2020-04-27

As long as the copyright police don't come after me (hint: don't tell!!); I will post the daily WuMo comics that have COVID-19 themes. I am absolutely positively certain that this experience is shared by us all!!! Don't step on the bathroom scale these days!!

I promise no more Frank Zappa! BTW, nobody responded to the contest question regarding Zappa's relation to the do-wop band. He and his colleagues released an album called 'Cruising with Reuben and the Jets,' consisting of do-wop songs, which received some mainstream radio play. The band leader, *Reuben Sano*, was the alter ego of Frank Zappa. You can find the album on YouTube.

I continue to look for inspiration in music during the pandemic and often return to Mahler. Today I offer a fine performance of the Symphony No. 2: https://www.youtube.com/watch?v=0-_CdYvJ6t4 I look past the religiosity of the choral verses and rather focus on the longing for relief. There is a good entry on Wikipedia discussing this symphony.

Before we move on to trials and other preprints, there are a couple of policy points that warrant consideration.

Yesterday, some readers and I had a discussion of vaccine trial policy and whether active challenge studies are useful. Here is the thorny ethical issue. This group is proposing to do challenge studies using live SARS-CoV-2 to help speed vaccine research (with a placebo arm!!!). At least one newspaper interviewed a couple of people who signed up and there have been some prominent scientists who have written on this and also here.. What I find baffling is that most often the rate limiting steps in vaccine development are the safety assessments. The 1 in 10,000 rare AE requires a trial of 30,000 to statistically show whether there is a concern. Are we gong to reach the point where a vaccine is not fully characterized, and a consent form will need to bd signed off on? I know these are times to think outside the box, but this particular idea does not strike me as wise. Ask yourself if you are sitting on an Institutional Review Board, would you approve a challenge trial such as this? A French group did an interesting survey of intent to participate in a vaccine clinical trial during the pandemic. Nearly 75 % and 48 % of the survey responders were likely to accept vaccination or participation in a clinical trial against COVID-19. Vaccine hesitancy will be the major barrier to COVID-19 vaccine uptake.

GREAT IDEA TIME!!!!! I have set up an anonymous Doodle Poll to see what the level of newsletter reader interest is in being part of a placebo-controlled SARS-CoV-2 vaccine challenge clinical trial. There wasn't enough room to go into all the informed consent stuff but if you are reading this newsletter you already know about that. The poll is here: https://doodle.com/poll/uv64527zfspav7kw and only aggregate yes/no results are compiled. VOTE TODAY (only if you are interested). I'll keep it open until Friday and then report back with the results.

CDER Director Janet Woodcock discusses clinical trials in this Centerwatch article. I tried to be nice in the paper on conducting clinical trials during a pandemic that I posted yesterday. Let me take of the gloves and be blunt! What is need is a Manhattan Project to this problem. Business as usual with lots of trial centers doing their own thing with slightly different versions of protocols won't cut it. Clinicians need information ASAP for both prophylactic and treatment approaches to SARS-CoV-2. Multiple groups coming up with master protocols will only lead to further delay while things are debated. This is all stuff that should have been done over a month ago!!! Contrast this slow walk to nowhere with how nimbly the OHDSI group has reacted. They held a week-long virtual study-a-thon at the end of March mapping out ways to respond to the pandemic. They have conducted two large observational studies and have a third very ambitious one on the drawing board (Project SCYLLA) that stands a good chance of providing important clinical information. I think I need to adopt a new mantra regarding clinical trial process, "Go Manhattan Project or Go Home."

The New York Times discusses possible treatment approach that addresses the sex differential of infection. Hormone patches for everyone? I don't know but there will be some trials scheduled.

MODELING

- This is a well thought out paper from a South African researcher on the strict lower bound for fatality rate. The Infection Fatality Rate (IFR) for COVID-19 is a poorly known, yet crucial, aspect of the disease. Counting only current deaths in a region and assuming everyone in that region is infected provides an absolute lower bound on the IFR. Using this estimator for New York City, Lombardy and Madrid yields strong bounds on the average IFR in overwhelmed health systems. Their combined 35,152 deaths implies IFR > 0.14% averaged over 25.1 million people. This is the best-case scenario and conclusively demonstrates that COVID-19 is more deadly than influenza. The actual value of the average COVID-19 IFR is likely to be higher than this bound. https://www.medrxiv.org/content/10.1101/2020.04.22.20076026v1

