envelope proteins that were treated with a range of concentrations of single agents and BromAc. For UV analysis of disulfide bonds reduction, both spike and envelope protein were treated with Acetylcysteine with the determination of loss of disulfide bonds. Results: Recombinant spike and envelope SARS-CoV-2 protein were fragmented by BromAc whilst single agents had minimal effect. Spike and envelope proteins disulphide bonds were reduced by Acetylcysteine. Conclusion: BromAc disintegrates the spike and envelope protein from SARS-CoV-2 and may render it non-infective. In vitro tests on live virus have been encouraging and clinical testing through nasal administration in patients with early SARS-CoV-2 infection is imminent. [**note: an interesting concept. N-acetylsysteine is in clinical trials but not with bromelain. I wonder if there will be a drug delivery problem in getting an enzyme into the body via the nasal route.**] https://www.biorxiv.org/content/10.1101/2020.09.07.286906v1
- To contain the coronavirus disease 2019 (COVID-19) pandemic, a safe and effective vaccine against the new severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is urgently needed in quantities sufficient to immunise large populations. In this study, we report the design, preclinical development, immunogenicity and anti-viral protective effect in rhesus

macaques of the BNT162b2 vaccine candidate. BNT162b2 contains an LNP-formulated nucleoside-modified mRNA that encodes the spike glycoprotein captured in its prefusion conformation. After expression of the BNT162b2 coding sequence in cells, approximately 20% of the spike molecules are in the one-RBD up, two-RBD down state. Immunisation of mice with a single dose of BNT162b2 induced dose level-dependent increases in pseudovirus neutralisation titers. Prime-boost vaccination of rhesus macaques elicited authentic SARS-CoV-2 neutralising geometric mean titers 10.2 to 18.0 times that of a SARS-CoV-2 convalescent human serum panel. BNT162b2 generated strong TH1 type CD4+ and IFNγ+ CD8+ T-cell responses in mice and rhesus macaques. The BNT162b2 vaccine candidate fully protected the lungs of immunised rhesus macaques from infectious SARS-CoV-2 challenge. BNT162b2 is currently being evaluated in a global, pivotal Phase 2/3 trial (NCT04368728). [**note: animal data for the Pfizer/BioNTech vaccine**] https://www.biorxiv.org/content/10.1101/2020.09.08.280818v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Convalescent plasma from SARS-CoV-2 infected individuals and monoclonal antibodies were shown to potently neutralize viral and pseudoviral particles carrying the S glycoprotein. However, a non-negligent proportion of plasma samples from infected individuals as well as S-specific monoclonal antibodies were reported to be non-neutralizing despite efficient interaction with the S glycoprotein in different biochemical assays using soluble recombinant forms of S or when expressed at the cell surface. How neutralization relates to binding of S glycoprotein in the context of viral particles remains to be established. Here we developed a pseudovirus capture assay (VCA) to measure the capacity of plasma samples or antibodies immobilized on ELISA plates to bind to membrane-bound S glycoproteins from SARS-CoV-2 expressed at the surface of lentiviral particles. By performing VCA and neutralization assays we observed a strong correlation between these two parameters. However, while we found that plasma samples unable to capture viral particles did not neutralize, capture did not guarantee neutralization, indicating that the capacity of antibodies to bind to the S glycoprotein at the surface of viral particles is required but not sufficient to mediate neutralization. Altogether, our results highlights the importance of better understanding the inactivation of S by plasma and neutralizing antibodies. [**note: not all antibodies are created equal when it comes to neutralizing properties.**] https://www.biorxiv.org/content/10.1101/2020.09.08.287482v1
- The human immune response to SARS-CoV-2 infection is highly variable, with less than 10% of infections resulting in severe COVID-19 requiring intensive care unit (ICU) treatment. Here we have analyzed the dynamics of the adaptive immune response in COVID-19 ICU patients at the level of single cell transcriptomes and B cell and T cell receptor (BCR, TCR) repertoires. Early after ICU admission, before seroconversion in response to SARS-CoV-2 spike protein, patients generate activated peripheral B cells with a type 1 interferon-induced gene expression signature. After seroconversion, patients display circulating activated B cells expressing an IL-21-induced gene expression signature and mainly IgG1 and IgA1, two isotypes induced by IL-21 and TGF-β, respectively. In sustained COVID-19, the persistent immune reaction is shifted to IgA2-expressing activated peripheral B cells, displaying somatic hypermutation, and expressing TGF-β-induced signature genes, like IgA germline transcripts. The switch from an IgG1 to an IgA2-dominated B cell response correlates with the appearance of SARS-CoV-2 reactive follicular T helper cells expressing IL-21 and/or TGF-β in the blood. Despite the continued presence of IgA2-

expressing B cells and IgA antibodies in the blood of progressed COVID-19 patients, IgA2 secreting cells were scarce in the lungs of deceased COVID-19 patients. In summary, in severely affected COVID-19 patients SARS-CoV-2 triggers chronic immune reactions which are controlled by TGF-β, with most of the activated B cells being no longer specific for the SARS-CoV-2 spike protein and its receptor binding domain, nor for nucleoprotein. TGF-β may candidate as a target to ameliorate detrimental immunopathology in those patients. [**note: more on the immue response from Germany in severe COVID-19 cases.**]
https://www.medrxiv.org/content/10.1101/2020.09.04.20188169v1

- Severe acute respiratory syndrome (SARS) coronavirus-2 (SARS-CoV-2) is a single-stranded, enveloped RNA virus and the etiological agent of the current COVID-19 pandemic. Efficient replication of the virus relies on the activity of nonstructural protein 1 (Nsp1), a major virulence factor shown to facilitate suppression of host gene expression through promotion of host mRNA degradation and interaction with the 40S ribosomal subunit. Here, we report the crystal structure of the globular domain of SARS-CoV-2 Nsp1, encompassing residues 13 to 127, at a resolution of 1.65 A. Our structure features a six-stranded, capped β-barrel motif similar to Nsp1from SARS-CoV and reveals how variations in amino acid sequence manifest as distinct structural features. Through comparative analysis of structural homologues, we identified a topological signature associated with this protein fold that facilitated modeling of Nsp1 from MERS-CoV. Combining our high-resolution crystal structure with existing data on the C-terminus of Nsp1 from SARS-CoV-2, we propose a model of the full-length protein. Our results provide unparalleled insight into the molecular structure of a major pathogenic determinant of SARS-CoV-2. [**note: crystal structure of one of the key SARS-CoV-2 virulence factors.**]
https://www.biorxiv.org/content/10.1101/2020.09.08.288191v1

DIAGNOSTIC DEVELOPMENT

- Nothing today

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

2020-09-10

We are going to stay with Bellini's 'Casta Diva' for little while longer; I'll conclude on Saturday with the version I believe to be the best from currently active singers. If you are not an opera fan, feel free to move one. Here is Renee Fleming from the Palace of the Tsars in St. Petersburg:
https://www.youtube.com/watch?v=Rg4L5tcxFcA

The Washington Post discusses the hot new corporate job, 'Head of Remote Work.' The amount of remote work being done during the COVID-19 pandemic is showing companies a new way to the future. You saw this in yesterday's link about the huge drop in office real estate space. I suspect a lot of companies will be moving to a remote office orientation with maybe 2 days or so per week in the office. Can you believe Austrian citizens with no or very remote linkage to the US received COVID-19 stimulus checks? Yes they did!!! Don't these people have any common sense? Apparently not. Did they really think college football could be played this fall without any COVID-19 worries?

Ed Yong has his usual great piece in The Atlantic, discussing why we are trapped in a pandemic spiral and what to do about it. This one is MANDATORY reading for all of you.

STAT argues that AstraZeneca should have been more transparent on the vaccine trial hold. I agree!

The Lancet has a short piece on tocilizumab and whether it might still be a good drug to treat COVID-19 despite the Roche trial data. There continue to be some observational trials that show possible efficacy and the UK RECOVERY trial is still studying it. I do not think we have the final answer on this drug.

JAMA have some 'good' news. There does not appear to be much chance of nosocomial COVID-19 in patients at a large academic medical center (Brigham & Women's in Boston). In this cohort study of 9149 patients admitted to a large US academic medical center over a 12-week period, 697 were diagnosed with COVID-19. In the context of a comprehensive and progressive infection control program, only 2 hospital-acquired cases were detected: 1 patient was likely infected by a presymptomatic spouse before visitor restrictions were implemented, and 1 patient developed symptoms 4 days after a 16-day hospitalization but without known exposures in the hospital. Young adults who require hospitalization for COVID-19 do experience adverse health outcomes. Young adults age 18 to 34 years hospitalized with COVID-19 experienced substantial rates of adverse outcomes: 21% required intensive care, 10% required mechanical ventilation, and 2.7% died. This in-hospital mortality rate is lower than that reported for older adults with COVID-19, but approximately double that of young adults with acute myocardial infarction.[4] Morbid obesity, hypertension, and diabetes were common and associated with greater risks of adverse events. Young adults with more than 1 of these conditions faced risks comparable with those observed in middle-aged adults without them. More than half of these patients requiring hospitalization were Black or Hispanic, consistent with prior findings of disproportionate illness severity in these demographic groups.

Nature have a good story on the 'underdog' COVID-19 vaccine candidates. As I have noted, we really do not know which of these dozens of vaccines will be the best one.

