2020-07-06

Welcome to Week 16 of a Newsletter that Shows no Sign of Ending

Let's stick with opera for today. Yesterday we had a nice presentation tale of love induced by magic potion. What about demented love you might ask? I have just piece for you, Richard Strauss's Salome. Based on a play by Oscar Wilde, the opera was shocking upon introduction and some opera houses refused to stage it. I have seen the final scene done in concert as well as on the opera stage but I must say, this version with German soprano Nadja Michael (who also studied at Indiana University!!!) is too over the top: https://www.youtube.com/watch?v=nan2WjNFVrU A semi-staged and well sung version is also good listening: https://www.youtube.com/watch?v=qa9ug0g0iAM

Germany has announced an aggressive serology project to look at the nationwide prevalence of SARS-CoV-2.

The Washington Post has a decent article on all of the RNA vaccines currently in trials. More on the letter from scientists urging the WHO to seriously address airborne spread of SARS-CoV-2 HERE. I linked to the New York Times article yesterday. I still think this is the old angels dancing on the head of pin problem. It's obvious that the virus is transmitted through the air and that enclosed spaces with poor ventilation present a risk. Avoid such places, but *if you must enter, wear a mask and keep the visit as brief as possible!*

The New York Times has an excellent story on the racial disparity of COVID-19 cases. Then there is this piece that may help you deal with people who have Coronavirus Fears and also why we might not be so great at risk assessment (not wearing masks is certainly one sign of *Coronavirus Dementia*).

This paper from STAT falls in the category of 'why did it take them so long?' Astute readers of this newsletter realize that I noted this problem way back in April (it seems like a COVID-eternity now). As I noted at the time, a lot of credible people's reputations are going to take a significant hit when the response to the pandemic is analyzed. I have been noticing in my review of newly registered clinical trials lots of small companies jumping on the bandwagon with therapies that will likely never enter trials and perhaps management is just trying to gain some notice and prop up stock prices. It's a sad thing to see. STAT also has an opinion piece on why we were late in responding to COVID-19.

The Guardian notes that even mild COVID-19 cases may be more complicated and dangerous.

Some my find this Science editorial on Surviving the Trauma of COVID-19 helpful.

Amid the doom and gloom, there is this feel-good story about a distiller who repurposed things to produce hand sanitizer. His grandmother was a teenage moonshiner back when the 1918 pandemic hit.

It is an extraordinary light day for reading, likely a result of the holiday weekend. I suspect tomorrow will be a lot different!

MODELING

- A large scale serology test in Spain. 35 883 households were selected from municipal rolls using two-stage random sampling stratified by province and municipality size, with all residents

invited to participate. From April 27 to May 11, 2020, 61 075 participants (75·1% of all contacted individuals within selected households) answered a questionnaire on history of symptoms compatible with COVID-19 and risk factors, received a point-of-care antibody test, and, if agreed, donated a blood sample for additional testing with a chemiluminescent microparticle immunoassay. Prevalences of IgG antibodies were adjusted using sampling weights and post-stratification to allow for differences in non-response rates based on age group, sex, and census-tract income. Using results for both tests, we calculated a seroprevalence range maximising either specificity (positive for both tests) or sensitivity (positive for either test). Seroprevalence was 5·0% (95% CI 4·7–5·4) by the point-of-care test and 4·6% (4·3–5·0) by immunoassay, with a specificity–sensitivity range of 3·7% (3·3–4·0; both tests positive) to 6·2% (5·8–6·6; either test positive), with no differences by sex and lower seroprevalence in children younger than 10 years (<3·1% by the point-of-care test). There was substantial geographical variability, with higher prevalence around Madrid (>10%) and lower in coastal areas (<3%). Seroprevalence among 195 participants with positive PCR more than 14 days before the study visit ranged from 87·6% (81·1–92·1; both tests positive) to 91·8% (86·3–95·3; either test positive). In 7273 individuals with anosmia or at least three symptoms, seroprevalence ranged from 15·3% (13·8–16·8) to 19·3% (17·7–21·0). Around a third of seropositive participants were asymptomatic, ranging from 21·9% (19·1–24·9) to 35·8% (33·1–38·5). Only 19·5% (16·3–23·2) of symptomatic participants who were seropositive by both the point-of-care test and immunoassay reported a previous PCR test. The majority of the Spanish population is seronegative to SARS-CoV-2 infection, even in hotspot areas. Most PCR-confirmed cases have detectable antibodies, but a substantial proportion of people with symptoms compatible with COVID-19 did not have a PCR test and at least a third of infections determined by serology were asymptomatic. *These results emphasise the need for maintaining public health measures to avoid a new epidemic wave*. https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)31483-5/fulltext and a good general commentary on the topic of seroprevalence in hotspots: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)31482-3/fulltext

- Coronavirus disease 2019 (COVID-19), which is caused by infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a global pandemic. It currently remains unclear whether convalescing patients have a risk of reinfection. We generated a rhesus macaque model of SARS-CoV-2 infection that was characterized by interstitial pneumonia and systemic viral dissemination mainly in the respiratory and gastrointestinal tracts. Rhesus macaques reinfected with the identical SARS-CoV-2 strain during the early recovery phase of the initial SARS-CoV-2 infection did not show detectable viral dissemination, clinical manifestations of viral disease, or histopathological changes. Comparing the humoral and cellular immunity between primary infection and rechallenge revealed notably enhanced neutralizing antibody and immune responses. Our results suggest that primary SARS-CoV-2 exposure protects against subsequent reinfection in rhesus macaques. [**note: let us hope this animal model is applicable to human infections!!!**] https://science.sciencemag.org/content/early/2020/07/01/science.abc5343

NEWLY REGISTERED CLINICAL TRIALS

- The following protocol proposes a mixed methods pilot study, whereby the immediate purpose is to generate quantifiable information regarding the feasibility of a virtual trial with the

Hexoskin Platform, collection of data quality in COVID-19 patients, and values for algorithm construction. In essence, sixteen participants with a confirmed COVID-19 diagnosis will be invited to wear the Hexoskin biometric garment during the course of several hours per week, over a period of 28 days. In addition to the biometric data collection, participants will be invited to complete four questionnaires. The biometric data will be combined to the sociodemographic information and patient symptomatology to construct an algorithm which can accurately predict patient outcomes. [**note: this looks way cool! I have no clue what this shirt does but you can click on the link above to find out.**] NCT04453891

CLINICAL TRIAL RESULTS

- Nothing today.

DRUG DEVELOPMENT

- Nothing today.

VIRUS BIOCHEMISTRY

- The COVID-19 pandemic is the most important public health threat in recent history. Here we study how its causal agent, SARS-CoV-2, has diversified genetically since its first emergence in December 2019. We have created a pipeline combining both phylogenetic and structural analysis to identify possible human-adaptation related mutations in a data set consisting of 4,894 SARS-CoV-2 complete genome sequences. Although the phylogenetic diversity of SARS-CoV-2 is low, the whole genome phylogenetic tree can be divided into five clusters/clades based on the tree topology and clustering of specific mutations, but its branches exhibit low genetic distance and bootstrap support values. *We also identified 11 residues that are high-frequency substitutions, with four of them currently showing some signal for potential positive selection. These fast-evolving sites are in the non-structural proteins nsp2, nsp5 (3CL-protease), nsp6, nsp12 (polymerase) and nsp13 (helicase), in accessory proteins (ORF3a, ORF8) and in the structural proteins N and S. Temporal and spatial analysis of these potentially adaptive mutations revealed that the incidence of some of these sites was declining after having reached an (often local) peak, whereas the frequency of other sites is continually increasing and now exhibit a worldwide distribution.* Structural analysis revealed that the mutations are located on the surface of the proteins that modulate biochemical properties. We speculate that this improves binding to cellular proteins and hence represents fine-tuning of adaptation to human cells. Our study has implications for the design of biochemical and clinical experiments to assess whether important properties of SARS-CoV-2 have changed during the epidemic. [**note: good work from these Chinese researchers looking at a large dataset of genome sequences.**] https://www.biorxiv.org/content/10.1101/2020.07.04.187435v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

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2020-07-07

It is looking like it may be an all opera week! Here is young love epitomized by the rose presentation scene from Richard Strauss's Der Rosenkavalier. This is a filmed version of the 1962 Vienna Opera production with Sena Jurniac and Anneliese Rothenberger: https://www.youtube.com/watch?v=G3nmm5l0-Ys IMO, this duet has never been done any better than this and the sumptuous staging is to die for. It was no secret that Strauss preferred women's voices and his best works were for them. However, the Italian Singer's aria from Rosenkavalier is splendid as Piotr Beczala shows in this Vienna Production: https://www.youtube.com/watch?v=K8OvpdGrWUE Beczala was in DC several years ago for a recital with Vocal Arts DC. I was invited to the after-recital dinner and was seated next to him! What a surprise. I asked him what the most difficult aria for him and he replied it was this Strauss piece. The tenor comes on right when the piece starts without any other warm up in the opera and departs right afterwards. He noted, "the high notes have to be very secure or you really end up fluffing your lines."

US COVID-19 STATISTICS - **Infection Rate: 0.9%; CRF: 4.4%** (I will be reporting these out based on what the Washington Post publishes each day. They should be taken with a grain of salt as both numbers are not precise. However, we should be able to see a trend over time and as I have noted I think the CRF will continue to drop.)

Elizabeth Rosenthal weighs in on the potential costs of a COVID-19 vaccine. Also in The New York Times, contact tracing in Florida may be impossible.

Speaking of vaccines, the Washington Post has a story on Ian Haydon, one of the volunteers in the Moderna trial. He had a very severe reaction to the vaccine. Let's hope this is an anomaly. Maryland biotech company, Novavax, received a $1.6B contract to develop a SARS-CoV-2 vaccine. They just finished a Phase 1 trial in Australia. Here is a very cool story on the man who invented the n95 mask! Though retired, he is still pitching in! Sticking with the Post, Florida decided to go ahead with reopening schools in the fall.

As noted in the past several days, there is a continuing debate over SARS-CoV-2 aerosols. The New York Times reviews what you should do now. Colleges are beginning to make decisions on how to reopen this fall. The spike in SARS-CoV-2 infections continues to result in test shortages and problems.

JAMA has a pair of articles on vaccine development HERE and HERE.

Of interest to clinicians is this Lancet viewpoint article on treating COVID-19 associated acute respiratory distress syndrome (ARDS).

Regeneron is moving into Phase 3 trials with its mAb cocktail.

MODELING

- The droplet has a limited travel distance. Nonetheless, especially in the indoor public space the air flow can propagate the droplet to travel long distance. Based on this situation, this paper aims to study the relationships of seat configuration-social distance-air flow-droplet dispersions. The analysis was based on the computational fluid dynamic (CFD) using lattice Boltzmann model

(LBM). The result confirms that by modifying public space configuration in this case by providing more space and increasing seating distance can reduce the vulnerability towards droplet dispersions. Whereas, providing shield including adding protection is far more effective in avoiding dispersions. The public space reconfiguration including increasing seat distance and reducing seating capacity also has an effect in reducing the indoor CO2. Capacity reduction from full capacity to 30% can decrease the CO2 from 5722 to 2144 ppm. [**note: I don't think this breaks any new ground but it's the first paper from Indonesia to be cited. We are all in this together!!!**] https://www.medrxiv.org/content/10.1101/2020.07.02.20145219v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- The current pandemic COVID-19 has affected most severely to the people with old age, or with comorbidities such as hypertension, diabetes mellitus, chronic kidney disease, COPD, and cancers. Cancer patients are twice more likely to contract the disease because of the malignancy or treatment-related immunosuppression; hence identification of the vulnerable population among these patients is essential. It is speculated that along with ACE2, other auxiliary proteins (DPP4, ANPEP, ENPEP, TMPRSS2) might facilitate the entry of coronaviruses in the host cells. We took a bioinformatics approach to analyze the gene and protein expression data of these coronavirus receptors in human normal and cancer tissues of multiple organs. Here, we demonstrated an extensive RNA and protein expression profiling analysis of these receptors across solid tumors and normal tissues. We found that among all, renal tumor and normal tissues exhibited increased levels of ACE2, DPP4, ANPEP, and ENPEP. Our results revealed that TMPRSS2 may not be the co-receptor for coronavirus in renal carcinoma patients. The receptors expression levels were variable in different tumor stages, molecular and immune subtypes of renal carcinoma. In clear cell renal cell carcinomas, coronavirus receptors were associated with high immune infiltration, markers of immunosuppression, and T cell exhaustion. Our study indicates that CoV receptors may play an important role in modulating the immune infiltrate and hence cellular immunity in renal carcinoma. As our current knowledge of pathogenic mechanisms will improve, it may help us in designing focused therapeutic approaches. [**note: it's a double whammy if you have renal carcinoma as the risk of COVID-19 progression is high.**] https://www.biorxiv.org/content/10.1101/2020.07.02.184663v1
- COVID-19 intensive care patients occasionally develop neurological symptoms. The absence of SARS-CoV-2 in most cerebrospinal fluid (CSF) samples suggests the involvement of further mechanisms including autoimmunity. We therefore determined whether anti-neuronal or anti-glial autoantibodies are present in eleven consecutive severely ill COVID-19 patients presenting with unexplained neurological symptoms. These included myoclonus, cranial nerve involvement, oculomotor disturbance, delirium, dystonia and epileptic seizures. Most patients showed signs of CSF inflammation and increased levels of neurofilament light chain. All patients had anti-neuronal autoantibodies in serum or CSF when assessing a large panel of autoantibodies against intracellular and surface antigens relevant for central nervous system diseases using cell-based assays and indirect immunofluorescence on murine brain sections. Antigens included proteins well-established in clinical routine, such as Yo or NMDA receptor, but also a variety of specific

undetermined epitopes on brain sections. These included vessel endothelium, astrocytic proteins and neuropil of basal ganglia, hippocampus or olfactory bulb. The high frequency of autoantibodies targeting the brain in the absence of other explanations suggests a causal relationship to clinical symptoms, in particular to hyperexcitability (myoclonus, seizures). While several underlying autoantigens still await identification in future studies, presence of autoantibodies may explain some aspects of multi-organ disease in COVID-19 and can guide immunotherapy in selected cases. [**note: here is some good work from Germany on the presence of autoantibodies in COVID-19 patients with neurological symptoms. This virus can be quite insidious.**] https://www.medrxiv.org/content/10.1101/2020.07.01.20143214v1

- Initially, the global outbreak of COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spared children from severe disease. However, after the initial wave of infections, clusters of a novel hyperinflammatory disease have been reported in regions with ongoing SARS-CoV-2 epidemics. While the characteristic clinical features are becoming clear, the pathophysiology remains unknown. Herein, we report on the immune profiles of eight Multisystem Inflammatory Syndrome in Children (MIS-C) cases. We document that all MIS-C patients had evidence of prior SARS-CoV-2 exposure, mounting an antibody response with normal isotype-switching and neutralization capability. We further profiled the secreted immune response by high-dimensional cytokine assays, which identified elevated signatures of inflammation (IL-18 and IL-6), lymphocytic and myeloid chemotaxis and activation (CCL3, CCL4, and CDCP1) and mucosal immune dysregulation (IL-17A, CCL20, CCL28). Mass cytometry immunophenotyping of peripheral blood revealed reductions of mDC1 and non-classical monocytes, as well as both NK- and T- lymphocytes, suggesting extravasation to affected tissues. Markers of activated myeloid function were also evident, including upregulation of ICAM1 and FcR1 in neutrophil and non-classical monocytes, well-documented markers in autoinflammation and autoimmunity that indicate enhanced antigen presentation and Fc-mediated responses. Finally, to assess the role for autoimmunity secondary to infection, we profiled the auto-antigen reactivity of MIS-C plasma, which revealed both known disease-associated autoantibodies (anti-La) and novel candidates that recognize endothelial, gastrointestinal and immune-cell antigens. *All patients were treated with anti- IL6R antibody or IVIG, which led to rapid disease resolution tracking with normalization of inflammatory markers*. [**note: more good work from Mt. Sinai looking at 8 cases of multisystem inflammatory syndrome in children. As noted they were able to successfully treat them.**]
  https://www.medrxiv.org/content/10.1101/2020.07.04.20142752v1
- Background: The COVID-19 pandemic due to SARS-CoV-2 infection can produce Acute Respiratory Distress Syndrome as a result of a pulmonary cytokine storm. Antihistamines are safe and effective treatments for reducing inflammation and cytokine release. Combinations of Histamine-1 and Histamine-2 receptor antagonists have been effective in urticaria, and might reduce the histamine-mediated pulmonary cytokine storm in COVID-19. Can a combination of Histamine-1 and Histamine-2 blockers improve COVID-19 inpatient outcomes? Methods: A physician-sponsored cohort study of cetirizine and famotidine was performed in hospitalized patients with severe to critical pulmonary symptoms. Pulmonologists led the inpatient care in a single medical center of 110 high-acuity patients that were treated with cetirizine 10 mg and famotidine 20 mg b.i.d. plus standard-of-care. Results: Of all patients, including those with Do Not Resuscitate directives, receiving the dual-histamine blockade for at least 48 hours, the

combination drug treatment resulted in a 16.4% rate of intubation, a 7.3% rate of intubation after a minimum of 48 hours of treatment, a 15.5% rate of inpatient mortality, and 11.0 days duration of hospitalization. The drug combination exhibited reductions in symptom progression when compared to published reports of COVID-19 patients. Concomitant medications were assessed and hydroxychloroquine was correlated with worse outcomes. Conclusions: This physician-sponsored cohort study of cetirizine and famotidine provides proof-of-concept of a new safe and effective method to reduce the progression in symptom severity, presumably by minimizing the histamine-mediated cytokine storm. Further clinical studies in COVID-19 are warranted of the repurposed off-label combination of two historically-safe histamine blockers. [**note: this is an interesting finding and of course these drugs have a long history of use. One of the authors has filed a patent on the use of this dual blockade approach. Good luck enforcing that one if it's granted.]**
https://www.medrxiv.org/content/10.1101/2020.06.30.20137752v1

- Background Viral shedding patterns and its correlation with the immune responses of mildly symptomatic COVID-19 patients are still poorly characterized. Methods: We enrolled the first five COVID-19 patients quarantined in our institution; none received immunomodulatory treatment. We monitored shedding of viral RNA and infectious virus by RT-PCR and cell culture from the upper respiratory tract, and characterized the kinetics of systemic innate and adaptive immune responses. Results Despite mild clinical disease, high viral loads and shedding of infectious virus were observed from the respiratory tract, with isolation of infectious virus and prolonged positivity by PCR up to day 7 and 19 post onset of symptoms, respectively. Robust innate responses characterized by an increase in activated CD14+CD16+ monocytes and cytokine responses were observed as early as 2 days after symptoms onset. Cellular and humoral SARS-CoV-2 specific adaptive responses were detectable in all patients. Conclusion Infectious virus shedding was limited to the first week of symptom onset in mild cases. A strong innate response, characterized by the mobilization of activated monocytes during the first days of infection, as well as SARS-CoV-2 specific antibodies were detectable, even in patients with mild disease. [**note: this Swiss group looked at viral shedding in mild COVID-19 cases. It's a very small number of patients but important information.**]
https://www.medrxiv.org/content/10.1101/2020.07.02.20143271v1