- I am a sucker for modest titles of research papers! This one, "A poor-man's approach to the effective reproduction number: the COVID-19 case" certainly meets the criterion!! It is shown that estimates of the effective reproduction number R_t for COVID-19 using standard packages such as EPIESTIM can be reproduced very accurately using the expression $R_t = c(t) / c(t + \tau)$, where $c(t)$ is the incidence at time t and τ the mean value of the series interval. [**note: I'll leave to others to read the paper.**] https://www.medrxiv.org/content/10.1101/2020.04.22.20076430v1
- Here is an interesting Russian modeling paper to detect superspreaders at the earliest moments. Most of epidemiological models applied for COVID-19 do not consider heterogeneity in infectiousness and impact of superspreaders, despite the broad viral loading distributions

amongst COVID-19 positive people (1-1 000 000 per mL). Also, mass group testing is not used regardless to existing shortage of tests. I propose new strategy for early detection of superspreaders with reasonable number of RT-PCR tests, which can dramatically mitigate development COVID-19 pandemic and even turn it endemic. Methods I used stochastic social-epidemiological SEIAR model, where S-suspected, E-exposed, I-infectious, A-admitted (confirmed COVID-19 positive, who are admitted to hospital or completely isolated), R-recovered. The model was applied to real COVID-19 dynamics in London, Moscow and New York City. Findings Viral loading data measured by RT-PCR were fitted by broad log-normal distribution, which governed high importance of superspreaders. The proposed full scale model of a metropolis shows that top 10% spreaders (100+ higher viral loading than median infector) transmit 45% of new cases. Rapid isolation of superspreaders leads to 4-8 fold mitigation of pandemic depending on applied quarantine strength and amount of currently infected people. High viral loading allows efficient group matrix pool testing of population focused on detection of the superspreaders requiring remarkably small amount of tests. Interpretation The model and new testing strategy may prevent thousand or millions COVID-19 deaths requiring just about 5000 daily RT-PCR test for big 12 million city such as Moscow. Though applied to COVID-19 pandemic the results are universal and can be used for other infectious heterogenous epidemics. [**note: how may COVID-19 *Typhoid Marys* are out there?**] https://www.medrxiv.org/content/10.1101/2020.04.22.20076166v1

- This one is for all the Public Health folks who read this newsletter!!! One of the more widely advocated solutions to slowing down the spread of COVID-19 has been automated contact tracing. Since proximity data can be collected by personal mobile devices, the natural proposal has been to use this for contact tracing as this provides a major gain over a manual implementation. In this work, we study the characteristics of automated contact tracing and its effectiveness for mapping the spread of a pandemic due to the spread of SARS-CoV-2. We highlight the infrastructure and social structures required for automated contact tracing to work for the current pandemic. We display the vulnerabilities of the strategy to inadequately sample the population, which results in the inability to sufficiently determine significant contact with infected individuals. Of crucial importance will be the participation of a significant fraction of the population for which we derive a minimum threshold. We conclude that a strong reliance on contact tracing to contain the spread of the SARS-CoV-2 pandemic can lead to the potential danger of allowing the pandemic to spread unchecked. A carefully thought out strategy for controlling the spread of the pandemic along with automated contact tracing can lead to an optimal solution. [**note: paging Dr. Tilson! Public Health people are needed to make this work!!**] https://www.medrxiv.org/content/10.1101/2020.04.22.20071043v1

NEWLY REGISTERED CLINICAL TRIALS

- Here is the estrogen patch trial I referred to in the intro to the newsletter. The purpose of this study is to find out if estrogen, a female sex hormone, given as a patch placed on skin of COVID19 positive or presumptive positive patients for 7 days can reduce the severity of COVID19 symptoms compared to regular care. This study has two study groups. One group will receive the study drug, a single-use Climara 25cm2 estrogen patch. The other group will receive standard of care. Participants will be asked questions about their symptoms for up 6 times in up

to 45 days. [**note: the abstract doesn't mention it is enrolling both males and females. I worry that the patient numbers may be too low to draw any conclusions.**] NCT04359329