The British Medical Journal has a large systematic study of COVID-19 in pregnant women. One in ten appear to be infected. Pregnant and recently pregnant women are less likely to manifest covid-19 related symptoms of fever and myalgia than non-pregnant women of reproductive age and are potentially more likely to need intensive care treatment for covid-19. Pre-existing comorbidities, high maternal age, and high body mass index seem to be risk factors for severe covid-19. Preterm birth rates are high in pregnant women with covid-19 than in pregnant women without the disease.

Eric Topol interviews Paul Offit on COVID-19 vaccine efforts. Offit discusses why it is unlikely we will have a vaccine before the end of the year. It pretty much is in line with the scenario I sketched out last week. Do read or listen to this!

Derek Lowe weighs in on the bradykinin work at Oak Ridge; the AZ/Oxford vaccine AE; and the Russian vaccine data which may or may not be questionable.

There are lots of stories on Bob Woodward's new book on President Trump's reaction to the SARS-CoV-2 pandemic. You all know where to find them and I won't waste any space in this newsletter providing commentary or links.

MODELING

- Nothing

NEWLY REGISTERED CLINICAL TRIALS

- I will check later today

CLINICAL TRIAL RESULTS

- Background: Gargling had been reported to have significant roles in the prevention and treatment of respiratory tract infections. The purpose of this study was to assess the ability of regular gargling to eliminate SARS-CoV-2 in the oropharynx and nasopharynx. Methodology: This pilot, open labeled, randomized, parallel study compared the effect of 30 seconds, 3 times/day gargling using 1% povidone-iodine (PVP-I), essential oils and tap water on SARS-CoV-2 viral clearance among COVID-19 patients in a tertiary hospital in Kuala Lumpur. Progress was monitored by day 4,6 and 12 PCR (Ct value), gargling and symptoms diary as well as clinical observations. Results: Five confirmed Stage 1 COVID-19 patients were recruited for each arm. The age range was from 22 to 56 years old. The majority were males. Two respondents had co-morbidities, which were asthma and obesity. Viral clearance was achieved at day 6 in 100%, 80%, 20% and 0% for 1% PVP-I, essential oils, tap water and control group respectively. Analysis of 1% PVP-I group versus control group showed significant p-value for comparison of PCR results on Day 4, Day 6 and Day 12. Conclusions: This preliminary study showed that gargling with 1% PVP-I and essential oils show great potential to be part of the treatment and management of Stage 1 COVID-19. Larger studies are required to ascertain the benefit of gargling for different stages of COVID-19 patients. This study was registered in clinicaltrial.gov (NCT04410159). [**note: this trial is from Malaysia looking at gargling with 1% povidone-iodine. There were only five patients in each of the trial arms so I would not take the 100% clearance rate of gargling with povidone-iodine seriously. There are some other trials going on with this agent both nasal lavage and gargling.**] https://www.medrxiv.org/content/10.1101/2020.09.07.20180448v1
- Background. Hydroxychloroquine has been shown to inhibit severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in vitro, but early clinical studies found no benefit treating patients with coronavirus disease 2019 (COVID-19). We set out to evaluate the effectiveness of hydroxychloroquine for prevention, as opposed to treatment, of COVID-19 mortality. Methods. We pre-specified and conducted an observational, population-based cohort study using national primary care data and linked death registrations in the OpenSAFELY platform, representing 40% of the general population in England. We used Cox regression to estimate the association between ongoing routine hydroxychloroquine use prior to the COVID-19 outbreak in England and risk of COVID-19 mortality among people with rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE). Model adjustment was informed by a directed acyclic graph. Findings. Of 194,637 patients with RA or SLE, 30,569 (15.7%) received ≥2 prescriptions of hydroxychloroquine in the six months prior to 1 March 2020. Between 1 March 2020 and 13 July 2020, there were 547 COVID-19 deaths, 70 among hydroxychloroquine users. Estimated standardised cumulative COVID-19 mortality was 0.23% (95% CI 0.18-0.29) among users and 0.22% (95% CI 0.20-0.25) among non-users; an absolute difference of 0.008% (95% CI -0.051-0.066). After accounting for age, sex, ethnicity, use of other immunuosuppressives, and geographic region, no association with COVID-19 mortality was observed (HR 1.03, 95% CI 0.80-1.33). We found no evidence of interactions with age or other immunosuppressives. Quantitative bias analyses indicated observed associations were robust to missing information regarding additional biologic treatments for rheumatological disease. We observed similar associations with the negative control outcome of non-COVID-19 mortality. Interpretation. *We*

found no evidence of a difference in COVID-19 mortality among patients who received hydroxychloroquine for treatment of rheumatological disease prior to the COVID-19 outbreak in England. [**note: this is an observational study from England looking at whether HCQ prevents COVID-19 mortality in patients who were taking the medicine for rheumatic disease. Mortality was the same in the control and HCQ groups.**]
https://www.medrxiv.org/content/10.1101/2020.09.04.20187781v1 **A Spanish cohort study showed pretty much the same non-protectiveness of chloroquine derivatives**:
https://www.medrxiv.org/content/10.1101/2020.09.03.20158121v1

- Background: We aimed to determine the impact of tocilizumab use in severe COVID-19 pneumonia mortality. Methods: We performed a multicentre retrospective cohort study in 18 tertiary hospitals in Spain, from March to April 2020. Consecutive patients admitted with severe COVID-19 treated with tocilizumab were compared to patients not treated with tocilizumab, adjusting by Inverse Probability of the Treatment Weights (IPTW). Tocilizumab effect in patients receiving steroids during the 48h following inclusion was analyzed. Results: During the study period, 506 patients with severe COVID-19 fulfilled inclusion criteria. Among them, 268 were treated with tocilizumab and 238 patients were not. Median time to tocilizumab treatment from onset of symptoms was 11 days (IQR 8-14). Global mortality was 23.7%. Mortality was lower in patients treated with tocilizumab than in controls (16.8% versus 31.5%, HR 0.514 [95CI 0.355-0.744], p<0.001; weighted HR 0.741 [95CI 0.619-0.887], p=0.001). Tocilizumab treatment reduced mortality by 14.7% relative to no tocilizumab treatment (RRR 46.7%). We calculated a number necessary to treat of 7. Among patients treated with steroids, mortality was lower in patients treated with tocilizumab than in those treated with steroids alone (10.9% versus 40.2%, HR 0.511 [95CI 0.352-0.741], p=0.036; weighted HR 0.6 [95CI 0.449-0.804], p<0.001) (Interaction p=0.094). Conclusions: These results show that survival of patients with severe COVID-19 is higher in patients treated with tocilizumab than in those not treated, and that tocilizumab effect adds to that of steroids administered to non-intubated cases with COVID-19 during the first 48 hours of presenting with respiratory failure despite of oxygen therapy. Randomised controlled studies are needed to confirm these results. [**note: here is a Spanish cohort study that shows a treatment effect with tocilizumab. The sponsor's clinical trial showed no difference and I think the big UK RECOVERY project may still be looking at this drug.**]
https://www.medrxiv.org/content/10.1101/2020.09.07.20189357v1
- Given that gastrointestinal (GI) symptoms are a prominent extrapulmonary manifestation of coronavirus disease 2019 (COVID-19), we investigated the impact of GI infection on disease pathogenesis in three large cohorts of patients in the United States and Europe. Unexpectedly, we observed that GI involvement was associated with a significant reduction in disease severity and mortality, with an accompanying reduction in key inflammatory proteins including IL-6, CXCL8, IL-17A and CCL28 in circulation. In a fourth cohort of COVID-19 patients in which GI biopsies were obtained, we identified severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) within small intestinal enterocytes for the first time in vivo but failed to obtain culturable virus. High dimensional analyses of GI tissues confirmed low levels of cellular inflammation in the GI lamina propria and an active downregulation of key inflammatory genes including IFNG, CXCL8, CXCL2 and IL1B among others. These data draw attention to organ-level heterogeneity in disease pathogenesis and highlight the role of the GI tract in attenuating SARS-CoV-2-associated inflammation with related mortality benefit. [**note: another paper from Mt. Sinai. This is a**

provocative finding in that GI symptoms may attenuate the inflammation accompanying the viral infection. I'll leave it up to the GI specialists to sort this one out but I guess this may be the one disease where having associated diarrhea is a good thing. Just stay hydrated.] https://www.medrxiv.org/content/10.1101/2020.09.07.20187666v1

- Purpose: The genetic locus 3p21.31 has been associated with severe coronavirus disease 2019 (COVID-19), but the underlying pathophysiological mechanism is unknown. Methods: To identify intermediate traits of the COVID-19 risk variant, we performed a phenome-wide association study (PheWAS) with 923 phenotypes in 310,999 European individuals from UK Biobank. For candidate target genes, we examined associations between their expression and the polygenic score (PGS) of 1,263 complex traits in a meta-analysis of 31,684 blood samples. Results: Our PheWAS identified and replicated multiple blood cell traits to be associated with the COVID-19 risk variant, including monocyte count and percentage (p = 1.07e-8, 4.09e-13), eosinophil count and percentage (p = 5.73e-3, 2.20e-3), and neutrophil percentage (p = 3.23e-3). The PGS analysis revealed positive associations between the expression of candidate genes and genetically predicted counts of specific blood cells: CCR3 with eosinophil and basophil (p = 5.73e-21, 5.08e-19); CCR2 with monocytes (p = 2.40e-10); and CCR1 with monocytes and neutrophil (p = 1.78e-6, 7.17e-5). Conclusions*: Multiple blood cell traits, especially monocyte, eosinophil, and neutrophil numbers, are associated with the COVID-19 risk variant and the expression of its candidate target genes, representing probable mechanistic links between the genetic locus 3p21.31 and severe COVID-19.* [**note: this paper looks at altered blood cell traits that may be predictive of severe COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.09.09.20191700v1