DRUG DEVELOPMENT

- There is an urgent need to find therapeutic agents that can help clear the virus to prevent the severe disease and death. Identifying effective and safer drugs can provide with more options to treat the COVID-19 infections either alone or in combination. Here we performed a high throughput screen of approximately 1700 US FDA approved compounds to identify novel therapeutic agents that can effectively inhibit replication of coronaviruses including SARS-CoV-2. Our two-step screen first used a human coronavirus strain OC43 to identify compounds with anti-coronaviral activities. The effective compounds were then screened for their effectiveness in inhibiting SARS-CoV-2. These screens have identified 24 anti-SARS-CoV-2 drugs including previously reported compounds such as hydroxychloroquine, amlodipine, arbidol hydrochloride, tilorone 2HCl, dronedarone hydrochloride, and merfloquine hydrochloride. Five of the newly identified drugs had a safety index (cytotoxic/effective concentration) of >600, indicating wide therapeutic window compared to hydroxychloroquine which had safety index of 22 in similar

experiments. Mechanistically, five of the effective compounds were found to block SARS-CoV-2 S protein-mediated cell fusion. These FDA approved compounds can provide much needed therapeutic options that we urgently need in the midst of the pandemic. [**note: another broad drug screening paper with not much new. It's pretty much a laundry list of compounds from different therapeutic categories and it's not clear that any of them will ever enter trials.**] https://www.biorxiv.org/content/10.1101/2020.07.06.188953v1

- The Mac1 domain of the multifunctional SARS-CoV-2 non-structural protein 3 (nsp3) is a potential COVID-19 drug target because it is suspected to enhance the ability of the virus to evade the human immune system. The SARS-CoV-2 Mac1 domain binds ADP-ribose and proteins harboring this important post-translational modification. Small molecules that bind the Mac1 domain in place of ADP-ribose might therefore be useful as molecular probes or scaffolds for antiviral drug discovery. Two high throughput screens were used here to identify such ligands in small libraries of drugs and drug-like compounds. The first screen used differential scanning fluorimetry (DSF, aka the thermal shift or ThermoFluor assay) to examine the melting temperature of SARS-CoV-2 Mac1 domain in the presence of various compounds. In the second screen, various high-resolution SARS-CoV-2 Mac1 structures were used with Autodock VINA to identify potential ligands. Numerous hit compounds were either steroids (estradiol valerate & flunisolide), beta-lactams (cefaclor & cefatrizine), or benzimidazoles (telmisartan, rabeprazole, omeprazole, & esomeprazole). Isothermal titration calorimetry was used to confirm that rabeprazole, omeprazole, and compounds in other chemical classes, such as irinotecan, nifedipine, trifluoperazine, bind SARS-CoV-2 Mac1 with an affinity similar to ADP-ribose. [**note: this is a new target that I've not seen before. It is not clear how one can do drug design here as modulators would not have any impact on *in vitro* virus inhibition. One would need to study them directly in an animal model looking for impact on the immune system which may not be present.**] https://www.biorxiv.org/content/10.1101/2020.07.06.190413v1
- Severe acute respiratory syndrome CoV-2 (SARS-CoV-2) is currently causing a worldwide pandemic with high morbidity and mortality. Development of animal models that recapitulate important aspects of coronavirus disease 2019 (COVID-19) is critical for the evaluation of vaccines and antivirals, and understanding disease pathogenesis. SARS-CoV-2 has been shown to use the same entry receptor as SARS-CoV-1, human angiotensin-converting enzyme 2 (hACE2)(1-3). Due to amino acid differences between murine and hACE2, inbred mouse strains fail to support high titer viral replication of SARS-CoV-2 virus. Therefore, a number of transgenic and knock-in mouse models, as well as viral vector-mediated hACE2 delivery systems have been developed. Here we compared the K18-hACE2 transgenic model to adenovirus-mediated delivery of hACE2 to the mouse lung. We show that K18-hACE2 mice replicate virus to high titers in both the lung and brain leading to lethality. In contrast, adenovirus-mediated delivery results in viral replication to lower titers limited to the lung, and no clinical signs of infection with a challenge dose of 104 plaque forming units. The K18-hACE2 model provides a stringent model for testing the ability of vaccines and antivirals to protect against disease, whereas the adenovirus delivery system has the flexibility to be used across multiple genetic backgrounds and modified mouse strains. [**note: good work in coming up with a mouse model for drug and vaccine development.**] https://www.biorxiv.org/content/10.1101/2020.07.06.190066v1

VIRUS BIOCHEMISTRY

- To identify features in the genome of the SARS-CoV-2 pathogen responsible for the COVID-19 pandemic that may contribute to its viral replication, host pathogenicity, and vulnerabilities, we investigated how and to what extent the SARS-CoV-2 genome sequence differs from other well-characterized human and animal coronavirus genomes. Our analyses suggest the presence of unique sequence signatures in the 3'-untranslated region (UTR) of betacoronavirus lineage B, which phylogenetically encompasses SARS-CoV-2, SARS-CoV, as well as multiple groups of bat and animal coronaviruses. In addition, we identified genome-wide patterns of variation across different SARS-CoV-2 strains that likely reflect the effects of selection. Finally, we provide evidence for a possible host microRNA-mediated interaction between the 3'-UTR and human microRNA hsa-miR-1307-3p based on predicted, yet extensive, complementary base-pairings and similar interactions involving the Influenza A H1N1 virus. This interaction also suggests a possible survival mechanism, whereby a mutation in the SARS-CoV-2 3'-UTR leads to a weakened host immune response. The potential roles of host microRNAs in SARS-CoV-2 replication and infection, and the exploitation of conserved features in the 3'-UTR as therapeutic targets, warrant further investigation. [**note: interesting finding but I don't see how it can be utilized in terms of drug design.**]
  https://www.biorxiv.org/content/10.1101/2020.07.06.190207v1

DIAGNOSTIC DEVELOPMENT

- The actual demand on SARS-CoV-2 diagnosis is a current challenge for clinical laboratories. Sample pooling may help to ameliorate workload in clinical laboratories. Objective: to evaluate the efficacy of sample pooling compared to the individual analysis for the diagnosis of CoVID-19, by using different commercial platforms for nucleic acid extraction and amplification. Design and settings: observational, prospective, multicentre study across 9 Spanish clinical microbiology laboratories including SARS-CoV-2 RNA testing performed in April 2020, during the first three days after acceptance to participate. Participants and Methods: 3519 naso-oro-pharyngeal samples received at the participating laboratories were processed individually and in pools (351 pools) according to the existing methodology in each of the centres. Results: We found that 253 pools (2519 samples) were negative, and 99 pools (990 samples) were positive; with 241 positive samples (6.85%), our pooling strategy would have saved 2167 PCR tests. For 29 pools (made out of 290 samples) we found discordant results when compared to their correspondent individual samples: in 24/29 pools (30 samples), minor discordances were found; for five pools (5 samples), we found major discordances. Sensitivity, specificity, positive and negative predictive values for pooling were 97.93%, 100%, 100% and 99.85% respectively; accuracy was 99.86% and kappa concordant coefficient was 0.988. As a result of the sample dilution effect of pooling, a loss of 2-3 Cts was observed for E, N or RdRP genes. Conclusion: we show a high efficiency of pooling strategies for SARS-CoV-2 RNA testing, across different RNA extraction and amplification platforms, with excellent performance in terms of sensitivity, specificity, and positive and negative predictive values. We believe that our results may help clinical laboratories to respond to the actual demand and clinical need on SARS-CoV-2 testing, especially for the screening of low prevalence populations. [**note: nice work from Spain showing how sample pooling can work in a real world situation!**]
  https://www.medrxiv.org/content/10.1101/2020.07.04.20146027v1

- Though this is only a meta-analysis of serology accuracy it is useful for the diagrams and presentation. There is still a lot to do but as others have reported out, using two tests in parallel can improve sensitivity and accuracy. Ultimately, clinical laboratories will have to validate tests to confirm the manufacturer's specifications. https://www.bmj.com/content/370/bmj.m2516 and there is this accompanying BMJ editorial on the topic.

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2020-07-08

In memoriam - Charlie Daniels passed away the other day (not COVID-19) related. He and his band always had a great time performing. His best-known hit was 'The Devil Went Down to Georgia.' Here is the band live at the Grand Ole opry: https://www.youtube.com/watch?v=J5j69aEZ8xE

US COVID-19 STATISTICS - **Infection Rate: 0.9%; CRF: 4.3%** (CRF drops by 0.1%)

Here is another cautionary and tragic tale of a teen who perished from COVID-19 and the Washington Post has an even bigger story on the random use of HCQ in a southeast Pennsylvania veterans center. Staying with the Post, here is a story of how Israel may have let things get out of control after having a great deal of early success……and then there is Florida.

Prayer may not be a way out of this pandemic as this New York Times article notes; churches are a major source of COVID-19 cases. The Times also discusses problems with virus tracking apps. I think the issues can be addressed but it is likely that public confidence is low and will affect widespread use.

Healthcare workers confront anti-maksers; too sad a story to see repeated.

The Atlantic's Ed Yong has a good story on public-health specialist burn out.

Derek Lowe explains T Cells and Antibody Levels as well as our ignorance of how the immune system functions in full.

A young psychiatry resident discusses his battle with COVID-19 in this STAT article. He is still experiencing symptoms 100 days post infection.

For those of my loyal readers who don't read the abstracts in depth, I recommend reading the paper in the MODELING section on how pickled vegetables may help stave of COVID-19. Yes, it is a crude observational study but these days, that is what passes for science. You can have a lot of fun making your own pickled foods; they tasted good; and hey, they may even have disease-fighting properties. I did provide a link to one DIY paper. There are many more and if you Google "pickle fermentation lids" you can find an assortment of different types of lids and weights to help you out.

MODELING

- University campuses present an ideal environment for viral spread and are therefore at extreme risk of serving as a hotbed for a COVID-19 outbreak. While active surveillance throughout the semester such as widespread testing, contact tracing, and case isolation, may assist in detecting

and preventing early outbreaks, these strategies will not be sufficient should a larger outbreak occur. It is therefore necessary to limit the initial number of active cases at the start of the semester. We examine the impact of pre-semester NAT testing on disease spread in a university setting. Methods: We implement simple dynamic transmission models of SARS-CoV-2 infection to explore the effects of pre-semester testing strategies on the number of active infections and occupied isolation beds throughout the semester. We assume an infectious period of 3 days and vary R0 to represent the effectiveness of disease mitigation strategies throughout the semester. We assume the prevalence of active cases at the beginning of the semester is 5%. The sensitivity of the NAT test is set at 90%. Results: If no pre-semester screening is mandated, the peak number of active infections occurs in under 10 days and the size of the peak is substantial, ranging from 5,000 active infections when effective mitigation strategies (R0 = 1.25) are implemented to over 15,000 active infections for less effective strategies (R0 = 3). When one NAT test is mandated within one week of campus arrival, effective (R0 = 1.25) and less effective (R0 = 3) mitigation strategies delay the onset of the peak to 40 days and 17 days, respectively, and result in peak size ranging from 1,000 to over 15,000 active infections. When two NAT tests are mandated, effective (R0 = 1.25) and less effective (R0 = 3) mitigation strategies delay the onset of the peak through the end of fall semester and 20 days, respectively, and result in peak size ranging from less than 1,000 to over 15,000 active infections. If maximum occupancy of isolation beds is set to 2% of the student population, then isolation beds would only be available for a range of 1 in 2 confirmed cases (R0 = 1.25) to 1 in 40 confirmed cases (R0 = 3) before maximum occupancy is reached. Conclusion: Even with highly effective mitigation strategies throughout the semester, inadequate pre-semester testing will lead to early and large surges of the disease and result in universities quickly reaching their isolation bed capacity. We therefore recommend NAT testing within one week of campus return. While this strategy is sufficient for delaying the timing of the outbreak, pre-semester testing would need to be implemented in conjunction with effective mitigation strategies to reduce the outbreak size. [**note: another model for university reopening management from Clemson University researchers.**] https://www.medrxiv.org/content/10.1101/2020.07.06.20147272v1

- The COVID-19 pandemic poses an existential threat to many US residential colleges: either they open their doors to students in September or they risk serious financial consequences. Objective: To define SARS-CoV-2 screening performance standards that would permit the safe return of students to campus for the Fall 2020 semester. Design: Decision and cost-effectiveness analysis linked to a compartmental epidemic model to evaluate campus screening using tests of varying frequency (daily-weekly), sensitivity (70%-99%), specificity (98%-99.7%), and cost ($10-$50/test). Reproductive numbers Rt = {1.5, 2.5, 3.5} defined three epidemic scenarios, with additional infections imported via exogenous shocks. We generally adhered to US government guidance for parameterization data. Participants: A hypothetical cohort of 5000 college-age, uninfected students. Main Outcome(s) and Measure(s): Cumulative tests, infections, and costs; daily isolation dormitory census; incremental cost-effectiveness; and budget impact. All measured over an 80-day, abbreviated semester. Results: With Rt = 2.5, daily screening with a 70% sensitive, 98% specific test produces 85 cumulative student infections and isolation dormitory daily census averaging 108 (88% false positives). Screening every 2 (7) days nets 135 (3662) cumulative infections and daily isolation census 66 (252) with 73% (4%) false positives. Across all scenarios, test frequency exerts more influence on outcomes than test sensitivity.

Cost-effectiveness analysis selects screening every {2, 1, 7} days with a 70% sensitive test as the preferred strategy for Rt = {2.5, 3.5, 1.5}, implying a screening cost of {$470, $920, $120} per student per semester. Conclusions & Relevance: Rapid, inexpensive and frequently conducted screening (even if only 70% sensitive) would be cost-effective and produce a modest number of COVID-19 infections. While the optimal screening frequency hinges on the success of behavioral interventions to reduce the base severity of transmission (Rt), this could permit the safe return of student to campus.[**note: and this study on testing of university students comes from Harvard and Yale investigators.**]
https://www.medrxiv.org/content/10.1101/2020.07.06.20147702v1

- The aim of this study is the characterization and genomic tracing by phylogenetic analyses of 59 new SARS-CoV-2 Italian isolates obtained from patients attending clinical centres in North and Central Italy until the end of April 2020. All but one of the newly characterized genomes belonged to the lineage B.1, the most frequently identified in European countries, including Italy. Only a single sequence was found to belong to lineage B. A mean of 6 nucleotide substitutions per viral genome was observed, without significant differences between synonymous and non-synonymous mutations, indicating genetic drift as a major source for virus evolution. tMRCA estimation confirmed the probable origin of the epidemic between the end of January and the beginning of February with a rapid increase in the number of infections between the end of February and mid-March. Since early February, an effective reproduction number (Re) greater than 1 was estimated, which then increased reaching the peak of 2.3 in early March, confirming the circulation of the virus before the first COVID-19 cases were documented. Continuous use of state-of-the-art methods for molecular surveillance is warranted to trace virus circulation and evolution and inform effective prevention and containment of future SARS-CoV-2 outbreaks. [**note: a thorough study of the phylogenetics of 59 SARS-CoV-2 isolates from patients in north and central Italy during the major part of the outbreak.**] https://www.medrxiv.org/content/10.1101/2020.07.06.20147140v1
- Simple plastic face shields have many advantages compared to regular medical masks. They are easily cleaned for reuse and comfortable to wear. In light of the spreading COVID-19 pandemic, the potential of face shields as a substitution for medical masks, as a recommendation to the general population, was tested. Testing the efficacy of the protective equipment utilized a cough simulator that was carefully tuned to replicate human cough in terms of droplet size distribution and outlet velocity. The tested protective equipment was worn on a manikin head simulating human breathing. An Aerodynamic Particle Sizer (APS) was used to analyze the concentration and size distribution of small particles that reach the manikin head respiration pathways. Additionally, Water sensitive papers were taped over and under the tested protective equipment, and were subsequently photographed and analyzed. For droplets larger than 3µm by diameter, the efficiency of shields to block cough droplets was found to be comparable to that of regular medical masks, with enhanced protection on face parts the mask does not cover. Additionally, for finer particles, of the order 0.3 to few microns, a shield was found to perform even better, blocking about 10 times more fine particles than the medical mask. This implies that for the general population that is not intendedly exposed to confirmed infected individuals, recommending the use of face shields as an alternative to medical masks should be considered. [**note: I saw a number of workers at Whole Foods yesterday morning wearing face shields.**

They must have gotten an early look at this paper. I'll have to price these out.]
https://www.medrxiv.org/content/10.1101/2020.07.06.20147090v1