CLINICAL TRIAL RESULTS

- Here is hospitalization data from Shenzhen, China. Using data from Shenzhen, China, where all cases were monitored in hospital and symptom profiles and clinical and lab results were available starting from early stages of clinical course, we characterized clinical progression of COVID-19 cases and determined important predictors for faster clinical progression to key clinical events and longer use of medical resources. Epidemiological, demographic, laboratory, clinical, and outcome data were extracted from electronic medical records. We found that those who progressed to the severe stage, developed acute respiratory distress syndrome, and were admitted to the intensive care unit (ICU) progressed on average 9.5 days (95%CI 8.7,10.3), 11.0 days (95%CI 9.7,12.3), and 10.5 days (95%CI 8.2,13.3) after symptom onset, respectively. We estimated that patients who were admitted to ICUs remained there for an average of 34.4 days (95%CI 24.1,43.2) and the average time on a ventilator was 28.5 days (95%CI 20.0,39.1) among those requiring mechanical ventilation. The median length of hospital stay was 21.3 days (95%CI, 20.5, 22.2) for the mild or moderate cases who did not progress to the severe stage, but increased to 52.1 days (95%CI, 43.3, 59.5) for those who required ICU admission. Clear characterization of clinical progression informs planning for healthcare resource allocation during COVID-19 outbreaks and provides a basis that helps assess the effectiveness of new treatment and therapeutics. [**note: there was a story in the New York Times on the heroic efforts required to save a single patient who progressed to ARDS.**] https://www.medrxiv.org/content/10.1101/2020.04.22.20076190v1
- ABSTRACT OBJECTIVE To investigate the dynamics of viral RNA, IgM, and IgG and their relationships in patients with SARS-CoV-2 pneumonia over an 8-week period. DESIGN Retrospective, observational case series. SETTING Wenzhou Sixth Peoples Hospital PARTICIPANTS Thirty-three patients with laboratory confirmed SARS-CoV-2 pneumonia admitted to hospital. Data were collected from January 27 to April 10, 2020. MAIN OUTCOME MEASURES Throat swabs, sputum, stool, and blood samples were collected, and viral load was measured by reverse transcription PCR (RT-PCR). Specific IgM and IgG against spike protein (S), spike protein receptor binding domain (RBD), and nucleocapsid (N) were analyzed. RESULTS At the early stages of symptom onset, SARS-CoV-2 viral load is higher in throat swabs and sputum, but lower in stool. The median (IQR) time of undetectable viral RNA in throat swab, sputum, and stool was 18.5 (13.25-22) days, 22 (18.5-27.5) days, and 17 (11.5-32) days, respectively. In sputum, 17 patients (51.5%) had undetectable viral RNA within 22 days (short persistence), and 16 (48.5%) had persistent viral RNA more than 22 days (long persistence). Three patients (9.1%) had a detectable relapse of viral RNA in sputum within two weeks of their discharge from the hospital. One patient had persistent viral RNA for 59 days or longer. The median (IQR) seroconversion time of anti-S IgM, anti-RBD IgM, and anti-N IgM was 10.5 (7.75-15.5) days, 14 (9-24) days, and 10 (7-14) days, respectively. The median (IQR) seroconversion time of anti-S IgG, anti-RBD IgG, and anti-N IgG was 10 (7.25-16.5) days, 13 (9-17) days, and 10 (7-14) days, respectively. By week 8 after symptom onset, IgM were negative in many of the previously positive patients, and IgG levels remained less than 50% of the peak levels in more than 20% of the patients. In about 40%

of the patients, anti-RBD IgG levels were 4-times higher in convalescence than in acute phase. SARS-CoV-2 RNA coexisted with antibodies for more than 50 days. Anti-RBD IgM and IgG levels, including anti-RBD IgM levels at presentation and peak time, were significantly higher in viral RNA short persistence patients than in long persistence patients. CONCLUSION This study adds important new information about the features of viral load and antibody dynamics of SARS-CoV-2. It is clear from these results that the viral RNA persists in sputum and stool specimens for a relatively long time in many patients. Anti-RBD may also serve as a potential protective antibody against SARS-CoV-2 infection, as viral persistence appears to be related to anti-RBD levels. Earlier treatment intervention also appears to be a factor in viral persistence. [**note: the number of patients is on the small side but the findings are interesting in that linkage between viral load and antibodies is studied. I hope the one patient with viral DNA for 59 days is an outlier!! Antibody presentation seems in line with other studies I have read.**] https://www.medrxiv.org/content/10.1101/2020.04.22.20071258v1

- Eosinopenia may predict a poor prognosis of COVID-19. However, to date, there is no detailed analysis of the clinical characteristics of COVID-19 patients with eosinopenia. Research question: The aim of this study was to describe clinical characteristics of COVID-19 patients with eosinopenia. Study Design and Methods: This was a multi-center retrospective study conducted in three tertiary hospitals. A total of 59 patients with COVID-19 were reviewed from January 23, 2020 to March 10, 2020. We described clincial characteristics of patients with COIVD-19 and eosinopenia phenotype. Results: The median age of patients with COVID-19 was 39 years old, and 32 (54,2%) were male. Patients with severe type had higher proportions of dyspnea (50%) and gastrointestinal symptoms (50%) compared with mild or moderate patients. Laboratory findings indicated that lower counts of lymphocyte and eosnophils were observed in patients with severe type. Cough, sputum, and fatigue were more common symptoms in eosinopenia patients compared with non-eosinopenia patients. High proportion of comorbidities was observed in eosinopenia patients. Laboratory findings indicated that lymphocyte counts (median: 101 cells/µl) in eosinopenia patients were significantly less than those of non-eosinopenia patients (median: 167 cells/µl, $p<0.001$). The use of corticosteroids therapy in COVID-19 patients with eosinopenia were notably higher than those in patients with non-eosinopenia (50% vs 13.8%, respectively, $p=0.005$). Compared with parameters in non-eosinopenia patients, eosinopenia patients were more inclined to have less lymphocyte counts (OR value 6.566, 95%CI[1.101-39.173], $p=0.039$). Interpretation Eosinopenia are very common in COVID-19 patient, particularly in severe patients. Common symptoms included fever, cough, sputum, and fatigue are frequent in eosinopenia patients. Eosinopenia may represent a novel phenotype in COVID-19, which needs further investigation. [**note: it's amazing all the different clinical symptoms this virus causes. I think gout is probably the only thing that has not been looked for.**] https://www.medrxiv.org/content/10.1101/2020.04.22.20071050v1

DRUG DEVELOPMENT

- Nothing new to report.