DRUG DEVELOPMENT

- We report the development and evaluation of safety and immunogenicity of a whole virion inactivated SARS-COV-2 vaccine (BBV152), adjuvanted with aluminium hydroxide gel (Algel), or a novel TLR7/8 agonist adsorbed Algel. We used a well-characterized SARS-CoV-2 strain and an established vero cell platform to produce large-scale GMP grade highly purified inactivated antigen, BBV152. Product development and manufacturing were carried out in a BSL-3 facility. Immunogenicity was determined at two antigen concentrations (3µg and 6µg), with two different adjuvants, in mice, rats, and rabbits. Our results show that BBV152 vaccine formulations generated significantly high antigen-binding and neutralizing antibody titers, at both concentrations, in all three species with excellent safety profiles. The inactivated vaccine formulation containing TLR7/8 agonist adjuvant-induced Th1 biased antibody responses with elevated IgG2a/IgG1 ratio and increased levels of SARS-CoV-2 specific IFN-γ+ CD4 T lymphocyte response. Our results support further development for Phase I/II clinical trials in humans. [**note: this is from India and uses traditional technology to generate an inactivated whole virion vaccine candidate.**] https://www.biorxiv.org/content/10.1101/2020.09.09.285445v1
- Recombinant forms of the spike protein of SARS-CoV-2 and related viruses have proven difficult to produce with good yields in mammalian cells. Given the panoply of potential COVID-19 diagnostic tools and therapeutic candidates that require purified spike protein and its importance for ongoing SARS-CoV-2 research, we have explored new approaches for spike production and purification. Three transient gene expression methods based on PEI-mediated transfection of CHO or HEK293 cells in suspension culture in chemically-defined media were compared for rapid production of full-length SARS-CoV-2 ectodomain. A high-cell-density

protocol using DXB11-derived CHOBRI/rcTA cells gave substantially better yields than the other methods. Different forms of the spike were expressed, including the wild-type SARS-CoV-2 sequence and a mutated/stabilized form (to favor expression of the full-length spike in pre-fusion conformation), with and without fusion to putative trimerization domains. An efficient two-step affinity purification method was also developed. Ultimately, we have been able to produce highly homogenous preparations of full-length spike, both monomeric and trimeric, with yields of 100-150 mg/L. The speed and productivity of this method support further development of CHO-based approaches for recombinant spike protein manufacturing. [**note: from Canada, this may be more of an important research finding. They can produce high levels of the Spike protein in CHO cells.**]
https://www.biorxiv.org/content/10.1101/2020.09.08.286732v1

- The SARS-CoV-2 pandemic poses an unprecedented public health crisis. Accumulating evidences suggest that SARS-CoV-2 infection causes dysregulation of immune system. However, the unique signature of early immune responses remains elusive. We characterized the transcriptome of rhesus macaques and mice infected with SARS-CoV-2. Alarmin S100A8 was robustly induced by SARS-CoV-2 in animal models as well as in COVID-19 patients. Paquinimod, a specific inhibitor of S100A8/A9, could reduce inflammatory response and rescue the pneumonia with substantial reduction of viral titers in SASR-CoV-2 infected animals. Remarkably, Paquinimod treatment resulted in 100% survival of mice in a lethal model of mouse coronavirus (MHV) infection. A novel group of neutrophils that contributed to the uncontrolled inflammation and onset of COVID-19 were dramatically induced by coronavirus infections. Paquinimod treatment could reduce these neutrophils and regain antiviral responses, unveiling key roles of S100A8/A9 and noncanonical neutrophils in the pathogenesis of COVID-19, highlighting new opportunities for therapeutic intervention. [**note: this is a drug designed to treat Lupus but I'm unsure whether it has been approved anywhere.**]
https://www.biorxiv.org/content/10.1101/2020.09.09.288704v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- SARS-CoV-2 specific IgG responses play critical roles for patients to recover from COVID-19, in-depth dissecting of the IgG responses on systems level is of great interest. Herein, we adopted a newly developed high-throughput epitope mapping technology (AbMap), analyzed 55 COVID-19 convalescent sera and 226 antibody samples enriched by specific proteins or peptides from these sera. We revealed three areas that are rich of IgG epitopes, two are on Spike protein but outside of RBD, and one is on Nucleocapsid protein. We identified 29 significant epitopes on Spike protein, from two of these significant epitopes, two critical epitope residues were found, i. e., D936 and P1263, which are highly related to the infectivity of SARS-CoV-2. In summary, we provided the first global map of IgG binding epitopes for SARS-CoV-2 at single amino acid resolution. This map will facilitate the precise development of therapeutic antibodies and vaccines. [**note: here is a useful paper from China on high-throughput epitope mapping technology. This is good stuff as it identifies down to a fairly precise level antibody interactions with the virus.**]
https://www.medrxiv.org/content/10.1101/2020.09.08.20190496v1
- With a rising incidence of COVID-19-associated morbidity and mortality worldwide, it is critical to elucidate the innate and adaptive immune responses that drive disease severity. We

performed longitudinal immune profiling of peripheral blood mononuclear cells from 45 patients and healthy donors. We observed a dynamic immune landscape of innate and adaptive immune cells in disease progression and absolute changes of lymphocyte and myeloid cells in severe versus mild cases or healthy controls. Intubation and death were coupled with selected natural killer cell KIR receptor usage and IgM+ B cells and associated with profound CD4 and CD8 T cell exhaustion. Pseudo-temporal reconstruction of the hierarchy of disease progression revealed dynamic time changes in the global population recapitulating individual patients and the development of an eight-marker classifier of disease severity. Estimating the effect of clinical progression on the immune response and early assessment of disease progression risks may allow implementation of tailored therapies. [**note: here is a nother immune profiling paper of mild and severe COVID-19 showing innate and adaptive immune dysfunction.**]
https://www.medrxiv.org/content/10.1101/2020.09.08.20189092v1

- The durability of infection-induced SARS-CoV-2 immunity has major implications for public health mitigation and vaccine development. Animal studies and the scarcity of confirmed re-infection suggests immune protection is likely, although the durability of this protection is debated. Lasting immunity following acute viral infection requires maintenance of both serum antibody and antigen-specific memory B and T lymphocytes and is notoriously pathogen specific, ranging from life-long for smallpox or measles4, to highly transient for common cold coronaviruses (CCC). Neutralising antibody responses are a likely correlate of protective immunity and exclusively recognise the viral spike (S) protein, predominantly targeting the receptor binding domain (RBD) within the S1 sub-domain. Multiple reports describe waning of S-specific antibodies in the first 2-3 months following infection. However, extrapolation of early linear trends in decay might be overly pessimistic, with several groups reporting that serum neutralisation is stable over time in a proportion of convalescent subjects. While SARS-CoV-2 specific B and T cell responses are readily induced by infection, the longitudinal dynamics of these key memory populations remains poorly resolved. Here we comprehensively profiled antibody, B and T cell dynamics over time in a cohort recovered from mild-moderate COVID-19. We find that binding and neutralising antibody responses, together with individual serum clonotypes, decay over the first 4 months post-infection, as expected, with a similar decline in S-specific CD4+ and circulating T follicular helper (cTFH) frequencies. In contrast, S-specific IgG+ memory B cells (MBC) consistently accumulate over time, eventually comprising a significant fraction of circulating MBC. Modelling of the concomitant immune kinetics predicts maintenance of serological neutralising activity above a titre of 1:40 in 50% of convalescent subjects to 74 days, with probable additive protection from B and T cells. *Overall, our study suggests SARS-CoV-2 immunity after infection is likely t 66 o be transiently protective at a population level. SARS-CoV-2 vaccines may require greater immunogenicity and durability than natural infection to drive long-term protection.* [**note: this is an Australian paper on the evolution of immunity to SARS-CoV-2. Note the argument for strong vaccines.**]
https://www.medrxiv.org/content/10.1101/2020.09.09.20191205v1
- SARS-CoV-2 entry in human cells is mediated by the interaction between the viral Spike protein and the human ACE2 receptor. This mechanism evolved from the ancestor bat coronavirus and is currently one of the main targets for antiviral strategies. However, there currently exist several Spike protein variants in the SARS-CoV-2 population as the result of mutations, and it is unclear if these variants may exert a specific effect on the affinity with ACE2 which, in turn, is

also characterized by multiple alleles in the human population. In the current study, the GBPM analysis, originally developed for highlighting host-guest interaction features, has been applied to define the key amino acids responsible for the Spike/ACE2 molecular recognition, using four different crystallographic structures. Then, we intersected these results with the current mutational status of these amino acids in the SARS-CoV-2 population. We identified several Spike mutations interacting with ACE2 and mutated in at least 10 distinct patients: N439K, G476S, S477N and N501Y. We also identified five ACE2 rare variants that may affect interaction with Spike and susceptibility to infection: S19P, E37K, M82I, E329G and G352V. [**note: this Italian group looks at several mutations in SARS-CoV-2 spike protein from patients. It will be useful to continue this line of research in other regions to track the virus and whether it is becoming less virulent over time.**]
https://www.biorxiv.org/content/10.1101/2020.09.09.289074v1

DIAGNOSTIC DEVELOPMENT

- Nothing

>>

2020-09-11

Continuing with Bellini's 'Casta Diva', the featured artist today is the great Maria Callas, the American-born Greek soprano who never met a role she couldn't sing. One of her debut roles in Italy was Brünnhilde in Wagner's Die Walküre! Her personal life was the stuff of soap opera, you really wondered what was really going on. Her prime singing years were during the 1950s and there are lots of conjecture about vocal deterioration that became prevalent during the latter part of the decade (rapid weight loss, singing too many heavy/light roles, lost confidence, early menopause; take your choice). I saw her in 1974 during the tour she was doing with Giuseppe di Stefano, and that was a total train wreck as neither singer had much to give. Here she is singing with a choir that cannot follow the conductor and she has to make things right: https://www.youtube.com/watch?v=TYl8GRJGnBY it is from a Paris concert in 1958. Tomorrow, my favorite current singer who is as good as the 'greats' who preceded her.