- The community lockdown measures implemented in the United States, during late March to end of May of 2020, resulted in a significant reduction in the community transmission of the COVID-19 pandemic throughout the country. However, a number of US states are currently experiencing an alarming post-lockdown resurgence of the pandemic, triggering the fear for a possible severe second wave of the pandemic in some US jurisdictions. We designed a mathematical model for addressing the key question of whether or not the universal use of face masks can halt or curtail such resurgence (and possibly avert a second wave, without having to undergo another cycle of major community lockdown) in the states of Arizona, Florida, New York and the entire US. The model was parametrized and fitted using cumulative mortality data from the four jurisdictions. Our study highlights the importance of early implementation of the community lockdown measures. In particular, a sizable reduction in the burden of the pandemic would have been recorded in each of the four jurisdictions if the community lockdown measures were implemented a week or two earlier. These reductions are greatly augmented if the early implementation of the lockdown measures is complemented with a public face masks use strategy. It is shown that the pandemic would have been almost completely suppressed from significantly taking off if the lockdown measures were implemented two weeks earlier, and if a sizable percentage of the residents of the four jurisdictions wore face masks during the respective lockdown periods. If the level of lifting of community lockdown is high (which entails allowing for greater community contacts and re-opening of businesses and social activities, in comparison to what was allowed during the community lockdown period), the states of Arizona and Florida will record a devastating second wave of the pandemic by the end of 2020, while the state of New York and the entire US will record milder second waves. If the level of lifting for the community lockdown was mild (i.e., only allowing very limited community contacts and business activities, in comparison to the lockdown period), only the state of Florida will experience a second wave. The severity of the projected second wave depends on the level of lifting of the community lockdown. For instance, the projected second wave for Arizona and Florida, associated with moderate and high level of lifting of lockdown, will be more severe than their respective first wave. For high level of lifting of lockdown measures, the increased use of face masks after the lockdown period greatly reduces the burden of the pandemic. In particular, for this high lifting scenario, none of the four jurisdictions will experience a second wave if half of their residents wear face masks consistently after their respective lockdown period). A testing strategy that increases the maximum detection rate of asymptomatic infected individuals (followed by contact tracing and self-isolation of the detected cases) greatly reduces the burden of the pandemic in all four jurisdictions, particularly if also combined with universal face mask use strategy. Universal use of face masks in public, with at least moderate level of compliance, could halt the post-lockdown resurgence of COVID-19, in addition to averting the potential for (or severity of) a second wave of the pandemic in each of the four jurisdictions. [**note: the authors of this paper come from Florida and Arizona and the title, "Could masks curtail the post-lockdown resurgence of COVID-19 in the US?" says it all. The answer is yes!**]
https://www.medrxiv.org/content/10.1101/2020.07.05.20146951v2
- Background Many foods have an antioxidant activity and nutrition may mitigate COVID-19. Some of the countries with a low COVID-19 mortality are those with a relatively high

consumption of traditional fermented foods. To test the potential role of fermented foods in COVID-19 mortality in Europe, we performed an ecological study. Methods The European Food Safety Authority (EFSA) Comprehensive European Food Consumption Database was used to study the country consumption of fermented vegetables, pickled/marinated vegetables, fermented milk, yoghurt and fermented sour milk. We obtained the COVID-19 mortality per number of inhabitants from the Johns Hopkins Coronavirus Resource Center. EuroStat data were used for data on potential confounders at the country level including Gross Domestic Product (GDP) (2019), population density (2018), percentage of people older than 64 years (2019), unemployment rate (2019) and percentage obesity (2014, to avoid missing values). Mortality counts were analyzed with quasi-Poisson regression models - with log of population as an offset - to model the death rate while accounting for over-dispersion. Results Of all the variables considered, including confounders, only fermented vegetables reached statistical significance with the COVID-19 death rate per country. For each g/day increase in the average national consumption of fermented vegetables, the mortality risk for COVID-19 decreased by 35.4% (95% CI: 11.4%, 35.5%). Adjustment did not change the point estimate and results were still significant. Discussion The negative ecological association between COVID-19 mortality and consumption of fermented vegetables supports the a priory hypothesis previously reported. The hypothesis needs to be tested in individual studies performed in countries where the consumption of fermented vegetables is common. [**note: damn, I was hoping yogurt would protect me. I guess I need to start fermenting some veggies now that the farm stands are plentiful. I need to know which vegetables are best for COVID protection. Knowing Google is my sometimes friend I found this DIY site: https://www.diynatural.com/fermented-vegetables/**] https://www.medrxiv.org/content/10.1101/2020.07.06.20147025v1

- Following widespread closures of food-related businesses due to efforts to curtail the spread of SARS-CoV-2, public health authorities reported increased sightings of rats in close vicinity of people. Because rats vector a number of pathogens transmissible to people, changes in their behavior has consequences for human health risks. To determine the extent of how stay-at-home measures influenced patterns of rat sightings we: 1) examined the number of rat-related public service requests before and during the period of lockdown in New York City (NYC) and Tokyo, Japan; 2) examined reports made in proximity to closed food service establishments in NYC; and 3) surveyed pest control companies in the United States, Canada, Japan, and Poland. During the month following lockdown, the overall number of reports decreased by 30% in NYC, while increasing 24% in Tokyo. However, new hotspots of 311 calls were observed in proximity of closed food service establishments in NYC; and there was a consistent positive association between kernel density estimates of food service establishments and location of 311 calls (r = 0.33 to 0.45). Similarly, more reports were observed in the restaurant-dense eastern side of Tokyo. Changes in clientele for pest control companies varied geographically, with 37% of pest-management companies surveyed in North America reporting 50-100% of their post-lockdown rat-related requests coming from new clients. In Warsaw, where there are no clusters of restaurants in densely-populated areas, there were no changes. In Tokyo, there were no changes in clients. We conclude that changes in public service calls are region-specific and localized, with increases in rat sightings more likely near restaurant-dense regions. Pest control companies surveyed in North America either lost much of their business or shifted clientele from old to new locations. We discuss possible mitigation measures including ramping up pest

control during re-opening of food-related establishments and the need for citywide rodent surveillance and disease monitoring. [**note: somebody had to do this type of study! Maybe it is time to put a call into the Pied Piper of Hamelin!**]
https://www.medrxiv.org/content/10.1101/2020.07.05.20146779v1

- We intranasally inoculated nine fruit bats (*Rousettus aegyptiacus*), ferrets (*Mustela putorius*), pigs (*Sus scrofa domesticus*), and 17 chickens (*Gallus gallus domesticus*) with 10^5 $TCID_{50}$ of a SARS-CoV-2 isolate per animal. Direct contact animals (n=3) were included 24 h after inoculation to test viral transmission. Animals were monitored for clinical signs and for virus shedding by nucleic acid extraction from nasal washes and rectal swabs (ferrets), oral swabs and pooled faeces samples (fruit bats), nasal and rectal swabs (pigs), or oropharyngeal and cloacal swabs (chickens) on days 2, 4, 8, 12, 16, and 21 after infection by quantitative RT-PCR (RT-qPCR). On days 4, 8, and 12, two inoculated animals (or three in the case of chickens) of each species were euthanised, and all remaining animals, including the contacts, were euthanised at day 21. All animals were subjected to autopsy and various tissues were collected for virus detection by RT-qPCR, histopathology immunohistochemistry, and in situ hybridisation. Presence of SARS-CoV-2 reactive antibodies was tested by indirect immunofluorescence assay and virus neutralisation test in samples collected before inoculation and at autopsy. Pigs and chickens were not susceptible to SARS-CoV-2. All swabs, organ samples, and contact animals were negative for viral RNA, and none of the pigs or chickens seroconverted. Seven (78%) of nine fruit bats had a transient infection, with virus detectable by RT-qPCR, immunohistochemistry, and in situ hybridisation in the nasal cavity, associated with rhinitis. Viral RNA was also identified in the trachea, lung, and lung-associated lymphatic tissue in two animals euthanised at day 4. One of three contact bats became infected. More efficient virus replication but no clinical signs were observed in ferrets, with transmission to all three direct contact animals. Mild rhinitis was associated with viral antigen detection in the respiratory and olfactory epithelium. Prominent viral RNA loads of $0–10^4$ viral genome copies per mL were detected in the upper respiratory tract of fruit bats and ferrets, and both species developed SARS-CoV-2-reactive antibodies reaching neutralising titres of up to 1/1024 after 21 days. [**note: this is a German study looking to see if some common farm animals are infected with SARS-CoV-2. Pigs and chickens did not show signs of infection though fruit bats and ferrets did. They suggest that ferrets might be a good test animal for studying vaccines and/or antivirals.**]
https://www.thelancet.com/journals/lanmic/article/PIIS2666-5247(20)30089-6/fulltext

NEWLY REGISTERED CLINICAL TRIALS

- I will report tomorrow.

CLINICAL TRIAL RESULTS

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly become a global pandemic. In addition to the acute pulmonary symptoms of COVID-19 (the disease associated with SARS-CoV-2 infection), pulmonary and distal coagulopathies have caused morbidity and mortality in many patients. Currently, the molecular pathogenesis underlying COVID-19 associated coagulopathies are unknown. While there are many theories for the cause of this pathology, including hyper inflammation and excess tissue damage, the cellular and molecular underpinnings are not yet clear. By analyzing transcriptomic data sets from experimental and

clinical research teams, we determined that changes in the gene expression of genes important in the extrinsic coagulation cascade in the lung epithelium may be important triggers for COVID-19 coagulopathy. This regulation of the extrinsic blood coagulation cascade is not seen with influenza A virus (IAV)-infected NHBEs suggesting that the lung epithelial derived coagulopathies are specific to SARS-Cov-2 infection. This study is the first to identify transcriptional changes at the level of the lung epithelium that have the to drive the COVID-19 associated coagulopathy. [**note: this is an intriguing finding but why does it only impact a small segment of COVID-19 infected patients?**] https://www.biorxiv.org/content/10.1101/2020.07.06.182972v1

- Acute cardiac related injury (ACRI) is common in hospitalized COVID-19 patients. Objective: To explain the pathological mechanism of ACRI and improve the treatment strategy by retrospectively observing the factors associated with ACRI and factors affecting the prognosis of ACRI with COVID-19 at an early stage. Methods: 619 COVID-19 patients were from Tongji Hospital, Wuhan. T test was used for continuous variables while Chi-square test for categorical factors. Univariable and multivariable logistic regression models were applied to estimate odds ratio (OR) with 95% confidence interval (CI). Results: Among the 619 OOS Level-I hospitalized COVID-19 patients, 102 (16.5%) were defined as ACRI (stage-1: 59 cases, stage-2: 43 cases). 50% of ACRI patients developed into severe cases and 25 patients died (CFR=24.5%), 42 times that of non-ACRI patients. Elderly (OR=2.83, P<0.001) , HTN (OR=2.09, P=0.005), γ-globulin (OR=2.08, P=0.004), TCM (OR=0.55, P=0.017), PLT (OR=2.94, P<0.001) and NLR (OR=2.20, P=0.004) were independently correlated with ACRI. SBP≥140, dyspnea, DM, smoking history were correlated with ACRI-stage2 only. In the prognostic subgroup analysis of ACRI patients,γ-globulin treatment could prolong LOS. TCM (OR=0.26, P=0.006), SBP≥160 (OR= 22.70, P=0.005), male (OR=2.66, P=0.044) were associated with severe illness while corticosteroids treatment (OR=3.34, P=0.033) and male (OR=4.303, P=0.008) with death. Surprisingly, we found the mortality of non-elderly patients is higher than elderly (32.4% VS 20.0%, P=0.164), and both IKF and RASI treatment were not correlated with any prognostic indicators including severe, death and LOS. Conclusion: This study observed that several non-traditional issues were associated with early cardiac injury in COVID-19 while many traditional cardiovascular risk factors were not. Besides elderly and male, hypertension was confirmed to be the most important risk factor. [**note: I haven't seen a paper from Wuhan in a while but here is one looking at acute cardiac related injury. Interesting and disturbing that they saw higher mortality in the non-elderly cohort.**] https://www.medrxiv.org/content/10.1101/2020.07.06.20147256v1
- There are plausible mechanisms by which angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) may increase the risk of COVID-19 infection or affect disease severity. To examine the association between these medications and COVID-19 infection or hospitalization, we conducted a retrospective cohort study within a US integrated healthcare system. Among people aged ≥18 years enrolled in the health plan for at least 4 months as of 2/29/2020, current ACEI and ARB use was identified from pharmacy data, and the estimated daily dose was calculated and standardized across medications. COVID-19 infections were identified through 6/14/2020 from laboratory and hospitalization data. We used logistic regression to estimate adjusted odds ratios (ORs) and 95% confidence intervals. Among 322,044 individuals, 720 developed COVID-19 infection. Among people using ACEI/ARBs, 183/56,105 developed COVID-19 (3.3 per 1000 individuals) compared with 537/265,939 without ACEI/ARB use (2.0 per 1000), yielding an adjusted OR of 0.94 (95% CI 0.75-1.16). For use of < 1 defined

daily dose vs. nonuse, the adjusted OR for infection was 0.89 (95% CI 0.62-1.26); for 1 to < 2 defined daily doses, 0.97 (95% CI 0.71-1.31); and for ≥2 defined daily doses, 0.94 (95% CI 0.72-1.23). The OR was similar for ACEIs and ARBs and in subgroups by age and sex. 29% of people with COVID-19 infection were hospitalized; the adjusted OR for hospitalization in relation to ACEI/ARB use was 0.92 (95% CI 0.54-1.57), and there was no association with dose. These findings support current recommendations that individuals on these medications continue their use. [**note: here's a large cohort study within Kaiser Permanente on ACEI/ARB therapy and COVID-19. Drug therapy seems to have little impact.**]
https://www.medrxiv.org/content/10.1101/2020.07.06.20120386v1

- Limited data are available for antiviral therapy efficacy especially for the most severe patients under mechanical ventilation suffering from Covid-19 related Acute Respiratory Distress Syndrome (ARDS). Methods Observational multicenter cohort of patients with moderate to severe Covid-19 ARDS, comparing antiviral strategies (none, hydroxychloroquine (HCQ), lopinavir/ritonavir (L/R), others (combination or remdesivir). The primary end-point was the day-28 ventilator free days (VFD), patients which died before d28 were considered as having 0 VFD. The variable was dichotomized in patients still ventilated or dead at day 28 vs patients being extubated and alive at day 28 (VFD = or > 0). Results We analyzed 376 patients (80 with standard of care (SOC), 49 treated with L/R, 197 with HCQ, and 50 others). The median number of d28-VFD was 0 (IQR 0-13) and was different across the different groups (P=0.01), the SOC patients having the highest d28-VFD. A multivariate logistic regression including antiviral strategies, showed that age (OR 0.95 CI95%:0.93-0.98), male gender (OR 0.53 CI95%:0.31-0.93), Charlson score (OR 0.85 CI95%:0.73-0.99) and plateau pressure (OR 0.94 CI95%:0.88-0.99) were associated with having 0 d28-VFD whereas P/F ratio (OR 1.005 CI95%:1.001-1.010) was associated with having > or = 1 d28-VFD (ie. being extubated and alive). Acute kidney injury (AKI) was frequent (64%), its incidence was different across the patients groups (P=0.01). In a post-hoc logistic multivariate regression apart from demographics characteristics and comorbidities, the use of L/R (administered to 81 of 376 patients) was associated with occurrence of AKI (OR 2.07 CI95%:1.17-3.66) and need for renal replacement therapy (RRT). Conclusion In this observational study of moderate to severe Covid-19 ARDS patients, we did not observed a benefit of treating patients with any specific antiviral treatment. We observed an association between L/R treatment and occurrence of AKI and need for RRT. [**note: an observational study showing not impact of either HCQ or lopinavir/ritonavir**]
https://www.medrxiv.org/content/10.1101/2020.06.28.20141911v1
- To assess the efficacy of hydroxychloroquine on mild-moderate COVID-19 patients in South Korea. Methods: A retrospective cohort study of the 358 laboratory-confirmed SARS-CoV-2 (COVID-19) patients was conducted. 226 patients met inclusion criteria for analysis. Propensity score matching (PSM) and Cox regression method were utilized to control and adjust for confounding factors. Mild to moderate COVID-19 patients were managed with hydroxychloroquine (HQ) plus antibiotics (n = 31) or conservative treatment (n = 195). Results: Kaplan-Meier curves drawn using propensity score-matched data revealed no differences between the length of time to viral clearance and duration of hospital stay between the two treatment arms (p=0.18, p=0.088). Multivariable Cox regression analysis similarly showed that time to viral clearance(Hazard ratio (HR) 0.97, [95%-confidence interval (CI): 0.57-1.67]) and symptom duration(HR 1.05, [95%-CI: 0.62-1.78]) were not different between groups. No severe

adverse event or death was observed in either group. Conclusions: HQ with antibiotics was not associated with better clinical outcomes in terms of time to viral clearance, length of hospital stay, and duration of symptoms compared to conservative treatment alone. Large prospective randomized trials are necessary for definitive conclusions. [**note: another negative report on HCQ with antibiotics**] https://www.medrxiv.org/content/10.1101/2020.07.04.20146548v1

DRUG DEVELOPMENT

- There is a need for safe and effective antiviral molecules with which to combat COVID-19 pandemics. Recently, in vitro inhibitory activity of favipiravir against SARS-CoV-2 was reported. Here, we used a Syrian hamster model to explore the pharmacokinetics of this molecule and its in vivo efficacy against SARS-CoV-2. Results revealed that high doses (700-1400mg/kg/day) significantly reduced virus replication in the lungs accompanied by clinical alleviation of the disease. However, these high doses were associated with significant toxicity in hamsters. Favipiravir pharmacokinetics displayed non-linear increase in plasma exposure between the doses and good lung penetration. Analysis of viral genomes in vivo showed that favipiravir induced a mutagenic effect. Whilst the plasma trough concentrations observed in this study were comparable with those previously found during human clinical trials, this potential toxicity requires further investigation to assess whether a tolerable dosing regimen can be found in humans that effectively reduces virus replication. [**note: favipiravir is only approved in Japan and there are some clinical trials for SARS-CoV-2 ongoing. The mutagentic effect shown here is troubling.**] https://www.biorxiv.org/content/10.1101/2020.07.07.191775v1
- A small interfering RNA (siRNA) inhibitors have demonstrated the novel modality for suppressing infectious diseases. Sixty-one siRNA molecules, predicted by the bioinformatics programs, were screened for the possibility of treating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using an in vitro plaque assay. Among six siRNA leads with the efficacy of reducing plaque number, the siRNA targeting RNA-dependent RNA polymerase (RdRp) showed a reduction in SARS-CoV-2 infection-induced fever and virus titer in the Golden Syrian hamster and rhesus macaque. These results suggest the potential for RdRp targeting siRNA as a new treatment for the coronavirus disease 2019 (COVID-19). [**note: the use of RNA as a drug has been tried and there are some antisense therapies approved. This is different approach and one worth more research.**] https://www.biorxiv.org/content/10.1101/2020.07.07.190967v1
- Background: Coronavirus disease 2019 (COVID-19) leads to inflammatory cytokine release, which can downregulate the expression of metabolizing enzymes. This cascade affects drug concentrations in the plasma. We investigated the association between lopinavir (LPV) and hydroxychloroquine (HCQ) plasma concentrations and the values of acute phase inflammation marker C-reactive protein (CRP). Methods: LPV plasma concentrations were prospectively collected in 92 patients hospitalized at our institution. Lopinavir/ritonavir was administered 12 hourly, 800/200 mg on day 1, and 400/100 mg on day 2 until day 5 or 7. HCQ was given at 800 mg, followed by 400 mg after 6, 24 and 48 hours. Hematological, liver, kidney, and inflammation laboratory values were analyzed on the day of drug level determination. Results: The median age of study participants was 59 (range 24 up to 85) years, and 71% were male. The median duration from symptom onset to hospitalization and treatment initiation was 7 days (IQR 4;10) and 8 days (IQR 5;10), respectively. The median LPV trough concentration on day 3 of treatment was 26.5 ug/mL (IQR 18.9;31.5). LPV plasma concentrations positively correlated with CRP

values (r=0.37, p<0.001), and were significantly lower when tocilizumab was preadministrated. No correlation was found between HCQ concentrations and CRP values. Conclusions: High LPV plasma concentrations were observed in COVID-19 patients. The ratio of calculated unbound drug fraction to published SARS-CoV2 EC50 values indicated insufficient LPV concentrations in the lung. CRP values significantly correlated with LPV but not HCQ plasma concentrations, implying inhibition of cytochrome P450 3A4 (CYP3A4) metabolism by inflammation. [**note: maybe lopinavir/ritonavir does not work because the drug doesn't reach the lung.. HCQ had no impact on C-reactive protein levels.**]
https://www.medrxiv.org/content/10.1101/2020.07.05.20146878v1