DIAGNOSTIC DEVELOPMENT

- Seattle investigators compare commercial and lab developed assays. Multiple laboratory developed tests and commercially available assays have emerged to meet diagnostic needs related to the SARS-CoV-2 pandemic. To date, there is limited comparison data for these different testing platforms. We compared the analytical performance of a laboratory developed test (LDT) developed in our clinical laboratory based on CDC primer sets and four commercially available, FDA emergency use authorized assays for SARS-CoV-2 (Cepheid, DiaSorin, Hologic Panther, and Roche Cobas) on a total of 169 nasopharyngeal swabs. The LDT and Cepheid Xpert Xpress SARS-CoV-2 assays were the most sensitive assays for SARS-CoV-2 with 100% agreement across specimens. The Hologic Panther Fusion, DiaSorin Simplexa, and Roche Cobas 6800 only failed to detect positive specimens near the limit of detection of our CDC-based LDT assay. All assays were 100% specific, using our CDC-based LDT as the gold standard. Our results provide initial test performance characteristics for SARS-CoV-2 RT-PCR and highlight the importance of having multiple viral detection testing platforms available in a public health emergency. [**note: the podcast I mentioned last week with the director of clinical microbiology at UCLA also noted the importance of having multiple test platforms.**]
  https://www.medrxiv.org/content/10.1101/2020.04.24.20074559v1
- Here is a clinical finding about the poor early linkage between antibody testing and CT chest examination. Background: We evaluated the clinical performance of an immunochromatographic (IC) IgM/IgG antibody assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) and chest computed tomography (CT) for the diagnosis of Coronavirus disease 2019 (COVID-19). Methods: We examined 139 serum specimens collected from 112 patients with COVID-19 and 48 serum specimens collected from 48 non-COVID-19 patients. The presence of IgM/IgG antibody for SARS-CoV2 was determined using the One Step Novel Coronavirus (COVID-19) IgM/IgG Antibody Test. Chest CT was performed in COVID-19 patients on admission. Findings: Of the 139 COVID-19 serum specimens, IgM was detected in 27.8%, 48.0%, and 95.8% of the specimens collected within 1 week, 1-2 weeks, and >2 weeks after symptom onset and IgG was detected in 3.3%, 8.0%, and 62.5%, respectively. Among the 48 non-COVID-19 serum specimens, 1 generated a false-positive result for IgM. Thirty-eight of the 112 COVID-19 patients were asymptomatic, of whom 15 were positive for IgM, and 74 were symptomatic, of whom 22 were positive for IgM and 7 were positive for IgG. The diagnostic sensitivity of CT scan alone and in combination with the IC assay was 57.9 % (22/38) and 68.4% (26/38) for the asymptomatic patients and 74.3% (55/74) and 82.4% (61/74) for the symptomatic patients, respectively. Conclusion: The IC assay had low sensitivity during the early phase of infection, and thus IC assay alone is not recommended for initial diagnostic testing for COVID-19. If RT-qPCR is not available, the combination of chest CT and IC assay may be useful for diagnosing COVID-19. https://www.medrxiv.org/content/10.1101/2020.04.22.20075564v1

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2020-04-28

This convinces me that it is not the right time to get a dog for companionship!

Here is a another fine piece of chamber music by Franz Schubert, the octet in F major, for strings, horn, bassoon & clarinet: https://www.youtube.com/watch?v=fnpVu8Eihj4 I'm constantly amazed at Schubert's musical output during his short life. By my count, I have sung maybe 35 or so of his lieder and have been working on and off on Winterreise. That recital is likely not to take place but as soon as things clear up, I'm planning on doing the Schumann Op. 39 songs (Eichendorff poems) with maybe Beethoven's An die ferne Geliebte. If it's safe for travel all my loyal readers will get an invite.

Saluting all the epidemiologists and thanks to a newsletter reader who sent me the link, here is a wonderful piece in The New Yorker on how scientists were regarded differently in New York and Seattle.

It looks like the first SARS-CoV-2 case in the Washington DC metro area was three weeks prior to the first publicized case. A woman who returned from Italy in early February came down with flu symptoms. An antibody test done recently showed the exposure to the virus.