The Washington Post has story about possible COVID-19 cases in Los Angeles last December. Count me as being skeptical about this one. From what we know about the infectivity, one would have expected far more hospitalizations. Enhanced coronavirus screening of oversees airline passengers is ending in the US. I hope it is not replaced by a wink and nod system.

The New York Times has the cautionary story about the University of Illinois reopening. The school had probably the best designed virus testing program of any major school but still couldn't control aberrant student behavior. As you know, I am following Mitch Daniels's Purdue University. In the past seven days they have conducted just over 5000 COVID-19 tests with 138 positives. I think Purdue is roughly the same size as Illinois, but they are doing far less testing. The latest fight over school reopening is taking place in Des Moines where the Governor wants all schools in the state to open but the school superintendent in the city wants to insure student and teacher safety. FDA regulators take the unprecedented step (to my knowledge) of defending their decisions during the COVID-19 pandemic. Here is the USA Today opinion column signed by the various center directors. China is engaging in

'vaccine diplomacy' by conducting clinical trials in a number of neighboring countries. China just approved a clinical trial for a nasally delivered COVID-19 vaccine. I know that a nasal vaccine has been developed by researchers at Washington Univ in St. Louis but it has not moved into trials.

The New Yorker has a good article on the state of the fight against COVID-19 and looks at vaccine development. Atul Gawande weighs in on the testing mess.

STAT note that persistent symptoms following viral infections may be common but difficult to study. This should not deter us and I will note the OHDSI group is soliciting volunteers to set up some protocols to do exactly this kind of work. Here is a nice opinion piece on real time gene sequencing as a tool to unravel the secrets of pandemic microorganisms.

The Lancet has an editorial on curing COVID-19. Yes, we need new oral anti-virals! Here is a commentary on vaccine confidence and a profile of the professor who set up the Vaccine Confidence Project.

Speaking of vaccines (and who isn't these days), Medscape has a piece on 'celebrating COVID vaccine development rather than criticizing it. I agree 100%! It's amazing to see the panoply of vaccines under development both in the research stage and in clinical trials.

Kaiser Health News discusses the risk of catching COVID-19 on an airplane.

MODELING

- CO2 is co-exhaled with aerosols containing SARS-CoV-2 by COVID-19 infected people and can be used as a proxy of SARS-CoV-2 concentrations indoors. Indoor CO2 measurements by low-cost sensors hold promise for mass monitoring of indoor aerosol transmission risk for COVID-19 and other respiratory diseases. We derive analytical expressions of CO2-based risk proxies and apply them to various typical indoor environments. Contrary to some earlier recommendations setting a single indoor CO2 threshold, we show that the CO2 level corresponding to a given infection risk varies by over 2 orders of magnitude for different environments and activities. Although large uncertainties, mainly from virus exhalation rates, are still associated with our infection risk estimates, our study provides more specific and practical recommendations for low-cost CO2-based indoor infection risk monitoring. [**note: this is an interesting paper that uses exhaled CO2 as and infection risk proxy for different indoor environments. They apply the model to some real life situations. It's a short paper and the math is not super difficult.]** https://www.medrxiv.org/content/10.1101/2020.09.09.20191676v1

NEWLY REGISTERED CLINICAL TRIALS

- Recent observations have suggested a role of neutrophil extracellular traps (NETs) in the pathophysiology of severe COVID-19. The aim of the study is to assess efficacy and safety of aerosolized DNase I to remove NETs and decrease respiratory distress in patients with COVID-19. [**note: this is a Swedish trial.**] NCT04541979
- This is a phase 2/3 study in which subjects with coronavirus disease 2019 (COVID-19) will receive VIR-7831 or placebo and will be assessed for safety, tolerability, efficacy, and pharmacokinetics. [**note: this is another mAb trial from Vir Biotechnology and GSK**] NCT04545060

CLINICAL TRIAL RESULTS

- Unfortunately, nothing new today.

DRUG DEVELOPMENT

- The COVID-19 pandemic caused by the SARS-CoV-2 requires a fast development of antiviral drugs. SARS-CoV-2 viral main protease (Mpro, also called 3C-like protease, 3CLpro) is a potential target for drug design. Crystal and co-crystal structures of the SARS-CoV-2 Mpro have been solved, enabling the rational design of inhibitory compounds. In this study we analyzed the available SARS-CoV-2 and the highly similar SARS-CoV-1 crystal structures. We identified within the active site of the Mpro, in addition to the inhibitory ligands interaction with the catalytic C145, two key H-bond interactions with the conserved H163 and E166 residues. Both H-bond interactions are present in almost all co-crystals and are likely to occur also during the viral polypeptide cleavage process as suggested from docking of the Mpro cleavage recognition sequence. We screened in silico a library of 6,900 FDA-approved drugs (ChEMBL) and filtered using these key interactions and selected 29 non-covalent compounds predicted to bind to the protease. Additional screen, using DOCKovalent was carried out on DrugBank library (11,414 experimental and approved drugs) and resulted in 6 covalent compounds. The selected compounds from both screens were tested in vitro by a protease activity inhibition assay. Two compounds showed activity at the 50microM concentration range. Our analysis and findings can facilitate and focus the development of highly potent inhibitors against SARS-CoV-2 infection. [**note: more work on identifying inhibitors to the Mpro enzyme from Israel. The two best inhibitors from this study were bicalutamide (already registered in a Univ of Florida clinical trial) and an investigational GSK drug that inhibits phosphodiesterase 4 and is nasally or orally delivered.}** https://www.biorxiv.org/content/10.1101/2020.09.10.288720v1
- The main protease (M^{pro}) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an attractive target for antiviral therapeutics. Recently, many high-resolution apo and inhibitor-bound structures of M^{pro}, a cysteine protease, have been determined, facilitating structure-based drug design. M^{pro} plays a central role in the viral life cycle by catalyzing the cleavage of SARS-CoV-2 polyproteins. In addition to the catalytic dyad His41-Cys145, M^{pro} contains multiple histidines including His163, His164, and His172. The protonation states of these histidines and the catalytic nucleophile Cys145 have been debated in previous studies of SARS-CoV M^{pro}, but have yet to be investigated for SARS-CoV-2. In this work we have used molecular dynamics simulations to determine the structural stability of SARS-CoV-2 M^{pro} as a function of the protonation assignments for these residues. We simulated both the apo and inhibitor-bound enzyme and found that the conformational stability of the binding site, bound inhibitors, and the hydrogen bond networks of M^{pro} are highly sensitive to these assignments. Additionally, the two inhibitors studied, the peptidomimetic N3 and an α-ketoamide, display distinct His41/His164 protonation-state-dependent stabilities. While the apo and the N3-bound systems favored N_δ (HD) and N_ϵ (HE) protonation of His41 and His164, respectively, the α-ketoamide was not stably bound in this state. Our results illustrate the importance of using appropriate histidine protonation states to accurately model the structure and dynamics of SARS-CoV-2 M^{pro} in both the apo and inhibitor-bound states, a necessary prerequisite for drug-design efforts. [**note: more work at identifying how various binding points of MPro inhibitors work.**] https://www.biorxiv.org/content/10.1101/2020.09.07.286344v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has caused substantially more infections, deaths, and economic disruptions than the 2002-2003 SARS-CoV. The key to understanding SARS-CoV-2's higher infectivity may lie in its host receptor recognition mechanism. This is because experiments show that the human ACE2 protein, which serves as the primary receptor for both CoVs, binds to CoV-2's spike protein 5-20 fold stronger than SARS-CoV's spike protein. The molecular basis for this difference in binding affinity, however, remains unexplained and, in fact, a comparison of X-ray structures leads to an opposite proposition. To gain insight, we use all-atom molecular dynamics simulations. Free energy calculations indicate that CoV-2's higher affinity is due primarily to differences in specific spike residues that are local to the spike-ACE2 interface, although there are allosteric effects in binding. Comparative analysis of equilibrium simulations reveals that while both CoV and CoV-2 spike-ACE2 complexes have similar interfacial topologies, CoV-2's spike protein engages in greater numbers, combinatorics and probabilities of hydrogen bonds and salt bridges with ACE2. *We attribute CoV-2's higher affinity to these differences in polar contacts, and these findings also highlight the importance of thermal structural fluctuations in spike-ACE2 complexation. We anticipate that these findings will also inform the design of spike-ACE2 peptide blockers that, like in the cases of HIV and Influenza, can serve as antivirals*. [**note: this is a decent model for affinity binding of SARS-CoV-2 to the ACE2 interface and it may inform development of potential inhibitors.**] https://www.biorxiv.org/content/10.1101/2020.09.10.291757v1
- Antibodies targeting the SARS-CoV-2 spike receptor-binding domain (RBD) are being developed as therapeutics and make a major contribution to the neutralizing antibody response elicited by infection. Here, we describe a deep mutational scanning method to map how all amino-acid mutations in the RBD affect antibody binding, and apply this method to 10 human monoclonal antibodies. The escape mutations cluster on several surfaces of the RBD that broadly correspond to structurally defined antibody epitopes. However, even antibodies targeting the same RBD surface often have distinct escape mutations. The complete escape maps predict which mutations are selected during viral growth in the presence of single antibodies, and enable us to design escape-resistant antibody cocktails--including cocktails of antibodies that compete for binding to the same surface of the RBD but have different escape mutations. Therefore, complete escape-mutation maps enable rational design of antibody therapeutics and assessment of the antigenic consequences of viral evolution. [**note: this is an interesting paper that looks at the possible mutations in the spike receptor binding domain and how this can help in rational design of antibody therapeutics.**] https://www.biorxiv.org/content/10.1101/2020.09.10.292078v1