- To address this need, we leveraged a biomaterial vaccine technology that consists of mesoporous silica rods (MSRs) that provide a sustained release of granulocyte-macrophage colony-stimulating factor (GM-CSF) and adjuvants to concentrate and mature antigen-presenting cells at the vaccine site. Here we explored the humoral responses resulting from the use of monophosphoryl lipid A (MPLA) as the adjuvant and SARS-CoV-2 spike proteins S1, S2, the nucleocapsid (N) protein, and receptor binding domain (RBD) as the target antigens. The dose of antigen and impact of pre-manufacturing of vaccines as versus loading antigen just-in-time was explored in these studies. Single shot MSR vaccines induced rapid and robust antibody titers to the presented antigens, even without the use of a boost, and sera from vaccinated animals demonstrated neutralizing activity against a SARS-CoV-2 pseudovirus. Overall, these results suggest the MSR vaccine system may provide potent protective immunity when utilized to present SARS-CoV-2 antigens. [**note: wait for it……..another new vaccine candidate!!! I have lost count now on the number of different approaches. This is a really cool technology. It's too bad they have missed out on Operation Warp Speed funding.**]
https://www.biorxiv.org/content/10.1101/2020.07.07.192203v1

VIRUS BIOCHEMISTRY

- The non-structural protein 1 (Nsp1), also referred to as the host shutoff factor, is the first viral protein that is synthesized in SARS-CoV-2 infected human cells to suppress host innate immune functions. By combining cryo-electron microscopy and biochemical experiments, we show that SARS-CoV-2 Nsp1 binds to the human 40S subunit in ribosomal complexes including the 43S pre-initiation complex. The protein inserts its C-terminal domain at the entrance to the mRNA channel where it interferes with mRNA binding. We observe potent translation inhibition in the presence of Nsp1 in lysates from human cells. Based on the high-resolution structure of the 40S-Nsp1 complex, we identify residues of Nsp1 crucial for mediating translation inhibition. We further show that the full-length 5' untranslated region of the genomic viral mRNA stimulates translation in vitro, suggesting that SARS-CoV-2 combines inhibition of translation by Nsp1 with efficient translation of the viral mRNA to achieve expression of viral genes. [**note: the virus gets more nefarious with every new preprint.]**
https://www.biorxiv.org/content/10.1101/2020.07.07.191676v1
- The Spike is a hallmark coronavirus protein that determines virus fusion, entry and spread in the host, and thus holds clues for the rapid spread of the SARS-CoV-2 pandemic. We have investigated the Spike from six β-coronaviruses, including the SARS-CoV-2, and find that their surface-exposed fusion peptides constituting the fusion loop are spatially organized contiguous to each other to work synergistically for triggering the virus-host membrane fusion process. The

SARS-CoV-2 fusion peptides have unique physicochemical properties, accrued in part from the presence of consecutive prolines that impart backbone rigidity which aids the virus fusogenicity. The specific contribution of these prolines has been inferred from comparative studies of their deletion mutant in a fellow murine β-coronavirus MHV-A59 that show significantly diminished fusogenicity in vitro and associated pathogenesis in vivo. The Spike cleavage-linked priming and fusogenic conformational transition steered by the fusion loop may be critical for the SARS-CoV-2 spread. [**note: I am not sure if fusogenicity is a word but this paper provides more information on the Spike protein and how the virus infects cells.**]
https://www.biorxiv.org/content/10.1101/2020.07.07.191973v1

DIAGNOSTIC DEVELOPMENT

- Wearable devices digitally measuring vital signs have been used for monitoring health and illness onset and have high potential for real-time monitoring and disease detection. As such they are potentially useful during public health crises, such as the current COVID-19 global pandemic. Using smartwatch data from 31 infected individuals identified from a cohort of over 5000 participants, we investigated the use of wearables for early, presymptomatic detection of COVID-19. From physiological and activity data, we first demonstrate that COVID-19 infections are associated with alterations in heart rate, steps and sleep in 80% of COVID-19 infection cases. Failure to detect these changes in the remaining patients often occurred in those with chronic respiratory/lung disease. Importantly the physiological alterations were detected prior to, or at, symptom onset in over 85% of the positive cases (21/24), in some cases nine or more days before symptoms. Through daily surveys we can track physiological changes with symptom onset and severity. Finally, we develop a method to detect onset of COVID-19 infection in real-time which detects 67% of infection cases at or before symptom onset. Our study provides a roadmap to a rapid and universal diagnostic method for the large-scale detection of respiratory viral infections in advance of symptoms, highlighting a useful approach for managing epidemics using digital tracking and health monitoring. [**note: looks like we all should be wearing smart watches and link our data to this Stanford group. Let's divert some of the BARDA funding to buy watches!!**] https://www.medrxiv.org/content/10.1101/2020.07.06.20147512v1
- To accurately measure seroprevalance in the population, boththe expected immune response as well as the assay performances have to be well characterised. Here, we describe the collection and initial characterisation of a blood and saliva biobank obtained after the initial peak of the SARS-CoV-2 pandemic in Switzerland. Methods: Two laboratory ELISA assays measuring IgA & IgG (Euroimmun), and IgM & IgG (Epitope Diagnostics) were used to characterise the biobank collected from 349 re- and convalescent patients from the canton of Basel-Landschaft. Findings: The antibody response in terms of recognized epitopes is diverse, especially in oligosymptomatic patients, while the average strength of the antibody response of the population does correlate with the severity of the disease at each time point. Interpretation: The diverse immune response presents a challenge when conducting epidemiological studies as the used assays only detect 90% of the oligosymptomatic cases. This problem cannot be rectified by using more sensitive assays or lower cut-offs as they concomitantly reduce specificity. [**note: this Swiss study points out some difficulties in using serology tests.**]
https://www.medrxiv.org/content/10.1101/2020.07.05.20145888v1

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2020-07-09

I was going to post a clip from the final scene of Der Rosenkavalier yesterday to close out the romantic love theme from that opera but wanted to acknowledge Charlie Daniel's passing. There are a number of good clips but I have always liked Renee Fleming in this role. Here is a Met production with Fleming, Susan Graham and Christine Schäfer with Edo de Waart conducting: https://www.youtube.com/watch?v=fi810zB3L04 Also, let's take a moment to remember the great violinist Ida Haendel who just passed away. Here she is playing an encore piece by Sarasate, 'Gypsy Airs' from 2008 when she was 85 years young: https://www.youtube.com/watch?v=uGXArQJA3Po

US COVID-19 STATISTICS - **Infection Rate: 0.9%; CRF: 4.2%** (CRF drops by 0.1%)

[**editorial warning**] I sometimes, no make that all the time, wonder why people do not seem to grasp basic public health concepts. We now have a story about Tennessee who thought increasing testing alone would solve the state's COVID-19 infection. WRONG! Testing is a tool and should never instill magical thinking in the populace. Now the CDC is apparently reconsidering its rules in light of comments from President Trump on school reopening. At this point, I need to put on the gloves and get in the ring! Have these people no clue? Yes, kids my be resistant to severe COVID-19 progression but stop and consider the teachers. Last I noticed, there are no teachers that fall in that age category. How is physical distancing going to be maintained in a crowed classroom of 30? Are all students going to have to wear masks? Will there be routine temperature checks at the door before students are admitted (many parents already send sick children to school)? How will special ed teachers deal with students that are often difficult to control? There are other questions that could be asked. It's time to put up or shut up! Secretaries Azar and de Vos, take some time out from your schedule and spend one week in a classroom this fall and see what the teachers are dealing with. That's it for the political editorial.

Straight out of a Philip K. Dick novel, robots are the future!!!!

At long last NIH has stepped up to the plate and established a network for testing vaccines and mAbs. I guess they looked at how well the UK was doing in setting up and running trials. Here is the Washington Post article on this. I seem to remember a white paper written back in April calling for this type of effort!

Alas, another sad mask story. Shoppers in Mobile still won't wear masks in stores even though the city requires it. One workers says management says just serve the customer and don't talk about the lack of a mask.

Derek Lowe weighs in on the really important stuff related to vaccines (yup, this is click bait but then I confront this every day with preprints that have teaser abstracts; I need to have a bit of fun with all my loyal readers!).

The BMJ weighs in on the issue of whether herd immunity is achievable. On the other hand there may be parts of New York City that have reached the herd immunity point albeit at a high cost in terms of mortality. So many unknowns!!!

I do not want to get into the broad debate about health insurance but will only note that there are large numbers of Americans who have either lost or will lose employer paid health insurance. This JAMA

insight article discusses the need to improve the Affordable Care Act market place so eligible people can easily transition.

STAT has a nice opinion piece on national wastewater testing for COVID-19. Staying with STAT, the zombie drug HCQ continues to be embroiled in a political fight and the CEO of Medicago, who have a plant-derived COVID-19 vaccine in development, comments on development issues (see the new clinical trials section for more on this vaccine). This story is worth a read.

This fashionista is saddened by the Chapter 11 bankruptcy filing of Brooks Brothers who also own the Southwick suit manufacturing facility that I had a long relationship with.

MODELING

- COVID-19 has rapidly affected mortality worldwide[1]. There is unprecedented urgency to understand who is most at risk of severe outcomes, requiring new approaches for timely analysis of large datasets. Working on behalf of NHS England, here we created OpenSAFELY: a secure health analytics platform covering 40% of all patients in England, holding patient data within the existing data centre of a major primary care electronic health records vendor. Primary care records of 17,278,392 adults were pseudonymously linked to 10,926 COVID-19-related deaths. COVID-19-related death was associated with: being male (hazard ratio (HR) 1.59, 95% confidence interval (CI) 1.53–1.65); older age and deprivation (both with a strong gradient); diabetes; severe asthma; and various other medical conditions. Compared with people with white ethnicity, Black and South Asian people were at higher risk even after adjustment for other factors (HR 1.48, 1.30–1.69 and 1.44, 1.32–1.58, respectively). We have quantified a range of clinical risk factors for COVID-19-related death in the largest cohort study conducted by any country to date. OpenSAFELY is rapidly adding further patients' records; we will update and extend results regularly. [**note: WOW, this is one large study!!! Only the UK could pull this one off. There is coverage by the New York Times.**] https://www.nature.com/articles/s41586-020-2521-4
- Asymptomatic or subclinical SARS-CoV-2 infections are often unreported, which means that confirmed case counts may not accurately reflect underlying epidemic dynamics. Understanding the level of ascertainment (the ratio of confirmed symptomatic cases to the true number of symptomatic individuals) and undetected epidemic progression is crucial to informing COVID-19 response planning, including the introduction and relaxation of control measures. Estimating case ascertainment over time allows for accurate estimates of specific outcomes such as seroprevalence, which is essential for planning control measures. Methods: Using reported data on COVID-19 cases and fatalities globally, we estimated the proportion of symptomatic cases (i.e. any person with any of fever >= to 37.5C, cough, shortness of breath, sudden onset of anosmia, ageusia or dysgeusia illness) that were reported in 210 countries and territories, given those countries had experienced more than ten deaths. We used published estimates of the case fatality ratio (CFR) as an assumed baseline. We then calculated the ratio of this baseline CFR to an estimated local delay-adjusted CFR to estimate the level of under-ascertainment in a particular location. We then fit a Bayesian Gaussian process model to estimate the temporal pattern of under-ascertainment. Results: We estimate that, during March 2020, the median

percentage of symptomatic cases detected across the 84 countries which experienced more than ten deaths ranged from 2.38% (Bangladesh) to 99.6% (Chile). Across the ten countries with the highest number of total confirmed cases as of 6th July 2020, we estimated that the peak number of symptomatic cases ranged from 1.4 times (Chile) to 17.8 times (France) larger than reported. Comparing our model with national and regional seroprevalence data where available, we find that our estimates are consistent with observed values. Finally, we estimated seroprevalence for each country. Despite low case detection in some countries, our results that adjust for this still suggest that all countries have had only a small fraction of their populations infected as of July 2020. Conclusions: We found substantial under-ascertainment of symptomatic cases, particularly at the peak of the first wave of the SARS-CoV-2 pandemic, in many countries. Reported case counts will therefore likely underestimate the rate of outbreak growth initially and underestimate the decline in the later stages of an epidemic. Although there was considerable under-reporting in many locations, our estimates were consistent with emerging serological data, suggesting that the proportion of each country's population infected with SARS-CoV-2 worldwide is generally low. [**note: this is a useful paper to take a look at to explore how infections are undercounted because of asymptomatic carriers who are never tested. It would really help identify what the true CRF is for this virus if we had better infections numbers. Death is "usually" easier to measure.** 😊]
https://www.medrxiv.org/content/10.1101/2020.07.07.20148460v1

- Timely identification of COVID-19 patients at high risk of mortality can significantly improve patient management and resource allocation within hospitals. This study seeks to develop and validate a data-driven personalized mortality risk calculator for hospitalized COVID-19 patients. Methods: De-identified data was obtained for 3,927 COVID-19 positive patients from six independent centers, comprising 33 different hospitals. Demographic, clinical, and laboratory variables were collected at hospital admission. The COVID-19 Mortality Risk (CMR) tool was developed using the XGBoost algorithm to predict mortality. Its discrimination performance was subsequently evaluated on three validation cohorts. Findings: The derivation cohort of 3,062 patients has an observed mortality rate of 26.84%. Increased age, decreased oxygen saturation (≤ 93%), elevated levels of C-reactive protein (≥ 130 mg/L), blood urea nitrogen (≥ 18 mg/dL), and blood creatinine (≥ 1.2 mg/dL) were identified as primary risk factors, validating clinical findings. The model obtains out-of-sample AUCs of 0.90 (95% CI, 0.87-0.94) on the derivation cohort. In the validation cohorts, the model obtains AUCs of 0.92 (95% CI, 0.88-0.95) on Seville patients, 0.87 (95% CI, 0.84-0.91) on Hellenic COVID-19 Study Group patients, and 0.81 (95% CI, 0.76-0.85) on Hartford Hospital patients. The CMR tool is available as an online application at covidanalytics.io/mortality_calculator and is currently in clinical use. Interpretation: The CMR model leverages machine learning to generate accurate mortality predictions using commonly available clinical features. This is the first risk score trained and validated on a cohort of COVID-19 patients from Europe and the United States. [**note: as I posited a couple of weeks ago it would be good if someone would try to correlate all the markers to see what the best predictors are. This mainly MIT group has done just that and it's online and being used! Good work.**] https://www.medrxiv.org/content/10.1101/2020.07.07.20148304v1
- Children (less than 19 years) account for 20% of the US population but currently represent less than 2% of coronavirus disease 2019 (COVID-19) cases. Because infected children often have few or no symptoms and may not be tested, the extent of infection in children is poorly

understood. METHODS During the March 18th-May 15th 2020 Louisiana Stay At Home Order, 1690 blood samples from 812 individuals from a Childrens Hospital were tested for antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein. Demographics, COVID-19 testing, and clinical presentation abstracted from medical records were compared with local COVID-19 cases. RESULTS In total, 62 subjects (7.6%) were found to be seropositive. The median age was 11 years with 50.4% female. The presenting complaint of seropositive patients was chronic illness (43.5%). Only 18.2% had a previous positive COVID-19 PCR or antibody test. Seropositivity was significantly associated with parish (counties), race, and residence in a low-income area. Importantly, seropositivity was linearly correlated with cumulative COVID-19 case number for all ages by parish. CONCLUSION In a large retrospective study, the seropositivity prevalence for SARS-CoV-2 in children in Louisiana during the mandated Stay At Home Order was 7.6%. Residence location, race, and lower socioeconomic factors were linked to more frequent seropositivity in children and correlated to regional COVID-19 case rates. Thus, a significant number of children in Louisiana had SARS-CoV-2 infections that went undetected and unreported and may have contributed to virus transmission. [**note: serology testing of stay at home youth in New Orleans during the hard hit period. Fairly high number of infections observed.**] https://www.medrxiv.org/content/10.1101/2020.07.07.20147884v1

NEWLY REGISTERED CLINICAL TRIALS

- Randomised, single-blinded trial. Patients with a diagnosis of COVID-19 infection within the past 96 hours and not requiring hospitalization will be recruited into a trial of BID Nasal irrigation for 14 days, followed by a 14 day observation period. Irrigation will be performed with either Probiorinse probiotic nasal irrigation solution or NeilMed Sinus rinse. Patients will be able to identify their treatments, but study staff will be blinded as to assignment. [**note: I had no idea there was a probiotic nasal rinse! I do use NeilMed Sinus rinse during allergy season and it seems to help alleviate symptoms. I've seen a bunch of nasal irrigation trials posted including those using Povidone/Iodine solutions. I'm skeptical that these will present infection but it would be great if they did as they are truly low cost.]** NCT04458519
- The study will be a randomized, partially-blinded, prime-boost, staggered dose-escalation Phase 1 study intended to assess the safety, tolerability, and immunogenicity of the Coronavirus-Like Particle COVID-19 Vaccine at three dose levels (3.75 µg, 7.5 µg, and 15 µg VLP) unadjuvanted or adjuvanted with either Adjuvant A or Adjuvant B in healthy adults 18 to 55 years of age, who have been tested for the absence of SARS-CoV-2 antibodies. [**note: another out of the blue vaccine trial. Medicago is based in Canada. It is a genetically engineered plant derived vaccine.**] NCT04450004

CLINICAL TRIAL RESULTS

- Development of antibody protection during SARS-CoV-2 (CoV-2) infection is a pressing question for public health and for vaccine development. We developed highly sensitive CoV-2-specific antibody and neutralization assays. CoV-2 Spike protein or Nucleocapsid protein specific IgG antibodies at titers more than 1:100,000 were detectable in all PCR+ subjects (n=87) and were absent in the negative controls. Other isotype antibodies (IgA, IgG1-4) were also detected. CoV-2 neutralization was determined in COVID-19 and convalescent plasma up to 10,000-fold dilution, using Spike protein pseudotyped lentiviruses, which was also blocked by neutralizing

antibodies (NAbs). Hospitalized patients had up to 3000-fold higher antibody and neutralization titers compared to outpatients or convalescent plasma donors. Further, subjects who donated plasma further out from the diagnosis of COVID-19 appeared to have lower titers. Interestingly, some COVID-19 patients also contained NAbs against SARS Spike protein pseudovirus. Together these results demonstrate the high specificity and sensitivity of our assays, which may impact understanding the quality or duration of the antibody response during COVID-19 and in determining the effectiveness of potential vaccines. [**note: more information on the immune response. What is troubling are the high levels of neutralizing antibodies in in hospitalized patients. Something else is going on with these patients relative to those who have lower antibody titers and are not admitted. This is one curious virus.]**
https://www.medrxiv.org/content/10.1101/2020.07.07.20148106v1