The latest treatment of the day is famotidine. A hospital system in New York are doing a trial (yet to be registered at NIH!!!) ± hydroxychloroquine against a placebo arm. Before you all drop what you are doing and run out to CVS to denude the shelves of Prilosec OTC be advised this protocol is using IV famotidine at concentration eight times the package labeled dose. This is really weird as I never saw this come up in any of the screening papers I've read. Apparently its based on some observational data coming out of China showing that patients taking famotidine had better clinical outcomes. I'll see if I can track that information down. Here is the money quote from the SCIENCE article:

- *The study's draft protocol was aimed only at evaluating famotidine's efficacy, but Trump's "game-changer" antimalarial drug was rapidly becoming the standard of care for hospitalized COVID-19 patients. That meant investigators would only be able to recruit enough subjects for a trial that tested a combination of famotidine and hydroxychloroquine. Those patients would be compared with a hydroxychloroquine-only arm and a historic control arm made up of hundreds of patients treated earlier in the outbreak. "Is it good science? No," Tracey says. "It's the real world."*

I've already suggested that the OHDSI group add this to the list of drugs they will be looking at. I am also surprised that there was no *in vitro* data regarding antiviral activity mentioned. Perhaps it works, but if it doesn't it will be another one for the garbage bin of bad science. [**note to self: stay on the fexofenadine, that one *in silico* study is convincing enough!**]

Regeneron and Sanofi announced that the mAb, sarilumab, was not effective in treating mild SARS-CoV-2 infections. The DSMB stopped that arm of the trial but permitted the use in seriously ill patients to continue. Derek Lowe weighs in on this as well.

There is an article in The New York Times on the race for a SARS-CoV-2 vaccine. Astute readers of the newsletter are aware that China have also published animal studies of one of their vaccine candidates showing extremely high immunogenicity and the Times article notes this. The Oxford vaccine is a disabled adenovirus vector and one problem with that approach is host immune response will be raised against both the SARS-CoV-2 and the adenovirus, potentially complicating things if a second challenge vaccine is needed. It's good to see a variety of vaccine platforms being tested to see what the best approach is.

MODELING

- Oh no!!! Maybe hot weather and humidity won't help us out!!! Say it ain't so. We study the effects of three types of variables on the early pace of spread of Covid-19: weather variables, temperature and absolute humidity; population density; the timeline of Covid-19 infection, as outbreak of disease occurs in different dates for different regions. The regions considered were all 50 U.S. states and 110 countries (those which had enough data available by April 10th. We looked for associations between the above variables and an estimate of the growth rate of cases, the exponential coefficient, computed using data for 10 days starting when state/country reached 100 confirmed cases. The results for U.S. states indicate that one cannot expect that higher temperatures and higher levels of absolute humidity would translate into slower pace of Covid-19 infection rate, at least in the ranges of those variables during the months of February and March of 2020 (-2.4 to 24C and 2.3 to 15g/m^3). In fact, the opposite is true: the higher the temperature and the absolute humidity, the faster the Covid-19 has expanded in the U.S. states, in the early stages of the outbreak. *Secondly, using the highest county population density for each state, there is strong positive association between population density and (early) faster spread of Covid-19. Finally, there is strong negative association between the date when a state reached 100 accumulated cases and the speed of Covid-10 outbreak (the later, the lower the estimate of growth rate). When these variables are considered together, only population density and the timeline variable show statistical significance.* We also develop the basic models for the collection of countries, without the demographic variable. Despite the evidence, in that case, that warmer and more humid countries have shown lower rates of Covid-19 expansion, the weather variables lose statistical significance when the timeline variable is added. [**note: models are only as good as the variables used for data input. I have no idea what the impact of weather will be and I doubt anyone else does at this point in time. Population density does appear to be one critical factor that is close to 'certainty.'**]
  https://www.medrxiv.org/content/10.1101/2020.04.21.20074450v1
- Of this we can be sure, social distancing works! It's even in my mantra. In early 2020, cities across China enacted strict social distancing measures to contain emerging coronavirus (COVID-

19) outbreaks. We estimated the speed with which these measures contained community transmission in each of 58 Chinese cities. On average, containment was achieved 7.83 days (SD 6.79 days) after the implementation of social distancing interventions, with an average reduction in the reproduction number (Rt) of 54.3% (SD 17.6%) over that time period. A single day delay in the implementation of social distancing led to a 2.41 (95% CI: 0.97, 3.86) day delay in containment. Swift social distancing interventions may thus achieve rapid containment of newly emerging COVID-19 outbreaks.
https://www.medrxiv.org/content/10.1101/2020.04.22.20075762v1

NEWLY REGISTERED CLINICAL TRIALS

- UC San Diego is launching a ramipril trial for treatment. [**note: this statement of exclusion criteria baffles me, "Participation in any other clinical trial of an experimental treatment for COVID-19 (*use of hydroxycholoroquine or compassionate use of choloroquine or azithromycin is allowed*)" Maybe they don't think this is a confounding factor.**] NCT04366050
- This is described by the French researchers as a counter-intuitive use of inhaled captopril. [**note: there is too much in the protocol to past here. Those interested should go to the clinicaltrials.gov website and do a search by the trial number.**] NCT04355429

CLINICAL TRIAL RESULTS

- Sadly no news on anything other than sarilumab that was already mentioned.