DIAGNOSTIC DEVELOPMENT

- Nothing New

>>

2020-09-12

I know that everyone was patiently waiting to find out who the star Norma of today is. It is none other than the wonderful Sondra Radvanovsky. I saw her live in the Washington Opera production of

Donizetti's Anna Bolena and she was simply spectacular. Here is a concert performance of 'Casta Diva': https://www.youtube.com/watch?v=RgUINdGLPFA and here she is on stage at the Met: https://www.youtube.com/watch?v=VF-NapCY8WM and why not also from Barcelona: https://www.youtube.com/watch?v=hqMBNYdpXEs

The Washington Post covers the politicization of convalescent plasma. Is anything associated with COVID-19 not political these days? The spectre of a double whammy of seasonal influenza and COVID-19 may pose problems for hospitals this winter.

The New York Times has a first-person account of being in a COVID-19 vaccine trial; she is even a Times reporter!

For those of you who would like a long form story on the China Syndrome, a rough history of what happened from the beginning, this series by Philippe Lemoine is excellent. Well worth reading!!

Science has a lengthy paper on the emergence of SARS-CoV-2 in Europe and North America that is well worth reading. For all my readers who are in the public health field here is the money quote: "Our analyses demonstrate the effectiveness of public health measures in preventing onward transmission **and show that intensive testing and contact tracing could have prevented SARS-CoV-2 from becoming established**." They got this one right!! Here is a nice genomic analysis of the early outbreak in Washington state that likely came from a single introduction in late January or early February. These researchers take an interesting approach to SARS-CoV-2 treatment by designing picomolar miniprotein inhibitors that can be delivered nasally. They conducted some inhibition studies and find that these selected compounds are more potent than the best of the current SARS-CoV-2 mAb. Get these into clinical trials, and I'll volunteer; I could just add this to my fluticasone spray!

STAT have an interesting article on how to find the causes of post-COVID-19 symptoms.

JAMA have a research study from Ohio State on the incidence of myocarditis in athletes who contracted COVID-19. Of 26 competitive athletes, 4 (15%) had CMR findings suggestive of myocarditis and 8 additional athletes (30.8%) exhibited LGE without T2 elevation suggestive of prior myocardial injury. COVID-19–related myocardial injury in competitive athletes and sports participation remains unclear. Cardiac magnetic resonance imaging has the potential to identify a high-risk cohort for adverse outcomes and may, importantly, risk stratify athletes for safe participation because CMR mapping techniques have a high negative predictive value to rule out myocarditis. [**note: this is something that should be followed as the number of college athletes is small and many of the universities have medical schools that could undertake this type of research.**] Here is a very nice viewpoint of COVID-19 and the road to immunity in an understandable format.

Medscape offer a summary of Dr. Fauci's briefing to the Society of Critical Care Medicine. 40-50% of the cases of COVID-19 are asymptomatic. Infectious SARS-CoV-2 may persist in the GI tract for weeks following infection. Put the toilet seat down before you flush and wash your hands!

Former CDC director, Julie Gerberding has a piece in the Annals of Internal Medicine on the importance of vital statistics in measuring the pandemic's impact.

Derek Lowe on drug repurposing, how often does it work? Not often at all and I've been skeptical of almost all the SARS-CoV-2 papers that have come out since mid-March. Drug development is hard work and there are lots of failures along the way.

MODELING

- Airborne transmission is an important transmission pathway for viruses, including SARS-CoV-2. Regions with a higher proportion of people wearing masks show better control of COVID-19, but the effectiveness of masks is still under debate due to their limited and variable efficiencies in removing respiratory particles. Here, we analyze experimental data and perform model calculations to show that this contrast can be explained by the different abundance regimes between particles and viruses. Upon short-term exposure, respiratory particles are usually in a particle-rich regime, but respiratory viruses are often in a virus-limited regime where the numbers of viruses inhaled by susceptible people are below or close to the infectious dose. This virus-limited regime ensures mask efficacy and synergy of multiple preventive measures in reducing the infection risk. [**note: I always enjoy reading papers that who why masks work for preventing COVID-19. This is one of those.**
https://www.medrxiv.org/content/10.1101/2020.09.10.20190348v1
- Despite considerable research progress on SARS-CoV-2, the direct zoonotic origin (intermediate host) of the virus remains ambiguous. The most definitive approach to identify the intermediate host would be the detection of SARS-CoV-2-like coronaviruses in wild animals. However, due to the high number of animal species, it is not feasible to screen all the species in the laboratory. Given that the recognition of the binding ACE2 proteins is the first step for the coronaviruses to invade host cells, we proposed a computational pipeline to identify potential intermediate hosts of SARS-CoV-2 by modeling the binding affinity between the Spike receptor-binding domain (RBD) and host ACE2. Using this pipeline, we systematically examined 285 ACE2 variants from mammals, birds, fish, reptiles, and amphibians, and found that the binding energies calculated on the modeled Spike-RBD/ACE2 complex structures correlate closely with the effectiveness of animal infections as determined by multiple experimental datasets. Built on the optimized binding affinity cutoff, we suggested a set of 96 mammals, including 48 experimentally investigated ones, which are permissive to SARS-CoV-2, with candidates from primates, rodents, and carnivores at the highest risk of infection. Overall, this work not only suggested a limited range of potential intermediate SARS-CoV-2 hosts for further experimental investigation; but more importantly, it proposed a new structure-based approach to general zoonotic origin and susceptibility analyses that are critical for human infectious disease control and wildlife protection. [**note: here is a nice study from Michigan on possible mammals that might be hosts for SARS-CoV-2. This may help identify the zoonotic origin of the virus.**]
https://www.biorxiv.org/content/10.1101/2020.09.11.293449v1

NEWLY REGISTERED CLINICAL TRIALS

- Did not check today.

CLINICAL TRIAL RESULTS

- Background: Sex-disaggregated data suggest that men with coronavirus disease 2019 (COVID-19) are more likely to die than women. Whether circulating testosterone or sex hormone-

binding globulin (SHBG) contributes to such sex differences remains unknown. Objective: To evaluate the associations of circulating total testosterone (TT), free testosterone (FT), and SHBG with COVID-19 mortality. Design: Prospective analysis. Setting: UK Biobank. Participants: We included 1306 COVID-19 patients (678 men and 628 women) who had serum TT and SHBG measurements and were free of cardiovascular disease or cancer at baseline (2006-2010). Main outcome measures: The death cases of COVID-19 were identified from National Health Service death records updated at 31 July 2020. Unconditional logistic regression was performed to estimate the odds ratio (OR) and 95% confidence intervals (CI) for mortality. Results: We documented 315 deaths of COVID-19 (194 men and 121 women). After adjusting for potential confounders, we did not find any statistically significant associations for TT (OR per 1-SD increase = 1.03, 95% CI: 0.85-1.25), FT (OR per 1-SD increase = 0.95, 95% CI: 0.77-1.17), or SHBG (OR per 1-SD increase = 1.09, 95% CI: 0.87-1.37) with COVID-19 mortality in men. Similar null results were observed in women (TT: OR per 1-SD increase = 1.10, 95% CI: 0.85-1.42; FT: OR per 1-SD increase = 1.10, 95% CI: 0.82-1.46; SHBG: OR per 1-SD increase = 1.16, 95% CI: 0.89-1.53). Conclusions: Our findings do not support a significant role of circulating testosterone or SHBG in COVID-19 prognosis. [**note: this is data from the UK biobank shows no association between circulating levels of testosterone and sex hormone binding globulin and COVID-19 mortality.**] https://www.medrxiv.org/content/10.1101/2020.09.11.20191783v1