- To date no effective therapy has been demonstrated for COVID-19. In vitro, studies indicated that ivermectin (IVM) has antiviral effect. Objectives: To assess the effectiveness of ivermectin (IVM) as add-on therapy to hydroxychloroquine (HCQ) and azithromycin (AZT) in treatment of COVID-19. Methods: This Pilot clinical trial conducted on hospitalized adult patients with mild to moderate COVID-19 diagnosed according to WHO interim guidance. Sixteen Patients received a single dose of IVM 200Mcg /kg on admission day as add on therapy to hydroxychloroquine (HCQ)and Azithromycin (AZT) and were compared with 71 controls received HCQ and AZT matched in age, gender, clinical features, and comorbidities. The primary outcome was percentage of cured patients, defined as symptoms free to be discharged from the hospital and 2 consecutive negative PCR test from nasopharyngeal swabs at least 24 hours apart. The secondary outcomes were time to cure in both groups and evaluated by measuring time from admission of the patient to the hospital till discharge. Results: Of 87 patients included in the study,t he mean age ± SD (range) of patients in the IVM group was similar to controls [44.87 ± 10.64 (28-60) vs 45.23 ± 18.47 (8-80) years, p=0.78] Majority of patients in both groups were male but statistically not significant [11(69%) versus 52 (73%), with male: female ratio 2.21 versus 2.7-, p=0.72) All the patients of IVM group were cured compared with the controls [16 (100 %) vs 69 (97.2 %)]. Two patients died in the controls. The mean time to stay in the hospital was significantly lower in IVM group compared with the controls (7.62 ±2.75 versus 13.22 ±.90 days, p=0.00005, effect size= 0.82). No adverse events were observed Conclusions : Add-on use of IVM to HCQ and AZT had better effectiveness, shorter hospital stay, and relatively safe compared with controls. however, a larger prospective study with longer follow up may be needed to validate these results. [**note: this is included because it is the first preprint from Iraq showing that we are all in this together. Unfortunately, the patient size is too low to draw any firm conclusions. A lot of developing countries are conducting ivermectin trials. Of course they are also using HCQ + azithromycin. As always, TIWWDCT**]
https://www.medrxiv.org/content/10.1101/2020.07.07.20145979v1
- Generally, children and teenagers do not become seriously ill with COVID-19. However, in countries with high rates of coronavirus disease, children with the syndrome COVID-19 associated inflammation syndrome referred to as PIMS-TS have been reported. Similarities noted between SARS-CoV-2 Spike protein sequences and those of other super antigens has prompted the suggestion that this might be the mechanism by SARS-CoV-ST triggers PIMS-TS. It has also been suggested that the D614G variant found more commonly in the US and across European countries may explain why PIMS-TS appears to be common in these countries. Here

we analysed viral sequences from 13 paediatric COVID-19 patients of whom five were diagnosed with PIMS-TS. This is the first characterisation of viruses from PIMS-TS patients. In contrast to what has been hypothesised, we found no evidence of unique sequences associated with the viruses from PIMS-TS patients. [**note: this is useful, showing that there is not a variant SARS-CoV-2 causing inflammatory syndrome in pediatric patients.**] https://www.medrxiv.org/content/10.1101/2020.07.07.20148213v1

- Chloroquine has been frequently administered for treatment of coronavirus disease 2019 but there are serious concerns about its efficacy and cardiac safety. Our objective was to investigate the pharmacokinetics and safety of chloroquine in hospitalized COVID-19 patients. Design: A prospective observational study. Setting: Dutch hospitals Patients: Patients admitted to the hospital for treatment of COVID-19. Interventions: Pharmacokinetic sampling Measurements: The plasma concentrations of chloroquine and desethylchloroquine and QTc time. Main Results: A total of 83 patients were included. The median (IQR) plasma concentration chloroquine during treatment was 1.05 µmol/L (0.63 - 1.55 µmol/L). None of the patients reached exposure exceeding the concentration to inhibit SARS-CoV-2 replication by 90% (IC90) of 6.9 µM. Furthermore, ΔQTc >60 milliseconds occurred after initiation of chloroquine treatment in 34% patients and during treatment QTc ≥500 milliseconds was observed in 46% of patients. Conclusions: Recommended dose chloroquine treatment results in plasma concentrations that are unlikely to inhibit viral replication. Furthermore, the incidence of QTc prolongation was high. The preclinical promise of chloroquine as antiviral treatment in patients with COVID-19 is overshadowed by its cardiac toxicity and lack of effective exposure. It is unlikely that a positive clinical effect will be found with chloroquine for treatment of COVID-19. [**note: from The Netherlands, pretty convincing evidence that chloroquine should not be used to treat COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.07.06.20147470v1

DRUG DEVELOPMENT

- Clinical presentations range from asymptomatic, mild respiratory tract infection, to severe cases with acute respiratory distress syndrome, respiratory failure, and death. Reports on a dysregulated immune system in the severe cases calls for a better characterization and understanding of the changes in the immune system. Here, we profiled whole blood transcriptomes of 39 COVID-19 patients and 10 control donors enabling a data-driven stratification based on molecular phenotype. Neutrophil activation-associated signatures were prominently enriched in severe patient groups, which was corroborated in whole blood transcriptomes from an independent second cohort of 30 as well as in granulocyte samples from a third cohort of 11 COVID-19 patients. Comparison of COVID-19 blood transcriptomes with those of a collection of over 2,600 samples derived from 11 different viral infections, inflammatory diseases and independent control samples revealed highly specific transcriptome signatures for COVID-19. Further, stratified transcriptomes predicted patient subgroup-specific drug candidates targeting the dysregulated systemic immune response of the host. [**note: good work from a mainly German group of researchers honing in neutrophil activation-associate signatures. They also show how it may be useful in drug discovery.**] https://www.medrxiv.org/content/10.1101/2020.07.07.20148395v1

VIRUS BIOCHEMISTRY

- Nothing today.

DIAGNOSTIC DEVELOPMENT

- This study was aimed to investigate the application of SARS- COV-2 IgM and IgG antibodies in diagnosis of COVID-19 infection. Method: This study enrolled a total of 178 patients at Huangshi Central Hospital from January to February, 2020. Among them, 68 patients were SARS-COV-2 infected confirmed with nucleic acid test (NAT) and CT imaging. 9 patients were in the suspected group (NAT negative) with fever and other respiratory symptoms. 101 patients were in the control group with other diseases and negative to SARS-COV-2 infection. After serum samples were collected, SARS-COV-2 IgG and IgM antibodies were tested by chemiluminescence immunoassay (CLIA) for all patients. Results: The specificity of serum IgM and IgG antibodies to SARS-COV-2 were 99.01% (100/101) and 96.04% (97/101) respectively, and the sensitivity were 88.24% (60/68) and 97.06% (66/68) respectively. The combined detection rate of SARS-COV-2 IgM and IgG antibodies were 98.53% (67/68). Conclusion: Combined detection of serum SARS-COV-2 IgM and IgG antibodies had better sensitivity compared with single IgM or IgG test, which can be used as an important diagnostic tool for SARS-COV-2 infection and a screening tool of potential SARS-COV-2 carriers in clinics, hospitals and accredited scientific laboratory. [**note: from China a report showing that combined detection of IgM and IgG antibodies was better than a single test.**] https://www.medrxiv.org/content/10.1101/2020.07.07.20146902v1

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2020-07-10

I have been giving short shrift to violinists and will remedy this today. One of my favorite pieces is Max Bruch's Scottish Fantasy. The first recording I had back in the vinyl days was the one by Kyung-Wha Chung that was recorded back in 1972. Here is a much later live performance from Seoul in 2012. The conductor is her brother Myung-Whun Chung and frequent collaborator in chamber music performances: https://www.youtube.com/watch?v=0Bux10fh3CI

US COVID-19 STATISTICS - **Infection Rate: 0.9%; CRF: 4.2%** (CRF no change)

The New York Times has a story on the Regeneron efforts to develop a monoclonal antibody treatment in the middle of a pandemic lockdown. Also from the Times and unrelated to COVID-19, a number of pharma companies have committed significant funding to the discovery of new antibiotics to combat growing resistance. $1 billion is pledged to this effort and more about it is on the AMR action fund website. 👍 to the pharma companies participating in this sorely needed project! Want to know if your state is meeting needed testing levels? Many states are not as can be seen by this Times graphic. WHO has updated the guidance on airborne transmission; the new Q&A from WHO is HERE.

The Washington Post offers several pieces on reopening schools, HERE, HERE & HERE. Some good commentary from teachers who will be directly impacted by decsisions.

Do you have the urge to get away? Ms. Goldhammer told me last night she is tired of being holed up in our humble abode and that we need to find a place to go. I just discovered the ideal getaway spot!!! This island on Casco Bay in Maine (and one of my loyal readers spends summers in Maine!!!) was once a quarantine facility. The owner of the luxury property is now marketing it as a virus-free get away

destination. All one needs is a cool $250K. Ms. G will be disappointed to hear that this is a bit out of our price range.

Derek Thompson on the various reasons the mortality rate has plateaued.

Here is commentary by Peter Marks, the FDA CBER director, on what it will take to get a COVID-19 vaccine licensed.

Excellent news, cartoonist Gary Larson of *The Far Side* fame is back! He has three new digital cactoons up on his 'New Stuff' page. Enjoy!

MODELING

- The evolution of the COVID-19 pandemic can be monitored through the detection of SARS-CoV-2 RNA in sewage. Here, we measured the amount of SARS-CoV-2 RNA at the inflow point of the main waste water treatment plant (WWTP) of Montpellier, France. We collected samples 4 days before the end of lockdown and up to 45 days post-lockdown. We detected increased amounts of SARS-CoV-2 RNA at the WWTP, which was not correlated with the number of newly diagnosed patients. Future epidemiologic investigations may explain such asynchronous finding. [**note: wastewater sampling from a city in France examines the impact of the lockdown. The presence of positive viral RNA samples did not corelate with the drop in SARS-CoV-2 infections which had declined. The authors go into reasons for this asynchronicity. It may be that wastewater sampling is useful to monitor presence of virus in new areas but not of utility to show a decrease in the population.]**
  https://www.medrxiv.org/content/10.1101/2020.07.08.20148882v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- The prevalence and significance of digestive manifestations in COVID-19 remain uncertain. Methods: Consecutive patients hospitalized with COVID-19 were identified across a geographically diverse alliance of medical centers in North America. Data pertaining to baseline characteristics, symptomatology, laboratory assessment, imaging, and endoscopic findings from the time of symptom onset until discharge or death were manually abstracted from electronic health records to characterize the prevalence, spectrum, and severity of digestive manifestations. Regression analyses were performed to evaluate the association between digestive manifestations and severe outcomes related to COVID-19. Results: A total of 1992 patients across 36 centers met eligibility criteria and were included. Overall, 53% of patients experienced at least one gastrointestinal symptom at any time during their illness, most commonly diarrhea (34%), nausea (27%), vomiting (16%), and abdominal pain (11%). In 74% of cases, gastrointestinal symptoms were judged to be mild. In total, 35% of patients developed an abnormal alanine aminotransferase or total bilirubin level; these were elevated to less than 5 times the upper limit of normal in 77% of cases. After adjusting for potential confounders, the presence of gastrointestinal symptoms at any time (odds ratio 0.93, 95% confidence interval 0.76-1.15) or liver test abnormalities on admission (odds ratio 1.31, 95% confidence interval

0.80-2.12) were not independently associated with mechanical ventilation or death. Conclusions: Among patients hospitalized with COVID-19, gastrointestinal symptoms and liver test abnormalities were common but the majority were mild and their presence was not associated with a more severe clinical course. [**note: there must be 100 authors on the manuscript as it is a multi-center study. Good news is GI sumptoms are mild and not associated with more severe disease progression. However, this is something that should be tracked post infection.**] https://www.medrxiv.org/content/10.1101/2020.07.07.20143024v1

- Patients infected with SARS-CoV-2 differ in the severity of disease. In this study, SARS-CoV-2 specific T-cells and antibodies were characterized in patients with different COVID-19 related disease severity. Despite severe lymphopenia affecting all major lymphocyte subpopulations, patients with severe disease mounted significantly higher levels of SARS-CoV-2 specific T-cells as compared to convalescent individuals. SARS-CoV-2 specific CD4 T-cells dominated over CD8 T-cells and closely correlated with the number of plasmablasts and SARS-CoV-2 specific IgA- and IgG-levels. Unlike in convalescents, SARS-CoV-2 specific T-cells in patients with severe disease showed marked alterations in phenotypical and functional properties, which also extended to CD4 and CD8 T-cells in general. Given the strong induction of specific immunity to control viral replication in patients with severe disease, the functionally altered phenotype may result from the need for contraction of specific and general immunity to counteract excessive immunopathology in the lung. [**note: damn this virus is hard to decipher. This German study shows higher level of virus induced T cells than convalescent patients. Does this blow the T cell theory out of the water or is something else going on with severe COVID-19? To quote the great Bob Dylan, "something is happening but you don't know what it is."**] https://www.medrxiv.org/content/10.1101/2020.07.08.20148718v1
- In 68 respiratory specimens from a cohort of 35 COVID-19 patients, 32 of them with mild disease, we found SARS coronavirus-2 virus culture and sub-genomic RNA was rarely detectable beyond 8 days after onset of illness although virus RNA by RT-PCR remained detectable for many weeks. [**note: I may have mentioned this a couple of weeks ago when there was a similar paper. Absolute virus disappears but virus RNA does not. It may be that people who think they have a relapse really don't but show the presence of virus RNA. The gold standard is to do a virus culture.**] https://www.medrxiv.org/content/10.1101/2020.07.08.20148783v1
- Preventing communicable diseases requires understanding the spread, epidemiology, clinical features, progression, and prognosis of the disease. Early identification of risk factors and clinical outcomes might help to identify critically ill patients, provide proper treatment and prevent mortality. Methods: We conducted a prospective study in patients with flu-like symptoms referred to the imaging department of a tertiary hospital in IRAN between 3 March 2020 and 8 April 2020. Patients with COVID-19 were followed up to check their health condition after two months. The categorical data between groups were analyzed by Fisher exact test and continuous data by Wilcoxon Rank-Sum Test. Findings: 319 patients (mean age 45.48 years, 177 women) were enrolled. Fever, dyspnea, weakness, shivering, C-reactive protein (CRP), fatigue, dry cough, anorexia, anosmia, ageusia, dizziness, sweating and age were the most important symptoms of COVID-19 infection. Traveling in past three months, asthma, taking corticosteroids, liver disease, rheumatological disease, cough with sputum, eczema, conjunctivitis, tobacco use, and chest pain did not have any relationship with COVID-19. Interpretation: Finding clinical symptoms for early diagnosis of COVID-19 is a critical part of prevention. These symptoms can

help in the assessment of disease progression. To the best of our knowledge, some of the effective features on the mortality due to COVID-19 are investigated for the first time in this research. [**note: I always like to give credit to clinicians doing work under difficult circumstances. Here is an Iranian team doing some follow up on patients from tertiary hospital.**] https://www.medrxiv.org/content/10.1101/2020.07.07.20148569v1

- Among our patients, the incidence of transmission through apparent exposure to a family cluster was lower than that in other cohorts, possibly because of the late lockdown in Italy. As compared with the other cohorts, fewer patients in our cohort had moderate-to-severe disease, possibly because chest radiography was predominantly used and chest computed tomography was rarely used. Thus, fewer cases of diagnosed (subclinical) pneumonia may have been identified. Bedside lung ultrasonography by experienced sonographers was performed in only 10% of the patients, 90% of whom received a diagnosis of lung interstitial syndrome without further radiographic imaging. [**note: this is a letter to the New England Journal of Medicine on pediatric cases of COVID-19 in Italy. There is a nice table summarizing the various characteristics and outcomes, along with a comparison to three other countries. There were no fatalities in this cohort of 100 patients.**]
https://www.nejm.org/doi/full/10.1056/NEJMc2007617
- In patients who died from Covid-19–associated or influenza-associated respiratory failure, the histologic pattern in the peripheral lung was diffuse alveolar damage with perivascular T-cell infiltration. The lungs from patients with Covid-19 also showed distinctive vascular features, consisting of severe endothelial injury associated with the presence of intracellular virus and disrupted cell membranes. Histologic analysis of pulmonary vessels in patients with Covid-19 showed widespread thrombosis with microangiopathy. *Alveolar capillary microthrombi were 9 times as prevalent in patients with Covid-19 as in patients with influenza (P<0.001). In lungs from patients with Covid-19, the amount of new vessel growth — predominantly through a mechanism of intussusceptive angiogenesis — was 2.7 times as high as that in the lungs from patients with influenza (P<0.001). In our small series, vascular angiogenesis distinguished the pulmonary pathobiology of Covid-19 from that of equally severe influenza virus infection*. The universality and clinical implications of our observations require further research to define. [**note: this is a small pathology study of seven patients compared to those who died from ARDS secondary to influenza A. Something different is going on with COVID-19.**]
https://www.nejm.org/doi/full/10.1056/NEJMoa2015432
- Since December 2019, Coronavirus Disease 2019(COVID-19) occurred in wuhan, China, and outbreaked rapidly into a global pandemic. This current poses great challenges to hemodialysis (HD) patients. Objective: To make a comprehensive evaluation and comparison between HD patients confirmed with COVID-19 and the general HD patients. Methods: HD patients confirmed with COVID-19 in Wuhan No.5 Hospital were admitted as confirmed group from Jan 10 to Mar 15, 2020. And HD patients not infected in our dialysis center were chosen as control group. General characteristics, laboratory indicators were retrospectively collected, analyzed and compared. Results: A total of 142 cases were admitted, including 43 cases in confirmed group and 99 in control group. Body mass index (BMI) was slightly lower in confirmed group than that in control group (P=0.011). The proportion of one or less underlying disease in confirmed group(51.16%) was higher than that in control group(14.14%)(P< 0.001), and the proportion of three or more underlying diseases in confirmed group(11.63%) was lower than

that in control group(52.53%)(P< 0.001). Patients in confirmed group exhibited significantly lower hemoglobin, lymphocyte count, and lymphocyte percentage, but higher neutrophil percentage, neutrophil-to-lymphocyte ratio (NLR), C-reactive protein, aspartate transaminase, and alkaline phosphatase. There was no significant difference in age, gender, dialysis age, primary disease, the using of ACEI/ARB, platelet-to-lymphocyte ratio (PLR) , and other indicators between the two groups. Conclusions: Faced with Severe Acute Respiratory Syndrome-CoV-2 (SARS-CoV-2), HD patients with lower BMI and hemoglobin were more susceptible to be infected, which might be related to malnutrition. Once confirmed with COVID-19, HD patients expressed obviously disregulated in inflammation and immune. [**note: this is from Wuhan, looking at dialysis patients who are thought to be at more risk from SARS-CoV-2**] https://www.medrxiv.org/content/10.1101/2020.07.06.20147827v1

DRUG DEVELOPMENT

- Nothing New

VIRUS BIOCHEMISTRY

- Nothing New

DIAGNOSTIC DEVELOPMENT

- Nothing New

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

2020-07-11

One of the finest singer/songwriters of our day is John Legend. Bet you didn't know that out of college (UPenn) he was a management consultant with Boston Consulting Group!! He clearly made the right choice to go into the entertainment industry. He is also one of fifteen to grab the EGOT (Emmy, Grammy, Oscar, Tony). Here is 'All of Me' in the official music video format: https://www.youtube.com/watch?v=450p7goxZqg The song was written for his soon to be wife, Crissy Teigen. She is in the video and it was filmed in Italy the day before their wedding (I think the end shots were of the actual wedding). Wow, my loyal readers do get a lot for their money reading this newsletter.