DRUG DEVELOPMENT

- Good work from the Max Planck Institute on getting the structure of the SARS-CoV-2 polymerase. The coronavirus SARS-CoV-2 uses an RNA-dependent RNA polymerase (RdRp) for the replication of its genome and the transcription of its genes. Here we present the cryo-electron microscopic structure of the SARS-CoV-2 RdRp in its replicating form. The structure comprises the viral proteins nsp12, nsp8, and nsp7, and over two turns of RNA template-product duplex. The active site cleft of nsp12 binds the first turn of RNA and mediates RdRp activity with conserved residues. Two copies of nsp8 bind to opposite sides of the cleft and position the RNA duplex as it exits. Long helical extensions in nsp8 protrude along exiting RNA, forming positively charged 'sliding poles' that may enable processive replication of the long coronavirus genome. Our results will allow for a detailed analysis of the inhibitory mechanisms used by antivirals such as remdesivir, which is currently in clinical trials for the treatment of coronavirus disease 2019 (COVID-19). [**note: this is really fascinating stuff from a enzymological point of view. The polymerase is a complex of three gene products: a catalytical subunit and two accessory subunits. I'm going to take a longer look at this paper.**]
https://www.biorxiv.org/content/10.1101/2020.04.27.063180v1
- Interesting data on remdesivir potency. We report that remdesivir (RDV), a monophosphoramidate prodrug of an adenosine analog, potently inhibits SARS-CoV-2

replication in human lung cells and primary human airway epithelial cultures (EC50 = 0.01 µM). Weaker activity was observed in Vero E6 cells (EC50 = 1.65 µM) due to their low capacity to metabolize RDV. To rapidly evaluate in vivo efficacy, we engineered a chimeric SARS-CoV encoding the viral target of RDV, the RNA-dependent RNA polymerase, of SARS-CoV-2. In mice infected with chimeric virus, therapeutic RDV administration diminished lung viral load and improved pulmonary function as compared to vehicle treated animals. These data provide substantial evidence that RDV is potently active against SARS-CoV-2 in vitro and in vivo, supporting its further clinical testing for treatment of COVID-19. [**note: most of the *in vitro* studies I have seen are done in Vero cell cultures. Which of these models is best? Is it time to do rapid testing in macaques which seem to be the best animal model?**] https://www.biorxiv.org/content/10.1101/2020.04.27.064279v1

- Here is a bit of good news for vaccine developers from the researchers at Walter Reed. Here we analyzed SARS-CoV-2 sequence diversity across 5,700 sequences sampled since December 2019. The Spike protein, which is the target immunogen of most vaccine candidates, showed 93 sites with shared polymorphisms; only one of these mutations was found in more than 1% of currently circulating sequences. The minimal diversity found among SARS-CoV-2 sequences can be explained by drift and bottleneck events as the virus spread away from its original epicenter in Wuhan, China. Importantly, there is little evidence that the virus has adapted to its human host since December 2019. Our findings suggest that a single vaccine should be efficacious against current global strains. https://www.biorxiv.org/content/10.1101/2020.04.27.064774v1
- Here is a nice study from China on the cytotoxicity of chloroquine and hydroxychloroquine in multiple cell lines. To further uncover the toxicity profile of CQ and HCQ in different tissues, we evaluated the cytotoxicity of them in 8 cell lines, and further adopted the physiologically-based pharmacokinetic models (PBPK) to predict the tissue risk respectively. Retina, myocardium, lung, liver, kidney, vascular endothelium and intestinal epithelium originated cells were included in the toxicity evaluation of CQ and HCQ respectively. The proliferation pattern were monitored in 0-72 hours by IncuCyte S3, which could perform long-term continuous image and video of cells upon CQ or HCQ treatment. CC50 and the ratio of tissue trough concentrations to CC50 (R_{TTCC}) were brought into predicted toxicity profiles. The CC50 at 24 h, 48 h, 72 h of CQ and HCQ decreased in the time-dependent manner, which indicates the accumulative cytotoxic effect. HCQ was found to be less toxic in 7 cell types except cardiomyocytes H9C2 cells (CC50-48 h=29.55 µM; CC50-72 h=15.26 µM). In addition, R_{TTCC} is significant higher in CQ treatment group compared to HCQ group, which indicates that relative safety of HCQ. Both CQ and HCQ have certain cytotoxicity in time dependent manner which indicates the necessity of short period administration clinically. HCQ has the less impact in 7 cell lines proliferation and less toxicity compared to CQ in heart, liver, kidney and lung. [**quote: the paper is more cautious than the abstract. Given the long half-life of hydroxychloroquine, studies optimally should be extended out. The authors write, "Our results revealed that both CQ and HCQ have shown certain cytotoxicity in 8 different types of cell lines in time and dose dependent manner *in vitro,* suggesting the necessity for short period administration clinically."** https://www.biorxiv.org/content/10.1101/2020.04.22.056762v1
- More interesting work on remdesivir from China. SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) provides a promising but challenging drug target due to its intrinsic proofreading exoribonuclease (ExoN) function. Nucleoside triphosphate (NTP) analogues added to the