- Objectives: The prevalence of SARS-CoV-2 antibodies in the general population is largely unknown. Since many infections, even among the elderly and other vulnerable populations, are asymptomatic, the prevalence of antibodies could help determine how far along the path to herd immunity the general population has progressed. Also, in order to clarify the clinical manifestations of current or recent past COVID-19 illness, it may be useful to determine if there are any common alterations in routine clinical laboratory values. Methods: We performed SARS-CoV-2 antibody tests on 50,130 consecutive life insurance applicants who were having blood drawn for the purpose of underwriting (life risk assessment). Subjects were also tested for lipids, liver function tests, renal function studies, as well as serum proteins. Other variables included height, weight, blood pressure at the time of the blood draw, and history of common chronic diseases (hypertension, heart disease, diabetes, and cancer). Results: The overall prevalence of SARS-CoV-2 was 3.0%, and was fairly consistent across the age range and similar in males and females. Several of the routine laboratory tests obtained were significantly different in antibody-positive vs. antibody-negative subjects, including albumin, globulins, bilirubin, and the urine albumin:creatinine ratio. The BMI was also significantly higher in the antibody-positive group. Geographical distribution revealed a very high level of positivity in the state of New York compared to all other areas (17.1%). Using state population data from the US Census, it is estimated that this level of seropositivity would correspond to 6.98 million (99% CI: 6.56-7.38 million) SARS-CoV-2 infections in the US, which is 3.8 times the cumulative number of cases in the US reported to the CDC as of June 1, 2020. Conclusions: The estimated number of total SARS-CoV-2 infections based on positive serology is substantially higher than the total number of cases reported to the CDC. Certain laboratory values, particularly serum protein levels, are associated with positive serology, though these associations are not likely to be clinically meaningful. [**note: I totally forgot that blood draws from life insurance policy applicants might be a good data source! Well, here is one example. 50K people in this sample and they do a reasonable job at extrapolating this to come up with the total number of infections as of June**

1. I find this number reasonable as the multiplier is close to 4]
https://www.medrxiv.org/content/10.1101/2020.09.09.20191296v1

- OBJECTIVE: Nearly 5 % of the patients with COVID-19 develop an acute respiratory distress syndrome (ARDS). Extravascular lung water index (EVLWI) is a marker of pulmonary oedema which is associated with mortality in ARDS. In this study we evaluate whether EVLWI is higher in patients with COVID-19 associated ARDS as compared to controls and whether EVLWI has the potential to monitor disease progression. METHODS: From the day of intubation, EVLWI, cardiac function were monitored by transpulmonary thermodilution in n=25 patients with COVID-19 and compared to a control group of 49 non-COVID-19 ARDS-patients. RESULTS: EVLWI in COVID-19-patients was noticeably elevated and significantly higher than in the control group (17 (11-38) vs. 11 (6-26) mL/kg; p<0.001). High pulmonary vascular permeability index values (2.9 (1.0-5.2) versus 1.9 (1.0-5.2); p=0.003) suggest inflammatory oedema. By contrast, the cardiac parameters SVI, GEF and GEDVI were comparable. High EVLWI values were associated with viral persistence, prolonged intensive care treatment and mortality (23.2±6.7% vs. 30.3±6.0%, p=0.025). CONCLUSIONS: Compared to the control group, COVID-19 results in markedly elevated EVLWI-values in patients with ARDS. EVLWI reflects a non-cardiogenic pulmonary oedema in COVID-19 associated ARDS and could serve as parameter to monitor ARDS progression. [**note: from Germany and another maker for COVID-19 acute respiratory distress syndrome.]**
https://www.medrxiv.org/content/10.1101/2020.09.11.20192526v1
- COVID-19 respiratory infections are associated with copious, adherent respiratory secretions that prolong chronic ventilation and contribute to the morbidity and mortality caused by the disease. We hypothesized that hyaluronan, an extracellular matrix glycosaminoglycan produced at sites of active inflammation that promotes edema in other settings, might be a component of these secretions. To interrogate this, we examined the respiratory secretions collected from eight intubated patients with COVID-19, six control patients with cystic fibrosis (CF), a different respiratory disease also associated with thick adherent secretions, and eight healthy controls. In this sample set we found that hyaluronan content is increased approximately 20-fold in both CF and COVID-19 patients compared to healthy controls. The hyaluronan in COVID-19 samples was comprised of low-molecular weight fragments, the hyaluronan form most strongly linked with pro-inflammatory functions. Hyaluronan is similarly abundant in histologic sections from cadaveric lung tissue from COVID-19 patients. These findings implicate hyaluronan in the thick respiratory secretions characteristic of COVID-19 infection. Therapeutic strategies targeting hyaluronan should be investigated further for potential use in patients with COVID-19. [**note: more information on disease progression, this time it is hyaluronan.**]
https://www.medrxiv.org/content/10.1101/2020.09.11.20191692v1

DRUG DEVELOPMENT

- The spread of SARS-CoV-2 confers a serious threat to the public health without effective intervention strategies. Its variant carrying mutated Spike (S) protein D614G (SD614G) has become the most prevalent form in the current global pandemic. We have identified a large panel of potential neutralizing antibodies (NAbs) targeting the receptor-binding domain (RBD) of SARS-CoV-2 S. Here, we focused on the top 20 potential NAbs for the mechanism study. Of them, the top 4 NAbs could individually neutralize both authentic SARS-CoV-2 and SD614G pseudovirus efficiently. Our epitope mapping revealed that 16/20 potent NAbs overlapped the

same steric epitope. Excitingly, we found that one of these potent NAbs (58G6) exclusively bound to a linear epitope on S-RBD (termed as 58G6e), and the interaction of 58G6e and the recombinant ACE2 could be blocked by 58G6. We confirmed that 58G6e represented a key site of vulnerability on S-RBD and it could positively react with COVID-19 convalescent patients plasma. We are the first, as far as we know, to provide direct evidences of a linear epitope that can be recognized by a potent NAb against SARS-CoV-2 S-RBD. This study paves the way for the applications of these NAbs and the potential safe and effective vaccine design. [**note: identification of the binding epitope of some neutralizing antibodies.**] https://www.biorxiv.org/content/10.1101/2020.09.11.292631v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- By interrogating metabolic programs in the peripheral blood mononuclear cells (PBMC) of acutely infected COVID-19 patients, we identified novel and distinct immune cell subsets Our studies identified a non-clonal population of T cells expressing high H3K27me3 and voltage-dependent anion channel (VDAC) with mitochondrial dysfunction and increased susceptibility to cell death. Characterized by dysmorphic mitochondria and increased cytoplasmic cytochrome c, apoptosis of these cells was inhibited by preventing VDAC aggregation or blocking caspase activation. *Further, we observed a marked increase in Hexokinase II+ polymorphonuclear-myeloid derived suppressor cells (PMN-MDSC). While PMN-MDSC were also found in the PBMC of patients with other viral infections, the Hexokinase II+ PMN-MDSC were found exclusively in the acute COVID-19 patients with moderate or severe disease. Finally, we identified a population of monocytic MDSC (M-MDSC) expressing high carnitine palmitoyltransferase I (CPT1a) and VDAC, which were present in the PBMC of the acute COVID-19 patients, but not recovered COVID-19 patients and whose presence correlated with severity of disease. Overall, these unique populations of immune cells provide insight into the pathogenesis of SARS-CoV-2 infection and provide a means to predict and track disease severity as well as an opportunity to design and evaluate novel therapeutic regimens*. [**note: here is another immune system finding that can predict and track disease severity.**] https://www.medrxiv.org/content/10.1101/2020.09.10.20186064v1
- Although human antibodies elicited by severe acute respiratory distress syndrome coronavirus-2 (SARS-CoV-2) nucleocapsid (N) protein are profoundly boosted upon infection, little is known about the function of N-directed antibodies. *Herein, we isolated and profiled a panel of 32 N protein-specific monoclonal antibodies (mAb) from a quick recovery coronavirus disease-19 (COVID-19) convalescent, who had dominant antibody responses to SARS-CoV-2 N protein rather than to Spike protein. The complex structure of N protein RNA binding domain with the highest binding affinity mAb nCoV396 reveals the epitopes and antigen's allosteric changes. Functionally, a virus-free complement hyper-activation analysis demonstrates that nCoV396 specifically compromises N protein-induced complement hyper-activation, a risk factor for morbidity and mortality in COVID-19,* thus paving the way for functional anti-N mAbs identification. [**note: this is an interesting paper from China showing the importance of antibodies against the nucleocapsid (N) protein.** https://www.biorxiv.org/content/10.1101/2020.09.10.292318v1
- The ongoing SARS-CoV-2 pandemic has devastated the global economy and claimed nearly one million lives, presenting an urgent global health crisis. To identify host factors required for infection by SARS-CoV-2 and seasonal coronaviruses, we designed a focused high-coverage

CRISPR-Cas9 library targeting 332 members of a recently published SARS-CoV-2 protein interactome. We leveraged the compact nature of this library to systematically screen four related coronaviruses (HCoV-229E, HCoV-NL63, HCoV-OC43 and SARS-CoV-2) at two physiologically relevant temperatures (33°C and 37°C), allowing us to probe this interactome at a much higher resolution relative to genome scale studies. This approach yielded several new insights, including unexpected virus and temperature specific differences in Rab GTPase requirements and GPI anchor biosynthesis, as well as identification of multiple pan-coronavirus factors involved in cholesterol homeostasis. This coronavirus essentiality catalog could inform ongoing drug development efforts aimed at intercepting and treating COVID-19, and help prepare for future coronavirus outbreaks. [**note: from Rockefeller University, a CRISPR study of the host protein interactome that may be useful for drug development.**]
https://www.biorxiv.org/content/10.1101/2020.09.11.291716v1

DIAGNOSTIC DEVELOPMENT

- Nothing new today

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

2020-09-13

For this reflection Sunday let us have a listen to Beethoven's Piano Concerto #5, The Emperor. Here is Vladimir Ashkenazy with the London Philharmonic, conducted by Bernard Haitink in a 1974 performance. Magisterial: https://www.youtube.com/watch?v=V4pJxad_aI8

I can attest to the veracity of this Washington Post story on time being broken in the pandemic era. Looking for a good investment? Companies that make robot cleaners might be the place to look. In Pennsylvania there is a rebellion going on regarding the reopening of bars and restaurants. This is a difficult issue without an adequate solution as we know the risk of infection by SARS-CoV-2 increases in indoor spaces where the density of people is increased.