US COVID-19 STATISTICS - **Infection Rate: 1.0%; CRF: 4.1%** (CRF down 0.1%)

HCQ is clearly a *ZOMBIE* refusing to go away quietly. As this Washington Post story notes there are some both in the administration and outside that are using the recent Henry Ford hospital study data to urge FDA to reverse course and grant the emergency use authorization (EUA). I only point out to these people that HCQ is an approved drug and physicians can already prescribe it as they see fit. An EUA does not change this at all! The issue is what to do with the 60 million doses that were added to the strategic stockpile? These cannot be distributed without the EUA. There is more at the link.

The Washington Post also has a story on the lack of impact hot summer weather is having on the spread of SARS-CoV-2. This might be a result of spending time in enclosed air-conditioned spaces where aerosol spread is a problem.

JAMA have a nice review article on the pathophysiology, transmission, diagnosis and treatment of COVID-19. It is well worth reading. There is also a brief letter from Italian clinicians on the persistence of symptoms after acute COVID-19. One hopes that more data collection on this facet of the virus is collected so we have a better idea of the full pathophysiology.

The Los Angeles Times has an interesting story about using immune plasma as a protective against SARS-CoV-2 infection. I offered this conjecture a while back about mAb therapy being used in a similar manner. In fact, Regeneron has a clinical trial for this exact use! There is a big caveat here. We don't have much data on the use of immune plasma as a therapeutic. The presence/absence of antibodies in COVID-19 patients is confounding. We know that a number of people who are viral positive via PCR testing have very low or no antibodies. Additionally, very ill patients who require hospitalization may have very high levels of antibodies, including strong neutralizing ones. Why is this? Maybe some immunologists know but I sure don't. Once patients go into cytokine storm other modes of therapy are required. In sum, I think some of those interviewed in the article are correct that antibody treatment of ill patients needs to be shown before we begin to think about this approach as a prophylactic. As I am fond of saying, TIWWDCT. I don't think there is anything that would prevent someone from doing a clinical trial in the same way Regeneron is. If HCQ can be put into trials, immune plasma which has a better scientific rationale does as well.

Here are an interesting set of questions from an infectious disease doc about the origin of the pandemic.

Lots of modeling papers today.

MODELING

- People of minority ethnic background may be disproportionately affected by severe COVID-19 for reasons that are unclear. We sought to examine the relationship between ethnic background and (1) hospital admission for severe COVID-19; (2) in-hospital mortality. Methods. We conducted a case-control study of 872 inner city adult residents admitted to hospital with confirmed COVID-19 (cases) and 3,488 matched controls randomly sampled from a primary healthcare database comprising 344,083 people resident in the same region. To examine in-hospital mortality, we conducted a cohort study of 1827 adults consecutively admitted with COVID-19. Data collected included hospital admission for COVID-19, demographics, comorbidities, in-hospital mortality. The primary exposure variable was self-defined ethnicity. Results. The 872 cases comprised 48.1% Black, 33.7% White, 12.6% Mixed/Other and 5.6% Asian patients. In conditional logistic regression analyses, Black and Mixed/Other ethnicity were associated with higher admission risk than white (OR 3.12 [95% CI 2.63-3.71] and 2.97 [2.30-3.85] respectively). Adjustment for comorbidities and deprivation modestly attenuated the association (OR 2.28 [1.87-2.79] for Black, 2.66 [2.01-3.52] for Mixed/Other). Asian ethnicity was not associated with higher admission risk (OR 1.20 [0.86-1.66]). In the cohort study of 1827 patients, 455 (28.9%) died over a median (IQR) of 8 (4-16) days. Age and male sex, but not Black (adjusted HR 0.84 [0.63-1.11]) or Mixed/Other ethnicity (adjusted HR 0.69 [0.43-1.10]), were associated with in-hospital mortality. Asian ethnicity was associated with higher in-hospital

mortality (adjusted HR 1.54 [0.98-2.41]). Conclusions. Black and Mixed ethnicity are independently associated with greater admission risk with COVID-19 and may be risk factors for development of severe disease. Comorbidities and socioeconomic factors only partly account for this and additional ethnicity-related factors may play a large role. The impact of COVID-19 may be different in Asians. [**note: some of the best research is coming out of the UK. I'm not sure why, maybe the data is easier to manage. Here is a good ethnicity study of sever COVID-19 cases. Interesting that Asians had higher in hospital mortality.**] https://www.medrxiv.org/content/10.1101/2020.07.08.20148965v1

- Socio-economic disparities quite often have a central role in the unfolding of large-scale catastrophic events. One of the most concerning aspects of the ongoing COVID-19 pandemics is that it disproportionately affects people from Black and African American backgrounds creating an unexpected infection gap. Interestingly, the abnormal impact on these ethnic groups seem to be almost uncorrelated with other risk factors, including co-morbidity, poverty, level of education, access to healthcare, residential segregation, and response to cures. A proposed explanation for the observed incidence gap is that people from African American backgrounds are more often employed in low-income service jobs, and are thus more exposed to infection through face-to-face contacts, but the lack of direct data has not allowed to draw strong conclusions in this sense so far. Here we introduce the concept of dynamic segregation, that is the extent to which a given group of people is internally clustered or exposed to other groups, as a result of mobility and commuting habits. By analysing census and mobility data on more than 120 major US cities, we found that the dynamic segregation of African American communities is significantly associated with the weekly excess COVID-19 incidence and mortality in those communities. The results confirm that knowing where people commute to, rather than where they live, is much more relevant for disease modelling. [**note: building on the previous modeling work, here is another paper from the UK using US data. This is not the first time I've seen foreign papers on US outbreaks. I find the curious.**] https://www.medrxiv.org/content/10.1101/2020.07.08.20148742v1
- Evidence-based public health approaches that minimize the introduction and spread of new SARS-CoV-2 transmission clusters are urgently needed in the United States and other countries struggling with expanding epidemics. Here we analyze 247 full-genome SARS-CoV-2 sequences from two nearby communities in Wisconsin, USA, and find surprisingly distinct patterns of viral spread. Dane County had the 12th known introduction of SARS-CoV-2 in the United States, but this did not lead to descendant community spread. Instead, the Dane County outbreak was seeded by multiple later introductions, followed by limited community spread. In contrast, relatively few introductions in Milwaukee County led to extensive community spread. We present evidence for reduced viral spread in both counties, and limited viral transmission between counties, following the statewide Safer-at-Home public health order, which went into effect 25 March 2020. Our results suggest that early containment efforts suppressed the spread of SARS-CoV-2 within Wisconsin. [**note: this paper is by American authors from University of Wisconsin who looked at two different outbreaks showing distinct patterns of viral transmission.**] https://www.medrxiv.org/content/10.1101/2020.07.09.20149104v1
- Residential colleges are considering re-opening under uncertain futures regarding the COVID-19 pandemic. We consider repeat SARS-CoV-2 testing models for the purpose of containing outbreaks in the residential campus community. The goal of repeat testing is to rapidly detect

and isolate new infections as they occur to block transmission that would otherwise occur on campus and, of arguably greater importance, off. The models allow for the evolution of test sensitivity with time from infection, scheduled on-campus resident screening at a given frequency, imported infections from off campus throughout the school year, and a lag from testing until student isolation due to laboratory turnaround and student relocation delay. For early- (late-) transmission of SARS-CoV-2 by age of infection, we find that weekly screening cannot reliably contain outbreaks with reproductive numbers above 1.4 (1.6) if more than one imported exposure per 10,000 students occurs daily. Screening every three days can contain outbreaks providing the reproductive number remains below 1.75 (2.3) if transmission happens earlier (later) with time from infection, but at the cost of greatly increased false positive rates requiring more isolation quarters for students testing positive. Testing frequently while minimizing the delay from testing until isolation for those found positive are the most controllable levers for preventing large residential college outbreaks. [**note: this one is from Yale and argues that more frequent testing and rapid isolation are required to prevent large residential college outbreaks. We should have proof in three or so months from those schools that reopen. My benchmark is Purdue and it's outspoken President, Mitch Daniels. President Daniels wants teachers behind plexiglass and students wearing face masks. That is a start; also check the ventilation system in all the buildings; Purdue is an engineering school after all.]** https://www.medrxiv.org/content/10.1101/2020.07.09.20149351v1

- To design effective disease control strategies, it is critical to understand the incidence of diseases. In the Covid-19 epidemic in the United States (caused by outbreak of the SARS-CoV-2 virus), testing capacity was initially very limited and has been increasing at the same time as the virus has been spreading. When estimating the incidence, it can be difficult to distinguish whether increased numbers of positive tests stem from increases in the spread of the virus or increases in testing. This has made it very difficult to identify locations in which the epidemic poses the largest public health risks. Here, we use a probabilistic model to quantify beliefs about testing strategies and understand implications regarding incidence. We apply this model to estimate the incidence in each state of the United States, and find that: (1) the Covid-19 epidemic is likely to be more widespread than reported by limited testing, (2) the Covid-19 epidemic growth in the summer months is likely smaller than it was during the spring months, and (3) the regions which are at highest risk of Covid-19 epidemic outbreaks are not always those with the largest number of positive test results. [**note: math heavy papers are the bane of my existence as a curator! I think their point number 2 is clearly wrong. It is summer in TX, AZ, and FL and ICU beds are all taken and cases of COVID-19 are increasing.**] https://www.medrxiv.org/content/10.1101/2020.07.09.20141762v1
- Obesity is an emerging risk factor for coronavirus disease-2019 (COVID-19). Simple measures of physical fitness, such as self-reported walking pace, could also be important risk factors, but have not been well documented. This analysis includes 414,201 UK Biobank participants with complete covariate and linked COVID-19 data. We analysed the risk of severe (in-hospital) COVID-19 across categories of obesity status and walking pace. As of June 20th 2020 there were 972 cases of severe COVID-19 that had occurred within the cohort. Compared to normal weight individuals, the adjusted odds ratio (OR) for severe COVID-9 in those with obesity was 1.49 (1.24, 1.78). Compared to those with a brisk walking pace, the OR in slow walkers was 1.84 (1.49, 2.27). Slow walkers had the highest risk of severe COVID-19 regardless of obesity status.

For example, compared to normal weight brisk walkers, the odds of severe COVID-19 in obese brisk walkers was 1.39 (0.99, 1.98), whereas the odds in normal weight slow walkers was 2.48 (1.56, 3.93). *Self-reported walking pace, a simple measure of functional fitness, appears to be a risk factor for severe COVID-19 that is independent of obesity. This may help inform simple pragmatic public health risk stratification and preventative strategies*. [**note: another study from the UK Biobank! As soon as I am finished getting this newsletter out I am going for a brisk walk!**] https://www.medrxiv.org/content/10.1101/2020.07.10.20150003v1

- Through analysis of the ideal gas, we construct a random walk that on average matches the standard susceptible-infective-removed (SIR) model. We show that the most widely referenced parameter, the 'basic reproduction number' (R_0), is fundamentally connected to the relative odds of increasing or decreasing the infectives population. As a consequence, for $R_0 > 1$ the probability that no outbreak occurs is $1/R_0$. In stark contrast to a deterministic SIR, when $R_0 = 1.5$ the random walk has a 67% chance of avoiding outbreak. Thus, an alternative, probabilistic, interpretation of R_0 arises, which provides a novel estimate of the critical population density γ/r without fitting SIR models. We demonstrate that SARS-CoV2 in the United States is consistent with our model and attempt an estimate of γ/r. In doing so, we uncover a significant source of bias in public data reporting. Data are aggregated on political boundaries, which bear no concern for dispersion of population density. We show that this introduces bias in fits and parameter estimates, a concern for understanding fundamental virus parameters and for policy making. Anonymized data at the resolution required for contact tracing would afford access to γ/r without fitting. The random walk SIR developed here highlights the intuition that any epidemic is stochastic and recovers all the key parameter values noted by Kermack and McKendrick in 1927. **[note: the title of this paper, "What can the ideal gas say about global pandemics" sucked me in. I took a quick look at the paper and as with almost every model, one can argue that this is 'the' approach to looking at SARS-CoV-2 infection. For me, it's easier to look at hospital bed use.**]
  https://www.medrxiv.org/content/10.1101/2020.07.09.20150128v1
- The real impact of SARS-CoV-2 on overall mortality remains uncertain and surveillance reports attributed to COVID-19 a limited amount of deaths during the outbreak. Aim of this study is to assess the excess mortality (EM) during COVID-19 outbreak in highly impacted areas of northern Italy. Methods: We analyzed data on deaths occurred in the first four months of 2020 in health protection agencies (HPA) of Bergamo and Brescia (Lombardy), building a time-series of daily number of deaths and predicting the daily standardized mortality ratio (SMR) and cumulative number of excess deaths (ED) through a Poisson generalized additive model of the observed counts in 2020, using 2019 data as a reference. Results: We estimated 5740 (95% Credible Set (CS): 5552-5936) ED in the HPA of Bergamo and 3703 (95% CS: 3535 - 3877) in Brescia, corresponding to 2.55 (95% CS: 2.50-2.61) and 1.93 (95% CS: 1.89-1.98) folds increase in the number of deaths. The ED wave started a few days later in Brescia, but the daily estimated SMR peaked at the end of March in both HPAs, roughly two weeks after the introduction of lock-down measures, with significantly higher estimates in Bergamo (9.4, 95% CI: 9.1-9.7). Conclusion: *EM was significantly larger than that officially attributed to COVID-19, disclosing its hidden burden likely due to indirect effects on health system. Time-series analyses highlighted the impact of lockdown restrictions, with a lower EM in the HPA where there was a smaller delay*

between the epidemic outbreak and their enforcement. [**note: excess mortality study from northern Italy.]** https://www.medrxiv.org/content/10.1101/2020.07.10.20150565v1

NEWLY REGISTERED CLINICAL TRIALS

- Have not checked today.

CLINICAL TRIAL RESULTS

- The remdesivir clinical trial interim analysis has finally been published in the New England Journal of Medicine. There is an accompanying editorial and some interesting letters to the editor. [**note: clearly more data are needed for remdesivir and the timing of treatment. We still do not have an oral anti-viral that can be given to moderate COVID-19 patients. Virtually all treatment for which we have data is hospital based.**]
- This case series illustrates that iloprost might be a useful adjunctive therapy for COVID-19 vasculopathy, improving digital ischaemia as well as cardiorespiratory parameters. Inhaled iloprost has been shown to improve ventilation parameters through its vasodilatory effects, thereby improving gas exchange. Furthermore, systemically infused iloprost might also improve ventilation and perfusion matching in the lung, leading to the effects observed in our patients. Although larger controlled studies are needed to confirm our observations and despite the limitations inherent to small case series, based on the pharmacological effects of iloprost in analogous pathological states and its favourable safety profile, we suggest that iloprost might be a useful adjunctive treatment in COVID-19. [**note: this is from a small number of patients. Iloprost is in clinical trials.**] https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(20)30232-0/fulltext
- A cohort of seven infants (aged ≤1 year) with severe Kawasaki-like disease were diagnosed and treated at five hospitals in the UK between February and March, 2020 (appendix p 1). All of the infants received prompt intravenous immunoglobulins and steroid treatment, but none responded and all required the addition of a biological agent because of continued inflammation, recurring fever, and progressive changes on echocardiography. Six patients (86%) developed coronary artery aneurisms (Z score >2·5) and one infant died as a result of a ruptured aneurysm, despite early aggressive treatment. Five infants (71%) had negative serology for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); therefore, any correlation with the COVID-19 pandemic and paediatric inflammatory multisystemic syndrome temporally associated with SARS-CoV-2 is unclear. Nonetheless, we would like to alert paediatricians to this new and very aggressive phenotype in infants. [**note: this is something worth continuing to look at as we see more births during the pandemic.]** https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(20)30231-9/fulltext and there is also a single case report from NYU Langonne Health of a 45 year old male who developed Kawasaki like symptoms https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)31526-9/fulltext
- Limited data are available for pregnant women affected by SARS-CoV-2. Serological tests are critically important to determine exposure and immunity to SARS-CoV-2 within both individuals and populations. We completed SARS-CoV-2 serological testing of 1,293 parturient women at two centers in Philadelphia from April 4 to June 3, 2020. We tested 834 pre-pandemic samples collected in 2019 and 15 samples from COVID-19 recovered donors to validate our assay, which

has a ~1% false positive rate. We found 80/1,293 (6.2%) of parturient women possessed IgG and/or IgM SARS-CoV-2-specific antibodies. We found race/ethnicity differences in seroprevalence rates, with higher rates in Black/non-Hispanic and Hispanic/Latino women. Of the 72 seropositive women who also received nasopharyngeal polymerase chain reaction testing during pregnancy, 46 (64%) were positive. Continued serologic surveillance among pregnant women may inform perinatal clinical practices and can potentially be used to estimate seroprevalence within the community. [**note: here is a UPenn study of seroprevalence among parturient women (I leaned a new word today!!).**]
https://www.medrxiv.org/content/10.1101/2020.07.08.20149179v1