growing RNA chain should supposedly terminate viral RNA replication, but ExoN can cleave the incorporated compounds and counteract their efficacy. Remdesivir targeting SARS-CoV-2 RdRp exerts high drug efficacy in vitro and in vivo. However, its underlying inhibitory mechanisms remain elusive. Here, we performed all-atom molecular dynamics (MD) simulations with an accumulated simulation time of 12.6 microseconds to elucidate the molecular mechanisms underlying the inhibitory effects of remdesivir in nucleotide addition (RdRp complex: nsp12-nsp7-nsp8) and proofreading (ExoN complex: nsp14-nsp10). We found that the 1'-cyano group of remdesivir possesses the dual role of inhibiting both nucleotide addition and proofreading. For nucleotide addition, we showed that incorporation of one remdesivir is not sufficient to terminate RNA synthesis. Instead, the presence of the polar 1'-cyano group of remdesivir at an upstream site causes instability via its electrostatic interactions with a salt bridge formed by Asp865 and Lys593, rendering translocation unfavourable. This may eventually lead to a delayed chain termination of RNA extension by three nucleotides. For proofreading, remdesivir can inhibit cleavage via the steric clash between the 1'-cyano group and Asn104. To further examine the role of 1'-cyano group in remdesivir's inhibitory effects, we studied three additional NTP analogues with other types of modifications: favipiravir, vidarabine, and fludarabine. Our simulations suggest that all three of them are prone to ExoN cleavage. Our computational findings were further supported by an in vitro assay in Vero E6 cells using live SARS-CoV-2. The dose-response curves suggest that among tested NTP analogues, only remdesivir exerts significant inhibitory effects on viral replication. Our work provides plausible mechanisms at molecular level on how remdesivir inhibits viral RNA replication, and our findings may guide rational design for new treatments of COVID-19 targeting viral replication. [**note: wow, this virus is damn clever. It may be that simple antiviral drug treatment may not be optimal unless one can somehow block the proofreading subunit as well.**https://www.biorxiv.org/content/10.1101/2020.04.27.063859v1

DIAGNOSTIC DEVELOPMENT

- Abbott Labs, Ortho , DiaSorin, and Autobio Diagnostics all have received an FDA Emergency Use Authorization for serology tests. HERE IS THE BIG NEWS: the Autobio test is a lateral flow rapid test!!! Before anyone gets super-excited, the test is only for CLIA lab use and not field detection. However, it is the first such test to pass FDA scrutiny. Here are the product use instructions.

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2020-04-29

No WuMo comic today as the COVID-19 theme may be finished.

Stephen Sondheim turned 90 in March and a 90th birthday celebration was held and it's worth watching just for the simple pleasure of seeing so many wonderful singers performing in the COVID-19 era. For a shorter enjoyment of Sonheim here is Neil Patrick Harris singing "Being Alive" from Company: https://www.youtube.com/watch?v=HnTu8IBWvTQ

Gilead issued a press release today saying that remdesivir has met the primary endpoint in an NIAID run trial. Results will be announced at a press conference. **It goes without saying that the results of this trial are eagerly awaited!!!**

Here is an op-ed by Osterholm and Olshaker from the New York Times on testing. I've had my issues with Dr. Osterholm over the years but he is mostly right here. I do take issue with the need for high specificity tests for field research. Such testing informs us of the scope of infection and allows one to use more precise methods for tying up the loose ends.

Derek Lowe does the work so I don't need to. Here he discusses monoclonal antibody research for SARS-CoV-2. I did a thought experiment based on using a mAb as a bridge to a vaccine. the mark up on the approved mAbs is quite high and we don't know how much. Price increases have been much higher on a year to year basis than for most other drugs. Let's say for sake of argument that the government can come in and be a big purchaser of these drugs and that an autoinjectable formulation can be made. You want to give this to everyone over 35 as an arbitrary cut off (younger than 35 get very mild symptoms) and this is about 1/2 of 330M which means 165M need to get this. The government negotiates a price of $750/month/person you end up with a total price tag for one month of about $120B. Now you might think this is a huge number but the economists I've read say we are losing $85B every week that things are shut down under the present conditions. You see that the $120B expenditure now seems pretty reasonable based on monthly savings. It is likely that the number needed to be treated would be smaller, and the savings greater.