The New York Times has more on the politics of COVID-19 vaccine policy. My guidance: shut up, let the trials go forward and allow the FDA to do its job! COVID-19 cases are spiking in the Midwest. This well-done article discusses the ramifications of remote learning for students.

This report from Politico is Orwellian. CDC reports should be done by CDC without outside interference.

STAT notes the AZ/Oxford vaccine trials have resumed after a brief hold to investigate one severe adverse reaction. This is good news from a scientific viewpoint; Pfizer and BioNTech announced plans to expand their COVID-19 vaccine trial. The trial size will be increased to 44K up from the initial recruitment goal of 30K in order to gather more safety and efficacy data.

The Lancet's editor, Richard Horton, has an editorial well worth reading. Science and not rhetoric should be the foundation of the fight against COVID-19. Here is a rebuttal letter to an earlier paper discounting the role of ordinary speech in transmission of SARS-Cov-2.

Medscape have this story about how cetirizine and famotidine in combination appear to reduce some COVID-19 symptoms. I've not read the original paper. Treatment using H1/H2 blockers has been

commonly used to treat a number of different allergic conditions. I will be more convinced of this approach when the data from the large famotidine trial at Northwell Health is released.

MODELING

- Ray and A. Reddy recently anticipated the implication of circadian rhythm in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is the causative agent of the coronavirus disease (Covid-19). In addition to its key role in the regulation of biological functions, the circadian rhythm has been suggested as a regulator of viral infections. Specifically, the time of day of infection was found critical for illness progression, as has been reported for influenza, respiratory syncytial and parainfluenza type 3 viruses. We analyzed circadian rhythm implication in SARS-CoV-2 virus infection of isolated human monocytes, key actor cells in Covid-19 disease, from healthy subjects. The circadian gene expression of Bmal1 and Clock genes was investigated with q-RTPCR. Monocytes were infected with SARS-CoV-2 virus strain and viral infection was investigated by One-Step qRT-PCR and immunofluorescence. Interleukin (IL)-6, IL-1β and IL-10 levels were also measured in supernatants of infected monocytes. Using Cosinor analysis, we showed that Bmal1and Clock transcripts exhibited circadian rhythm in monocytes with an acrophase and a bathyphase at Zeitgeber Time (ZT)6 and ZT17. After forty-eight hours, the amount of SARS-CoV-2 virus increased in the monocyte infected at ZT6 compared to ZT17. The high virus amount at ZT6 was associated with significant increased release in IL-6, IL-1β and IL-10 compared to ZT17. Our results suggest that time day of SARS-CoV-2 infection affects viral infection and host immune response. They support consideration of circadian rhythm in SARS-CoV-2 disease progression and we propose circadian rhythm as a novel target for managing viral progression. [**note: time to map your circadian rhythm so you know what time it is safe to go out. Hey, maybe you don't even need to wear a mask because it is so safe! Count me as skeptical about this paper.**] https://www.biorxiv.org/content/10.1101/2020.09.09.290718v1
- In a previous paper [10] a model of the distribution of respiratory droplets and aerosols by Lagrangian turbulent air-flow was developed. It is used to show how the SARS-CoV-2 Coronavirus can be spread by the breathing of single infected person. The model shows that the concentration of viruses in the cloud, exhaled by one person, can increase to infectious levels within a certain amount of time, in a confined space where the air re-circulates. In [10] the model was used to analyze the air-flow and SARS-CoV-2 Coronavirus build-up in a restaurant in Guangzhou, China [19,18]. *In this paper, we add the analysis of two more cases, an outbreak among lay-Buddhists, on a bus [26], traveling to a ceremony in Zhejiang province, China, and an outbreak in a Call Center in Seoul, Korea [20]. The analysis and comparison of these three cases, leads to the conclusion that the SARS-CoV-2 Coronavirus attacks in two steps: The first step is a linear spread between individuals with a couple of days delay. The second step is an exponential spread effected by the air-conditioning system affecting a much larger number of people. Thus in the second step, the ventilation becomes the super-spreader*. [**note: anyone from my undergrad alma mater always gets a citation in the newsletter! This is further work on aerosol transmission and shows a two-step attack by SARS-CoV-2. Yes, indoor ventilation is important! You can control for this by masking up BUT, if you are in a bar or restaurant the mask comes down and you are vulnerable.**]
  https://www.medrxiv.org/content/10.1101/2020.09.11.20192997v1

- Contact tracing is a well-known tool for public health professionals to trace and isolate contacts of known infectious persons. During a pandemic contact tracing is critical to ending an outbreak, but the volume of cases makes tracing difficult without adequate staffing tools. Hospitals equipped with electronic medical records can utilize these databases to automatically link cases into possible transmission chains and surface potential new outbreaks. While this automatic contact tracing does not have the richness of contact tracing interviews, it does provide a way for health systems to highlight potential super-spreader events and support their local health departments. Additionally, these data provide insight into how a given infection is spreading locally. These insights can be used to inform policy at the local level. [**note: here is an interesting approach to track and trace using hospital electronic medical records.**] https://www.medrxiv.org/content/10.1101/2020.09.08.20190876v1
- Background Masks have been widely recommended as a precaution against COVID-19 transmission. Several studies have shown the efficacy of masks at curbing droplet dispersion in lab settings. Using individual response data from the Imperial College London-YouGov personal measures survey, this study investigates reported mask use and its association with the spread of COVID-19. Methods We examine the association of reported mask-wearing in a population within a country with the growth rate of active cases COVID-19 cases. The analysis uses country-wide data from weekly surveys of individuals mask-wearing behavior in public places, as well as other concurrently implemented nonpharmaceutical interventions, mobility changes, new cases of COVID-19 data and other control variables. The mask-wearing data were obtained from the Imperial College-YouGov multi-country survey of peoples personal habits before and during COVID-19. Because mask-wearing showed substantial variation across countries and over time, we use a reduced form econometric model to relate population-wide changes in mask-wearing to the growth rate in COVID-19. Results The results indicate that reported mask-wearing could play an important role in mitigating the growth of COVID-19. Widespread mask-wearing within a country associates with an expected 7% (95% CI: 3.94%-9.99%) decline in the growth rate of daily active cases of COVID-19 in the country. This daily decline equates to an expected 88.5% drop in the growth of daily active cases over a 30-day period when compared to zero percent mask-wearing, all else held equal. The decline in daily growth rate due to the combined effect of reported mask-wearing, reduced social mobility, and non-pharmaceutical interventions averages 28.1% (95% CI: 24.2%-32%). These estimates remain robust across multiple sensitivity analyses. Conclusions Face mask usage as a preventative measure against the transmission of COVID-19 varies widely across countries. This observational study uses data from 24 countries and finds that reported face mask usage associates with a decline in new COVID-19 cases. Even though the model controls for a large variety of variables that affect spread, and we run a number of robustness checks, it remains possible that some of the decline associated with face masks is related to confounding variables not included in the model. In summary, due to the confounding variables and the variations in the type of mask and its usage, randomized control trials of mask usage in populations are needed to determine the true effect of mask wearing on mitigating the transmission of infectious respiratory diseases. [**note: more information on mask wearing**] https://www.medrxiv.org/content/10.1101/2020.09.11.20192971v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow

CLINICAL TRIAL RESULTS

- Background: SARS-CoV-2 induced coagulopathy can lead to thrombotic complications such as stroke. Cerebral venous sinus thrombosis (CVST) is a less common type of stroke which might be triggered by COVID-19. We present a series of CVST cases with SARS-CoV-2 infection. Methods: In a multinational retrospective study, we collected all cases of CVST in SARS-CoV-2 infected patients admitted to nine tertiary stroke centers from the beginning of the pandemic to June 30th, 2020. We compared the demographics, clinical and radiological characteristics, risk factors, and outcome of these patients with a control group of non-SARS-CoV-2 infected CVST patients in the same seasonal period of the years 2012-2016 from the country where the majority of cases were recruited. Results: A total of 13 patients fulfilled the inclusion criteria (62% women, mean age 50.9 years). Six patients were discharged with good outcomes (mRS≤2) and three patients died in hospital. Compared to the control group, the SARS-CoV-2 infected patients were significantly older (50.9 versus 36.7 years, p<0.001), had a lower rate of identified CVST risk factors (23.1% versus 84.2%, p<0.001), had more frequent cortical vein involvement (38.5% versus 10.5%, p: 0.025), and a non-significant higher rate of in-hospital mortality (23.1% versus 5.3%, p: 0.073). Conclusion: CVST should be considered as potential comorbidity in SARS-CoV-2 infected patients presenting with neurological symptoms. *Our data suggest that compared to non-SARS-CoV-2 infected patients, CVST occurs in older patients, with lower rates of known CVST risk factors and might lead to a poorer outcome in the SARS-CoV-2 infected group*. [**note: this is a multi-national cohort study looking at cerebral venous thrombosis triggered by COVID-19. The looked at data from nine tertiary stroke centers and identified 13 patients.**] https://www.medrxiv.org/content/10.1101/2020.09.12.20186106v1
- Background: COVID-19 can lead to acute respiratory failure and an exaggerated inflammatory response. Studies have suggested promising outcomes using monoclonal antibodies targeting IL-1β (Anakinra) or IL6 (Tocilizumab), however no head to head comparison was done between the two treatments. Herein, we report our experience in treating COVID-19 pneumonia associated with cytokine storm with either subcutaneous Anakinra given concomitantly with intravenous immunoglobulin (IVIG), or intravenous Tocilizumab. Methods: Comprehensive clinical and laboratory data from patients with COVID-19 pneumonia admitted at our hospital between March and May 2020 were collected. Patients who received either Anakinra/ IVIG or Tocilizumab were selected. Baseline characteristics including oxygen therapy, respiratory status evaluation using ROX index, clinical assessment using NEWS score and laboratory data were collected. Outcomes included mortality, intubation, ICU admission and length of stay. In addition, we compared the change in ROX index, NEWS score and inflammatory markers at days 7 and 14 post initiation of therapy. Results: 84 consecutive patients who received either treatment (51 in the Anakinra/ IVIG group and 33 in the Tocilizumab group) were retrospectively studied. Baseline inflammatory markers were similar in both groups. There was no significant difference regarding to death (21.6% vs 15.2%, p 0.464), intubation (15.7% vs 24.2%, p 0.329), ICU need (57.1% vs 48.5%, p 0.475) or length of stay (13+9.6 vs 14.9+11.6, p 0.512) in the Anakinra/IVIG and Tocilizumab, respectively. Additionally, the rate of improvement in ROX index, NEWS score and inflammatory markers was similar in both groups at days 7 and 14. Furthermore, there was no difference in the incidence of superinfection in both groups. Conclusion: *Treating COVID-19 pneumonia associated with cytokine storm features with either subcutaneous Anakinra/IVIG or intravenous Tocilizumab is associated with improved clinical*

outcomes in most subjects. The choice of treatment does not appear to affect morbidity or mortality. Randomized controlled trials are needed to confirm our study findings. **[note: more confounding information on tocilizumab. Anakinra is in some clinical trials but I do not think results have been published yet. Obviously randomized clinical trials are the best approach to substantiate these findinfs.**]
https://www.medrxiv.org/content/10.1101/2020.09.11.20192401v1

- ABSTRACT BACKGROUND: Currently, there is no proven effective therapy nor vaccine for the treatment of SARS-CoV-2. Evidence regarding the potential benefit of early administration of hydroxychloroquine (HCQ) therapy in symptomatic patients with Coronavirus Disease (COVID-19) is not clear. METHODS: This observational prospective cohort study took place in 238 ambulatory fever clinics in Saudi Arabia, which followed the Ministry of Health (MOH) COVID-19 treatment guideline. This guideline included multiple treatment options for COVID-19 based on the best available evidence at the time, among which was Hydroxychloroquine (HCQ). Patients with confirmed COVD-19 (by reverse transcriptase polymerase chain reaction (PCR) test) who presented to these clinics with mild to moderate symptoms during the period from 5-26 June 2020 were included in this study. Our study looked at those who received HCQ-based therapy along with supportive care (SC) and compared them to patients who received SC alone. The primary outcome was hospital admission within 28-days of presentation. The secondary outcome was a composite of intensive care admission (ICU) and/or mortality during the follow-up period. Outcome data were assessed through a follow-up telephonic questionnaire at day 28 and were further verified with national hospitalisation and mortality registries. Multiple logistic regression model was used to control for prespecified confounders. RESULTS: Of the 7,892 symptomatic PCR-confirmed COVID-19 patients who visited the ambulatory fever clinics during the study period, 5,541 had verified clinical outcomes at day 28 (1,817 patients in the HCQ group vs 3,724 in the SC group). At baseline, patients who received HCQ therapy were more likely to be males who did not have hypertension or chronic lung disease compared to the SC group. No major differences were noted regarding other comorbid conditions. All patients were presenting with active complaints; however, the HCQ groups had higher rates of symptoms compared to the SC group (fever: 84% vs 66.3, headache: 49.8 vs 37.4, cough: 44.5 vs 35.6, respectively). Early HCQ-based therapy was associated with a lower hospital admission within 28-days compared to SC alone (9.4% compared to 16.6%, RRR 43%, p-value <0.001). The composite outcome of ICU admission and/or mortality at 28-days was also lower in the HCQ group compared to the SC (1.2% compared to 2.6%, RRR 54%, p-value 0.001). Adjusting for age, gender, and major comorbid conditions, a multivariate logistic regression model showed a decrease in the odds of hospitalisation in patients who received HCQ compared to SC alone (adjusted OR 0.57 [95% CI 0.47-0.69], p-value <0.001). The composite outcome of ICU admission and/or mortality was also lower for the HCQ group compared to the SC group controlling for potential confounders (adjusted OR 0.55 [95% CI 0.34-0.91], p-value 0.019). CONCLUSION: Early intervention with HCQ-based therapy in patients with mild to moderate symptoms at presentation is associated with lower adverse clinical outcomes among COVID-19 patients, including hospital admissions, ICU admission, and/or death. [**note: another HCQ study, this time from Saudi Arabia. This is an observational study and lacking the appropriate control group. I guess HCQ works sometimes and sometimes it does not.**]
https://www.medrxiv.org/content/10.1101/2020.09.09.20184143v1

DRUG DEVELOPMENT

- Vaccines and antiviral agents are in urgent need to stop the COVID-19 pandemic. To facilitate antiviral screening against SARS-CoV-2 without requirement for high biosafety level facility, we developed a bacterial artificial chromosome (BAC)-vectored replicon of SARS-CoV-2, nCoV-SH01 strain, in which secreted Gaussia luciferase (sGluc) was encoded in viral subgenomic mRNA as a reporter gene. The replicon was devoid of structural genes spike (S), membrane (M), and envelope (E). Upon transfection, the replicon RNA replicated in various cell lines, and was sensitive to interferon alpha (IFN-α), remdesivir, but was resistant to hepatitis C virus inhibitors daclatasvir and sofosbuvir. Replication of the replicon was also sensitive overexpression of zinc-finger antiviral protein (ZAP). We also constructed a four-plasmid in-vitro ligation system that is compatible with the BAC system, which makes it easy to introduce desired mutations into the assembly plasmids for in-vitro ligation. This replicon system would be helpful for performing antiviral screening and dissecting virus-host interactions. [**note: this is fascinating paper from China on the creation of a bacterial artificial non-infectious replicon of SARS-CoV-2. It may be useful in looking at viral protein interactions and drug discovery.**]
https://www.biorxiv.org/content/10.1101/2020.09.11.294330v1
- The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly become a global public health threat due to the lack of effective drugs or vaccines against SARS-CoV-2. The efficacy of several repurposed drugs has been evaluated in clinical trials. Among these drugs, a relatively new antiandrogen agent, enzalutamide, was proposed because it reduces the expression of transmembrane serine protease 2 (TMPRSS2), a key component mediating SARS-CoV-2-driven entry into host cells, in prostate cancer cells. However, definitive evidence for the therapeutic efficacy of enzalutamide in COVID-19 is lacking. Here, we evaluated the antiviral efficacy of enzalutamide in prostate cancer cells, lung cancer cells, human lung organoids and SARS-CoV-2-infected Ad-ACE2-transduced Tmprss2 knockout (Tmprss2-KO) and wild-type (WT) mice. TMPRSS2 knockout significantly inhibited SARS-CoV-2 infection in vivo. Enzalutamide effectively inhibited SARS-CoV-2 infection in human prostate cancer cells (LNCaP) but not in human lung cancer cells or patient-derived lung organoids. Although Tmprss2 knockout effectively blocked SARS-CoV-2 infection in ACE2-transduced mice, enzalutamide showed no antiviral activity due to the AR independence of TMPRSS2 expression in mouse and human lung epithelial cells. Moreover, we observed distinct AR binding patterns between prostate cells and lung cells and a lack of direct binding of AR to TMPRSS2 in human lung cells. Thus, our findings do not support the postulated protective role of enzalutamide in treating COVID-19. [**note: another paper from China, this one looking at whether enzalutamide is useful in treating COVID-19. It looks as if it wouldn't but there is a Swedish trial ongoing with this drug.**]
https://www.biorxiv.org/content/10.1101/2020.09.11.293035v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Nothing today

DIAGNOSTIC DEVELOPMENT

- Nothing today