- Risk factors associated with severe disease and mortality include advanced age,hypertension, diabetes, and obesity. Clear mechanistic understanding of how these comorbidities converge to enable severe infection is lacking. Notably each of these risk factors pathologically disrupts the lipidome and this disruption may be a unifying feature of severe COVID-19. Here we provide the first in depth interrogation of lipidomic changes, including structural-lipids as well as the eicosanoids and docosanoids lipid mediators (LMs), that mark COVID-19 disease severity. Our data reveal that progression from moderate to severe disease is marked by a loss of specific immune regulatory LMs and increased pro-inflammatory species. Given the important immune regulatory role of LMs, these data provide mechanistic insight into the immune balance in COVID-19 and potential targets for therapy with currently approved pharmaceuticals. [**note: this is a new observation. As an old lipid biochemist, I find this research fascinating. There are some new markers for severe COVID-19 and possible drug targets.**]
https://www.medrxiv.org/content/10.1101/2020.07.09.20149849v1
- Pregnancy is known to increase the risk of severe illnesses in response to viral infections. Therefore, the impact of SARS−CoV−2 infection during gestational ages might be detrimental and the potential vertical transmission should be thoroughly studied. Herein, we investigated whether SARS−CoV−2 vertical transmission is possible and, in case, whether this results in a fetal involvement. Additionally, we analyzed the role of the antibody and the inflammatory responses in placenta and plasma from SARS−CoV−2−positive pregnant women and fetuses. 31 SARS−CoV−2 pregnant women were enrolled. Real−time PCR was performed to detect the virus on maternal and newborns nasopharyngeal swabs, vaginal swabs, maternal and umbilical cord plasma, placenta and umbilical cord biopsies, amniotic fluids and milk. Maternal and umbilical cord plasma, and milk were tested for specific anti−SARS−CoV−2 antibodies. RNA expression quantification of genes involved in the inflammatory response was performed on four selected placentas. On maternal and umbilical cord plasma of the same subjects, secreted cytokines/chemokines were quantified. SARS−CoV−2 is found in at-term placentae and in the umbilical cord blood, in the vaginal mucosa of pregnant women and in milk. Furthermore, we report the presence of specific anti−SARS−CoV-2 IgM and IgG antibodies in the umbilical cord blood of pregnant women, as well as in milk specimens. Finally, a specific inflammatory response is triggered by SARS−CoV−2 infection in pregnant women at both systemic and placental level, and in umbilical cord blood plasma. Our data strongly support the hypothesis that in−utero vertical transmission is possible in SARS−CoV−2 positive pregnant women. This is essential for defining proper obstetric management of COVID−19 pregnant women, or putative indications for mode and timing of delivery. [**note: from Milan, maternal to fetal transmission**

of SARS-CoV-2. This has been in the news this past week.]
https://www.medrxiv.org/content/10.1101/2020.07.09.20149591v1

- Understanding innate immune responses in COVID-19 is important for deciphering mechanisms of host responses and interpreting disease pathogenesis. Natural killer (NK) cells are innate effector lymphocytes that respond to acute viral infections, but might also contribute to immune pathology. Here, using 28-color flow cytometry, we describe a state of strong NK cell activation across distinct subsets in peripheral blood of COVID-19 patients, a pattern mirrored in scRNA-seq signatures of lung NK cells. Unsupervised high-dimensional analysis identified distinct immunophenotypes that were linked to disease severity. Hallmarks of these immunophenotypes were high expression of perforin, NKG2C, and Ksp37, reflecting a high presence of adaptive NK cell expansions in circulation of patients with severe disease. Finally, arming of CD56bright NK cells was observed in course of COVID-19 disease states, driven by a defined protein-protein interaction network of inflammatory soluble factors. This provides a detailed map of the NK cell activation-landscape in COVID-19 disease. [**note: from Sweden, some good work on natural killer cells and disease severity. I read most of the paper and as a non-immunologist found it fascinating. Someone will put all this together and figure out why what should be a decent immune response goes haywire.**]
https://www.medrxiv.org/content/10.1101/2020.07.07.20148478v1
- Objective In this study, we evaluated the efficacy of hydroxychloroquine (HCQ) against coronavirus disease 2019 (COVID-19) via a randomized controlled trial (RCT) and a retrospective study. Methods Subjects admitted to 11 designated public hospitals in Taiwan between April 1 and May 31, 2020, with COVID-19 diagnosis confirmed by pharyngeal real-time RT-PCR for SARS-CoV-2, were randomized at a 2:1 ratio and stratified by mild or moderate illness. HCQ 400 mg twice for 1 d and HCQ 200 mg twice daily for 6 days were administered. Both study group and controlled group received standard of care (SOC). Pharyngeal swabs and sputum were collected every other day. The proportion and time to negative viral PCR were assessed on day 14. In the retrospective study, medical records were reviewed for patients admitted before March 31, 2020. Results There were 33 and 37 cases in the RCT and retrospective study, respectively. In the RCT, the median times to negative rRT-PCR from randomization to hospital day 14 were 5 days (95% CI; 1-9 days) and 10 days (95% CI; 2-12 days) for the HCQ and SOC groups, respectively (p = 0.40). On day 14, 81.0% (17/21) and 75.0% (9/12) of the subjects in the HCQ and SOC groups, respectively, had undetected virus (p = 0.36). In the retrospective study, 12 (42.9%) in the HCQ group and 5 (55.6%) in the control group had negative rRT-PCR results on hospital day 14 (p = 0.70). Conclusions Neither study demonstrated that HCQ shortened viral shedding in mild to moderate COVID-19 subjects. [**note: maybe I am turning into a Zombie!! I've lost track of all the HCQ trials. Anyway, this one is from Taiwan. It's a small number of patients but Taiwan pretty much has the pandemic under control and maybe this is all they could find within 11 hospitals. Bottom line, no effect on viral shedding which was the study endpoint.**] https://www.medrxiv.org/content/10.1101/2020.07.08.20148841v1
- *Our understanding of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still developing*. We investigated seroprevalence and immune responses in subjects professionally exposed to SARS-CoV-2 and their family members (155 individuals; ages 5-79 years). Seropositivity for SARS-CoV-2 spike glycoprotein aligned with PCR results that confirmed previous infection. Anti-spike IgG titers remained high 60 days post-infection and did not

associate with symptoms, but spike-specific IgM did associate with malaise and fever. We found limited household transmission, with children of infected individuals seldomly seropositive, highlighting professional exposure as the dominant route of infection in our cohort. We analyzed PBMCs from a subset of seropositive and seronegative adults. TLR7 agonist- activation revealed an increased population of IL-6^{+}TNF^{-}IL-1β^{+} monocytes, while SARS-CoV-2 peptide stimulation elicited IL-33, IL-6, IFNa2, and IL-23 expression in seropositive individuals. IL-33 correlated with CD4^{+} T cell activation in PBMCs from convalescent subjects, and was likely due to T cell-mediated effects on IL-33- producing cells. IL-33 is associated with pulmonary infection and chronic diseases like asthma and COPD, but its role in COVID-19 is unknown. Analysis of published scRNAseq data of bronchoalveolar lavage fluid (BALF) from patients with mild to severe COVID-19 revealed a population of IL-33-producing cells that increases with disease. Together these findings show that IL-33 production is linked to SARS-CoV- 2 infection and warrant further investigation of IL-33 in COVID-19 pathogenesis and immunity. [**note: the more I read, I find that the first sentence is this abstract is a big understatement. I had no idea that there was an IL-33! It seems to be another marker for both pathogenesis and immunity.**] https://www.medrxiv.org/content/10.1101/2020.07.09.20148056v1

DRUG DEVELOPMENT

- Passive transfer of antibodies from COVID-19 convalescent patients is being used as an experimental treatment for eligible patients with SARS-CoV-2 infections. The United States Food and Drug Administration's (FDA) guidelines for convalescent plasma recommends target antibody titers of 160. We evaluated SARS-CoV-2 neutralizing antibodies in sera from recovered COVID-19 patients using plaque reduction neutralization tests (PRNT) at low (PRNT50) and high (PRNT90) stringency thresholds. We found that neutralizing activity increased with time post symptom onset (PSO), reaching a peak at 31-35 days PSO. At this point, the number of sera having neutralizing titers of at least 160 was ~93% (PRNT50) and ~54% (PRNT90). Sera with high SARS-CoV-2 antibody levels (≥960 ELISA titers) showed maximal activity, but not all high titer sera contained neutralizing antibody at FDA recommended levels, particularly at high stringency. These results underscore the value of serum characterization for neutralization activity. [**note: some good work from the Mt. Sinai and NY state lab on looking at neutralizing antibodies from convalescent plasma. Not all high titer sera contained enough neutralizing antibody.**] https://www.medrxiv.org/content/10.1101/2020.07.10.20150557v1

VIRUS BIOCHEMISTRY

- Nothing today.

DIAGNOSTIC DEVELOPMENT

- These last months, dozens of SARS-CoV-2 serological tests have become available with varying performances. A major effort was completed to compare 17 serological tests. Methods In a preliminary phase, we compared 17 IgG, IgM, IgA and pan Ig serological tests including ELISA, LFA, CLIA and ECLIA on a panel of 182 sera, comprising 113 sera from hospitalized patients with a positive RT-PCR, and 69 sampled before 1st November 2019, expected to give a positive and negative results, respectively. In a second phase, the five best performing and most available tests were further evaluated on a total of 582 sera (178 and 404 expected positive and negative,

respectively), allowing the assessment of 20 possible cross-reactions with other virus. Results In the preliminary phase, among eight IgG/pan-Ig ELISA or CLIA/ECLIA tests, four had a sensitivity and specificity above 90% and 98% respectively, and on six IgM/IgA tests, only one was acceptable. Only one LFA test on three showed good performances for both IgG and IgM. For all the tests IgM and IgG aroused concomitantly. In the second phase, no tests showed particular cross-reaction. We observed an important heterogeneity in the development of the antibody response, and that anti-nucleocapside (anti-N) antibodies appeared earlier than the anti-spike (anti-S) proteins. Conclusions The identified SARS-CoV-2 serology tests may be used for the diagnostic of CoviD-19 for negative RT-PCR patients presenting severe to mild suggestive symptoms or particular clinical presentation. Detection of both anti-N and anti-S could be complementary to increase the sensitivity of the analysis. [**note: I have not seen a serology test comparison in a couple of weeks and the Swiss test is quite thorough. Clinical labs need to do their own validation!**] https://www.medrxiv.org/content/10.1101/2020.07.09.20149864v1

- Emergence of a new variant of spike protein (D614G) with increased infectivity and transmissibility has prompted many to analyze the potential role of this variant in the SARS-CoV-2 pandemic. When a new variant emerges, there is a concern regarding whether an individual exposed to one variant of a virus will have cross-reactive immune memory to the second variant. Accordingly, we analyzed the serologic reactivity of D614 (original) and G614 variant spike proteins. We found that antibodies from a high-incidence population in New York City reacted both toward the original D614 spike and the G614 spike variant. These data suggest that patients who have been exposed to either SARS-CoV-2 variant have humoral immunity that can respond against both variants. This is an important finding both for SARS-CoV-2 disease biology and for potential antibody-based therapeutics. [**note: good news in that both the original SARS-CoV-2 and the variant behave similarly with regard to antibodies from high-incidence population in New York City.**]
  https://www.medrxiv.org/content/10.1101/2020.07.08.20147371v1
- The Eurofins Covid-19 SentinelTM program was developed to monitor the evolution of the pandemic and for early detection of outbreaks. The study objective was to develop a wastewater testing method to analyze SARS-CoV-2 as an indicator of community infection rate resurgence of COVID-19 or in well-defined sites such as production facilities or nursing homes. Eurofins performed >700 tests on 78 unique samples from 18 sites in Denmark, France and Belgium. Ten variant test protocols were trialed. Protocol variations trialed included centrifugation, precipitation of the SARS-CoV-2 RNA, agitation prior to precipitation, cooling, and pasteurization of the samples. A method was succesfully developed and reliability was supported by stability, reproducibility, and dilution & linearity studies. Results obtained showed a direct link to number of RNA copies in the sample using a calibration curve with synthetic SARS-CoV-2. Analysis was performed on both the liquid phase and solid phase of wastewater samples, with virus RNA detected in both phases but more frequently in the liquid phase. The virus was present in a sample from a Danish community wastewater treatment plant collected on February 24, 3 days before the first COVID-19 case was officially reported in the country. The greatest concentration of virus detected corresponded to when the COVID-19 crisis was at its peak in Denmark. Based on studies carried out in a Danish hospital, the wastewater testing method is expected to be able to detect a community COVID-19 prevalence rate as low as a 0,02%-0,1% (i.e. between 2 virus shedders per 10000 and 1 virus shedder per 1000). The

wastewater testing method was used to monitor a Danish Community after a COVID-19 outbreak and it was shown that the method can be used as a semi-quantitative method to monitor the development of an outbreak. [**note: interesting paper on the development of a wastewater treatment diagnostic test for SARS-CoV-2 RNA.**]
https://www.medrxiv.org/content/10.1101/2020.07.10.20150573v1

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2020-07-12

For reflection Sunday I offer two pieces. The famous London recital venue Wigmore Hall hosted empty-seat lunchtime pandemic recitals during the Month of June. I will link to some of these in future newsletters but one piece that seems apt for reflection is Franz Schubert's great song cycle, 'Die Winterreise'. Yes, it is bleak, but I can think of no other cycle where the music and poetry are so intertwined. The cycle has been done by both men and women of differing voice ranges though it was original written in the tenor range. Here we have Mitsuko Uchida (a great recitalist in her own right) and tenor, Mark Padmore: https://www.youtube.com/watch?v=2en3ZugteLI Padmore's voice sounds better than I remember and his interpretation of the cycle is sublime. The second selection is by the great John Coltrane, another phenomenal artist taken away from us at the height of his talent. His composition 'Alabama' was written shortly after the 1963 bombing of a church in Birmingham that killed three teenage girls. We are fortunate that he performed this on Ralph Gleason's Jazz Casual show late in the same year: https://www.youtube.com/watch?v=saN1BwlxJxA Coltrane is with his great sidemen: McCoy Tyner, piano; Jimmy Garrison, bass; and Elvin Jones, drums. Schubert's bleak outlook and Coltrane's sense of optimism (his great recording 'A Love Supreme' which crystalized his sense of spirituality was recorded a year later) seem like a good pairing.

US COVID-19 STATISTICS - **Infection Rate: 1.0%; CRF: 4.1%** (CRF unchanged)

A surgeon weighs in on face masks. "Your mask feels uncomfortable? Get over it." Bad behavior by frat boys (nothing new here) imperils reopening of some universities.

The New York Times has a good overview on school reopening.

What is wrong with this picture from Pittsburgh that was taken last month?

MODELING

- With no known treatments or vaccine, COVID-19 presents a major threat, particularly to older adults, who account for the majority of severe illness and deaths. The age-related susceptibility is partly explained by increased comorbidities including dementia and type II diabetes. While it is unclear why these diseases predispose risk, we hypothesize that increased biological age, rather than chronological age, may be driving disease-related trends in COVID-19 severity with age. To test this hypothesis, we applied our previously validated biological age measure (PhenoAge) composed of chronological age and nine clinical chemistry biomarkers to data of 347,751 participants from a large community cohort in the United Kingdom (UK Biobank), recruited between 2006 and 2010. Other data included disease diagnoses (to 2017), mortality data (to 2020), and the UK national COVID-19 test results (to May 31, 2020). Accelerated aging 10-14 years prior to the start of the COVID-19 pandemic was associated with test positivity (OR=1.15 per 5-year acceleration, 95% CI: 1.08 to 1.21, p=3.2x10-6) and all-cause mortality with test-confirmed COVID-19 (OR=1.25, per 5-year acceleration, 95% CI: 1.09 to 1.44, p=0.002) after adjustment for demographics including current chronological age and pre-existing diseases or conditions. The corresponding areas under the curves were 0.669 and 0.803, respectively. Biological aging, as captured by PhenoAge, is a better predictor of COVID-19 severity than chronological age, and may inform risk stratification initiatives, while also elucidating possible underlying mechanisms, particularly those related to inflammaging. [**note: the UK Biobank is indeed a treasure trove! These researcher posit that biological age rather than chronological age is the important factor in sever disease. Do any of my loyal readers know what their PhenoAge is? I sure don't but have downloaded this paper for further study. I like to think that I am 30 years younger than I am.**]
  https://www.medrxiv.org/content/10.1101/2020.07.10.20147777v1
- Antibody (Ab) responses to SARS-CoV-2 can be detected in most infected individuals 10-15 days following the onset of COVID-19 symptoms. However, due to the recent emergence of this virus in the human population it is not yet known how long these Ab responses will be maintained or whether they will provide protection from re-infection. Using sequential serum samples collected up to 94 days post onset of symptoms (POS) from 65 RT-qPCR confirmed SARS-CoV-2-infected individuals, we show seroconversion in >95% of cases and neutralizing antibody (nAb) responses when sampled beyond 8 days POS. We demonstrate that the magnitude of the nAb response is dependent upon the disease severity, but this does not affect the kinetics of the nAb response. Declining nAb titres were observed during the follow up period. Whilst some individuals with high peak ID_{50} (>10,000) maintained titres >1,000 at >60 days POS, some with lower peak ID_{50} had titres approaching baseline within the follow up period. A similar decline in nAb titres was also observed in a cohort of seropositive healthcare workers from Guy's and St Thomas' Hospitals. We suggest that this transient nAb response is a feature shared by both a SARS-CoV-2 infection that causes low disease severity and the circulating seasonal coronaviruses that are associated with common colds. This study has important implications when considering widespread serological testing, Ab protection against re-infection with SARS-CoV-2 and the durability of vaccine protection. [**note: more data on antibody response and longevity, this**

time from the UK. I would like someone skilled in digesting immunology papers to review all of this. It appears confounding to me.]
https://www.medrxiv.org/content/10.1101/2020.07.09.20148429v1

- Humoral immunity in asymptomatic infections with SARS-CoV-2 has not been well established. 63 healthy contacts, 63 asymptomatic individuals, and 51 mild patients were enrolled in this study and screened using nucleic acid testing (NAT) and commercial kits of serum IgM and IgG antibodies against recombinant nucleoprotein (N) and spike (S) proteins of SARS-CoV-2. Asymptomatic and mild patients were classified into at least four types based on NAT and serological tests, especially 81% and 25.4% negative NAT but positive IgM/IgG responses, respectively. Antibody dynamics were further demonstrated by IgM and IgG profile responses to SARS-CoV-2 proteome. IgM antibody responses against S1 were elicited in asymptomatic individuals as early to the seventh day after exposure and peaked on days from 17d to 25d, which might be used as early diagnostic biomarkers. Moreover, asymptomatic individuals evoked weaker S1 specific IgM and neutralizing antibody responses than mild patients. Most importantly, S1 specific IgM/IgG responses and the titers of neutralizing antibody in asymptomatic individuals gradually vanished in two months. Our findings might have important implications for serological survey, public health and immunization strategy. [**note: following up on the previous paper, here is one from China. I particularly like that part of the funding came from the 13th Five-year Plan of China; I thought those were a thing of the distant past. No matter, my comment above also holds for this paper.**]
https://www.medrxiv.org/content/10.1101/2020.07.09.20149633v1
- To accurately interpret COVID-19 seroprevalence surveys, knowledge of serum-IgG responses to SARS-CoV-2 with a better understanding of patients who do not seroconvert, is imperative. This study aimed to describe serum-IgG responses to SARS-CoV-2 in a cohort of patients with both severe and mild COVID-19, including extended studies of patients who remained seronegative more than 90 days post symptom onset. Results: Forty-seven patients (mean age 49 years, 38% female) were included. All (15/15) patients with severe symptoms and 29/32 (90.6%) patients with mild symptoms of COVID-19 developed SARS-CoV-2-specific IgG antibodies in serum. Time to seroconversion was significantly shorter (median 11 vs. 22 days, P=0.04) in patients with severe compared to mild symptoms. Of the three patients without detectable IgG-responses after >90 days, all had detectable virus-neutralizing antibodies and in two, spike-protein receptor binding domain-specific IgG was detected with an in-house assay. Antibody titers were preserved during follow-up and all patients who seroconverted, irrespective of the severity of symptoms, still had detectable IgG levels >75 days post symptom onset. Conclusions: Patients with severe COVID-19 both seroconvert earlier and develop higher concentrations of SARS-CoV-2-specific IgG than patients with mild symptoms. Of those patients who not develop detectable IgG antibodies, all have detectable virus-neutralizing antibodies, suggesting immunity. Our results showing that not all COVID-19 patients develop detectable IgG using two validated commercial clinical methods, even over time, are vital for the interpretation of COVID-19 seroprevalence surveys and for estimating the true infection prevalence in populations. {**note: and here is long term data from Sweden. What is interesting is that neutralizing antibodies are present after more than 90 days in some patients who had no detectable IgG response.**]
https://www.medrxiv.org/content/10.1101/2020.07.11.20151324v1

- Background The COVID-19 pandemic has spread worldwide, affecting millions of people and exposing them to home quarantine, isolation, and social distancing. While recent reports showed increased distress and depressive/anxiety state related to COVID-19 crisis, we investigated how home quarantine affected sleep parameters in healthy individuals. Methods 160 healthy individuals who were in home quarantine in April 2020 for at least one month participated in this study. Participants rated and compared their quantitative sleep parameters (time to go to bed, sleep duration, getting-up time) and sleep quality factors, pre-and during home quarantine due to the COVID-19 pandemic. Furthermore, participants chronotype was determined to see if sleep parameters are differentially affected in different chronotypes. Results The time to fall asleep and get-up in the morning were significantly delayed in all participants, indicating a significant circadian misalignment. Sleep quality was reported to be significantly poorer in all participants and chronotypes, and included more daily disturbances (more sleep disturbances, higher daily dysfunctions due to low quality of sleep) and less perceived sleep quality (lower subjective sleep quality, longer time taken to fall asleep at night, more use of sleep medication for improving sleep quality) during home quarantine. Conclusions Home quarantine due to COVID-19 pandemic has a detrimental impact on sleep quality. Online interventions including self-help sleep programs, stress management, relaxation practices, stimulus control, sleep hygiene, and mindfulness training are available interventions in the current situation. [**note: kudos to these Iranian researchers for looking into sleep quality! I can attest to their findings.**] https://www.medrxiv.org/content/10.1101/2020.07.09.20149138v1
- Males are at a higher risk of dying from COVID-19. Older age and cardiovascular disease are also associated with COVID-19 mortality. We compared the male-to-female (sex) ratios in mortality by age for COVID-19 with cardiovascular mortality and cancer mortality in the general population. Methods: We obtained data from official government sources in the US and five European countries: Italy, Spain, France, Germany, and the Netherlands. We analyzed COVID-19 deaths by sex and age in these countries and similarly analyzed their deaths from cardiovascular disease (coronary heart disease or stroke) and cancer, the two leading age-related causes of death in middle-to-high income countries. Findings: In both the US and European countries, the sex ratio of deaths from COVID-19 exceeded one throughout adult life. The sex ratio increased up to a peak in midlife, and then declined markedly in later life. This pattern was also observed for the sex ratio of deaths from cardiovascular disease, but not cancer, in the general populations of the US and European countries. Interpretation: The sex ratios of deaths from COVID-19 and from cardiovascular disease exhibit similar patterns across the adult life course. The underlying mechanisms are poorly understood, but could stem partially from sex-related biological differences that underlie the similar pattern for cardiovascular disease. These include, we propose, comparatively longer telomeres in females, ovarian hormones, and X chromosome mosaicism. [**note: more on the male/female disparity in severe COVID-19 mortality.**] https://www.medrxiv.org/content/10.1101/2020.07.10.20149013v1

NEWLY REGISTERED CLINICAL TRIALS

- This Phase 1 single-dose, dose-escalation study is an open label trial evaluating the safety of CPI-006, a humanized monoclonal antibody targeting the CD73 cell-surface ectonucleotidase, as immunotherapy for stable hospitalized mild or moderately symptomatic COVID-19 patients. [**note: trial is sponsored by Corvus Pharmaceuticals see the link for info.**] NCT04464395

- This study will assess the safety and immunogenicity of AG0301-COVID19 in healthy adult volunteers. [**note: and yet another vaccine trial! This is a DNA vaccine by AnGes, Inc. They are headquartered in Japan.**] NCT04463472
- The primary purpose is to evaluate the safety and tolerability of OP-101 and secondary purpose is to determine the effect of OP-101 reducing proinflammatory cytokines after a single dose in severe COVID-19 Patients. [**note: sponsor is Orpheris, Inc which is a subsidiary of Ashvattha Therapeutics**] NCT04458298
- This study aims to evaluate the safety and reactogenicity profile after 1 and 2 dose administrations of CVnCoV at different dose levels. [**note: and another vaccine trial! This one is from CureVac AG; another mRNA vaccine. Are all the eggs going in one basket?**] NCT04449276

CLINICAL TRIAL RESULTS

- The clinical presentation of hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome is one of the more severe forms of preeclampsia. COVID-19 infection exhibits signs that are shared with preeclampsia and HELLP syndrome, which may lead to needless interventions and iatrogenic preterm delivery. Objective: We evaluated the prevalence of HELLP-like signs in pregnant women admitted for COVID-19 and the value of angiogenic factors to rule out preeclampsia. Methods: a consecutive series of 27 pregnant women beyond 20 weeks of gestation, with symptomatic COVID-19. Clinical and analytical features were recorded and those cases with signs of HELLP syndrome were tested for sFlt-1/PlGF ratio. Results: Seven patients (25.9%) presented at least one sign of suspected HELLP syndrome, of which 2 (7.4%) were diagnosed clinically with PE because of hypertension and high transaminases and 5 (18.5%) had only elevated transaminases. sFlt-1/PlGF ratio was normal in 6 of 7. Conclusion: Symptomatic COVID-19 may simulate severe preeclampsia in pregnancy. Angiogenic factors may be essential to avoid false diagnosis and needless interventions. [**note: the impact of SARS-CoV-2 infection on pregnant women is of concern and this paper points to another risk that must be monitored.**] https://www.medrxiv.org/content/10.1101/2020.07.10.20133801v1
- Coronavirus disease 2019 (COVID-19) deteriorates suddenly primarily due to excessive inflammatory injury, and insulin-like growth factor-1 (IGF-1) is implicated in endocrine control of the immune system. However, the effect of IGF-1 levels on COVID-19 prognosis remains unknown. Objective: To investigate the association between circulating IGF-1 concentrations and mortality risk among COVID-19 patients. Design: Prospective analysis. Setting: UK Biobank. Participants: 1425 COVID-19 patients who had pre-diagnostic serum IGF-1 measurements at baseline (2006-2010). Main outcome measures: COVID-19 mortality (available death data updated to 22 May 2020). Unconditional logistic regression was performed to estimate the odds ratio (OR) and 95% confidence intervals (CIs) of mortality across the IGF-1 quartiles. Results: Among 1425 COVID-19 patients, 365 deaths occurred due to COVID-19. Compared to the lowest quartile of IGF-1 concentrations, the highest quartile was associated with a 37% lower risk of mortality (OR: 0.63, 95% CI: 0.43-0.93, P-trend=0.03). The association was stronger in women and nonsmokers (both P-interaction=0.01). Conclusions: Higher IGF-1 concentrations are associated with a lower risk of COVID-19 mortality. Further studies are required to determine whether and how targeting IGF-1 pathway might improve COVID-19 prognosis. [**note: yet**

another possible biomarker, in this case insulin-like growth factor-1. Interesting that this is a UK Biobank data study by Chinese researchers.]
https://www.medrxiv.org/content/10.1101/2020.07.09.20149369v1

- SARS-CoV-2 causes multiple immune-related reactions at various stages of the disease. The wide variety of skin presentations has delayed linking these to the virus. Previous studies had attempted to look at the prevalence and timing of SARS-COV-2 rashes but were based on mostly hospitalized severe cases and had little follow up. Using data collected on a subset of 336,847 eligible UK users of the COVID Symptom Study app, we observed that 8.8% of the swab positive cases (total: 2,021 subjects) reported either a body rash or an acral rash, compared to 5.4% of those with a negative swab test (total: 25,136). Together, these two skin presentations showed an odds ratio (OR) of 1.67 (95% confidence interval [CI]: 1.41-1.96) for being swab positive. Skin rashes were also predictive in the larger untested group of symptomatic app users (N=54,652), as 8.2% of those who had reported at least one classical COVID-19 symptom, i.e., fever, persistent cough, and/or anosmia, also reported a rash. Data from an independent online survey of 11,546 respondents with a rash showed that in 17% of swab positive cases, the rash was the initial presentation. Furthermore, in 21%, the rash was the only clinical sign. Skin rashes cluster with other COVID-19 symptoms, are predictive of a positive swab test and occur in a significant number of cases, either alone or before other classical symptoms. Recognising rashes is important in identifying new and earlier COVID-19 cases. [**note: add skin rash to the list of SARS-CoV-2 symptoms to be on the lookout for.**]
https://www.medrxiv.org/content/10.1101/2020.07.10.20150656v1
- Current COVID-19 pandemic poses an unprecedented threat to global health and healthcare systems. At least in western countries, the most amount of the death toll is accounted by old people affected by age-related diseases. In this regard, we proposed that COVID-19 severity may be tightly related to inflammaging, i.e. the age-related onset of inflammation, which is responsible for age-related diseases. It has been reported that systemic hyper-inflammation may turn to be detrimental in COVID-19 patients. Objective. Here, we exploited a recently closed clinical trial (NCT04315480) on the anti-IL-6 drug tocilizumab to assess whether microRNAs regulating inflammaging can be assessed as biomarkers of drug response and outcome. Methods. Serum levels of miR-146a-5p, -21-5p, and -126-3p were quantified by RT-PCR and Droplet Digital PCR by two independent laboratories on 30 patients with virologically confirmed COVID-19, characterized by multifocal interstitial pneumonia confirmed by CT-scan and requiring oxygen therapy, and 29 age- and gender-matched healthy control subjects. COVID-19 patients were treated with a single-dose intravenous infusion of 8 mg/kg tocilizumab and categorized into responders and non-responders. Results. We showed that COVID-19 patients who did not respond to tocilizumab have lower serum levels of miR-146a-5p after the treatment (p=0.007). Moreover, among non-responders, those with the lowest serum levels of miR-146a-5p experienced the most adverse outcome (p=0.008). Conclusion. Our data show that blood-based biomarkers, such as miR-146a-5p, can provide a molecular link between inflammaging and COVID-19 clinical course, thus allowing to enlarge the drug armory against this worldwide health threat. [**note: more biomarker work and this is potentially useful as it is linked to tocilizumab therapy response. I also learned that inflammaging may be a real word even though the Microsoft dictionary says otherwise.**]
https://www.medrxiv.org/content/10.1101/2020.07.11.20151365v1

- We aimed to measure SARS-CoV-2 serologic responses in children hospitalized with multisystem inflammatory syndrome (MIS-C) compared to COVID-19, Kawasaki Disease (KD) and other hospitalized pediatric controls. Methods: From March 17, 2020 - May 26, 2020, we prospectively identified hospitalized children at Children's Healthcare of Atlanta with MIS-C (n=10), symptomatic PCR-confirmed COVID-19 (n=10), KD (n=5), and hospitalized controls (n=4). With IRB approval, we obtained prospective and residual blood samples from these children and measured SARS-CoV-2 spike (S) receptor binding domain (RBD) IgM and IgG binding antibodies by quantitative ELISA and SARS-CoV-2 neutralizing antibodies by live-virus focus reduction neutralization assay. We statistically compared the log-transformed antibody titers among groups and performed correlation analyses using linear regression. Results: All children with MIS-C had high titers of SARS-CoV-2 RBD IgG antibodies, which correlated strongly with neutralizing antibodies (R2=0.667, P<0.001). Children with MIS-C had significantly higher SARS-CoV-2 RBD IgG antibody titers (geometric mean titer [GMT] 6800, 95%CI 3495-13231) than children with COVID-19 (GMT 626, 95%CI 251-1563, P<0.001), children with KD (GMT 124, 95%CI 91-170, P<0.001) and other hospitalized pediatric controls (GMT 85 [all below assay limit of detection], P<0.001). All children with MIS-C also had detectable RBD IgM antibodies, indicating recent SARS-CoV-2 infection. RBD IgG titers correlated with erythrocyte sedimentation rate (ESR) (R2=0.512, P<0.046) and with hospital and ICU lengths of stay (R2=0.590, P=0.010). Conclusion: Quantitative SARS-CoV-2 RBD antibody titers may have a role in establishing the diagnosis of MIS-C, distinguishing it from other similar clinical entities, and stratifying risk for adverse outcomes. [**note: yes, there are a small number of children who get multisystem inflammatory syndrome. Here is a serology study of this population from Atlanta.**] https://www.medrxiv.org/content/10.1101/2020.07.10.20150755v1

DRUG DEVELOPMENT

- Nothing today

VIRUS BIOCHEMISTRY

- Nothing today

DIAGNOSTIC DEVELOPMENT

- Rapid large-scale testing is essential for controlling the ongoing pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The standard diagnostic pipeline for testing SARS-CoV-2 presence in patients with an ongoing infection is predominantly based on pharyngeal swabs, from which the viral RNA is extracted using commercial kits followed by reverse transcription and quantitative PCR detection. As a result of the large demand for testing, commercial RNA extraction kits may be limited and alternative, non-commercial protocols are needed. Here, we provide a magnetic bead RNA extraction protocol that is predominantly based on in-house made reagents and is performed in 96-well plates supporting large-scale testing. Magnetic bead RNA extraction was benchmarked against the commercial QIAcube extraction platform. Comparable viral RNA detection sensitivity and specificity were obtained by fluorescent and colorimetric RT-LAMP using N primers, as well as RT-qPCR using E gene primers showing that the here presented RNA extraction protocol can be combined with a variety of detection methods at high throughput. Importantly, the presented diagnostic workflow can be

quickly set up in a laboratory without access to an automated pipetting robot. [**note: this may only be applicable to in house laboratories as I don't know how easily it might be to automate it.**] https://www.medrxiv.org/content/10.1101/2020.07.08.20147561v1

- RT-qPCR utilising upper respiratory swabs are the diagnostic gold standard for SARS-CoV-2 despite reported low sensitivity and limited scale up due to global shortages. Saliva is a non-invasive, equipment independent alternative to swabs. We collected 145 paired saliva and nasal/throat (NT) swabs at diagnosis (day 0) and repeated on day 2 and day 7 dependent on inpatient care and day 28 for study follow up. Laboratory cultured virus was used to determine the analytical sensitivity of spiked saliva and swabs containing amies preservation media. *Self-collected saliva samples were found to be consistent, and in some cases superior when compared to healthcare worker collected NT swabs from COVID-19 suspected participants. We report for the first time the analytical limit of detection of 10-2 and 100 pfu/ml for saliva and swabs respectively. Saliva is a easily self-collected, highly sensitive specimen for the detection of SARS-CoV-2*. [**note: is it time to end these silly drive through lines for nasal swab testing and just have drop off points for self-collected saliva? This Liverpool study points in that direction and there have been others as well.**]
https://www.medrxiv.org/content/10.1101/2020.07.09.20149534v1
- The ongoing COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) requires a significant, coordinated public health response. Assessing case density and spread of infection is critical and relies largely on clinical testing data. However, clinical testing suffers from known limitations, including test availability and a bias towards enumerating only symptomatic individuals. Wastewater-based epidemiology (WBE) has gained widespread support as a potential complement to clinical testing for assessing COVID-19 infections at the community scale. The efficacy of WBE hinges on the ability to accurately characterize SARS-CoV-2 concentrations in wastewater. To date, a variety of sampling schemes have been used without consensus around the appropriateness of grab or composite sampling. Here we address a key WBE knowledge gap by examining the variability of SARS-CoV-2 concentrations in wastewater grab samples collected every 2 hours for 72 hours compared with corresponding 24-hour flow-weighted composite samples. Results show relatively low variability (mean for all assays = 741 copies 100 mL-1, standard deviation = 508 copies 100 mL-1) for grab sample concentrations, and good agreement between most grab samples and their respective composite (mean deviation from composite = 159 copies 100 mL-1). When SARS-CoV-2 concentrations are used to calculate viral load, the discrepancy between grabs (log10 difference = 12.0) or a grab and its associated composite (log10 difference = 11.8) are amplified. A similar effect is seen when estimating carrier prevalence in a catchment population with median estimates based on grabs ranging 62-1853 carriers. Findings suggest that grab samples may be sufficient to characterize SARS-CoV-2 concentrations, but additional calculations using these data may be sensitive to grab sample variability and warrant the use of flow-weighted composite sampling. These data inform future WBE work by helping determine the most appropriate sampling scheme and facilitate sharing of datasets between studies via consistent methodology. [**note: an approach to wastewater monitoring for SARS-CoV-2 from the Hampton Roads Sanitation District.**]
https://www.medrxiv.org/content/10.1101/2020.07.10.20150607v1

- Currently it is unknown whether a positive serology results correlates with protective immunity against SARS-CoV-2. There are also concerns regarding the low positive predictive value of SARS-CoV-2 serology tests, especially when testing populations with low disease prevalence. Methods. A neutralization assay was validated in a set of PCR confirmed positive specimens and in a negative cohort. 9,530 specimens were screened using the Diazyme SARS-CoV-2 IgG serology assay and all positive results (N=164) were reanalyzed using the neutralization assay, the Roche total immunoglobin assay, and the Abbott IgG assay. The relationship between the magnitude of a positive SARS-CoV-2 serology result and the levels of neutralizing antibodies detected was correlated. Neutralizing antibody titers (ID50) were also longitudinally monitored in SARS-CoV-2 PCR confirmed patients. Results. The SARS-CoV-2 neutralization assay had a PPA of 96.6% with a SARS-CoV-2 PCR test and a NPA of 98.0% across 100 negative controls. ID50 neutralization titers positively correlated with all three clinical serology platforms. Longitudinal monitoring of hospitalized PCR confirmed COVID-19 patients demonstrates they made high neutralization titers against SARS-CoV-2. PPA between the Diazyme IgG assay alone and the neutralization assay was 50.6%, while combining the Diazyme IgG assay with either the Roche or Abbott platforms increased the PPA to 79.2% and 78.4%, respectively. Conclusions. For the first time, we demonstrate that three widely available clinical serology assays positively correlate with SARS-CoV-2 neutralization activity observed in COVID-19 patients. When a two-platform screen and confirm approach was used for SARS-CoV-2 serology, nearly 80% of two-platform positive specimens had neutralization titers (ID50 >50). [**note: these UC San Diego researchers look at three serology tests and measure neutralizing antibody titers.**]
  https://www.medrxiv.org/content/10.1101/2020.07.10.20150946v1