The other thing we don't know is whether humans on mAb therapy would still generate an immune response with subsequent infection. If so, the mAb prophylaxis might not be needed for more than a few months. Of course, this is all conjecture based on an ideal case.

Lots of abstracts to plough through today and an explosion of clinical trials using sera-enriched antibodies from recovered patients. I'm not listing them as they are a limiting technology.

MODELING

- I like modeling studies from countries where they are looking at something interesting. Here is one from South Africa trying to untangle issues related to vaccine availability. Background: COVID-19 has emerged and spread at great speed globally and has presented one of the greatest public health challenges in modern times with no proven cure or vac-cine. Africa is still early in this epidemic, therefore the spectrum of disease severity is not yet clear. Methods: We used a mathematical model to fit to the observed cases of COVID-19 in South Africa to estimate the basic reproductive number and critical vaccination coverages to con-trol the disease for different hypothetical vaccine efficacy scenarios. We also estimated the percentage reduction in effective contacts due to the social distancing measures imple-mented. Results: Early model estimates show that COVID-19 outbreak in South Africa had a basic reproductive number of 2.95 (95% credible interval [CrI] 2.83-3.33). A vaccine with 70% effi-cacy had the capacity to contain COVID-19 outbreak but at very higher vaccination cover-age 94.44% (95% CrI 92.44-99.92%)

with a vaccine of 100% efficacy requiring 66.10% (95% CrI 64.72-69.95%) coverage. Social distancing measures put in place have so far reduced the number of social contacts by 80.31% (95% CrI 79.76-80.85%). Conclusions: Findings suggest a highly efficacious vaccine would have been required to con-tain COVID-19 in South Africa. Therefore, the current social distancing measures to reduce contacts will remain key in controlling the infection in the absence of vaccines and other therapeutics. [**note: interesting conjecture about the need for a high efficacy vaccine.**] https://www.medrxiv.org/content/10.1101/2020.04.23.20077297v1

- This one was too good to pass up!!! Several ecological studies of the coronavirus disease 2019 (COVID-19) have reported correlations between group-level aggregated exposures and COVID-19 outcomes. While some studies might be helpful in generating new hypotheses related to COVID-19, results of such type of studies should be interpreted with cautions. To illustrate how ecological studies and results could be biased, we conducted an ecological study of COVID-19 outcomes and the distance to Brussels using European country-level data. We found that, the distance was negatively correlated with COVID-19 outcomes; every 100 km away from Brussels was associated with approximately 6% to 17% reductions (all P<0.01) in COVID-19 cases and deaths in Europe. Without cautions, such results could be interpreted as the closer to the Europe Union headquarters, the higher risk of COVID-19 in Europe. However, these results are more likely to reflect the differences in the timing of and the responding to the outbreak, etc. between European countries, rather than the 'effect' of the distance to Brussels itself. Associations observed at the group level have limitations to reflect individual-level associations - the so-called ecological fallacy. Given the public concern over COVID-19, ecological studies should be conducted and interpreted with great cautions, in case the results would be mistakenly understood. [**note to self: don't worry that I live only about 12 miles away from the US Capitol. This study only applies to the EU headquarters.**] https://www.medrxiv.org/content/10.1101/2020.04.23.20077008v1
- More on BCG vaccination. We tested the hypothesis that the vaccination against tuberculosis with BCG correlates with better outcomes for COVID-19 patients. To this end, we combined the information on demography, economy, major chronic diseases, and immunization policies with the COVID-19 outcomes. We filtered out at a fixed date all countries that were below a predetermined value for population size and number of COVID-19 deaths per million (DPM). Altogether, 55 countries, covering 62.9% of the world population, met our criteria and were analyzed. To allow a reliable comparison between countries, each was aligned to a critical alignment threshold date (0.5 DPM). We found that the years of BCG admission are negatively correlated with DPM at varying times post alignment. Results from multivariable regression tests with 22 quantitative properties of each country and its population substantiate the dominant contribution of BCG admission years to the COVID-19 outcomes. Analyzing countries according to an age group partition across several time-points, reveals that the strongest correlation is attributed to the coverage in BCG vaccination of the young population (<25 years), to a lesser degree the middle age group (25-64 years), while BCG coverage status of the elderly (>65 years) was insignificant. More specifically, the signal is attributed to recent immunizations (last 15 years) rather than past policies. We propose that BCG immunization coverage, especially among the most recently vaccinated, may contribute to attenuation of the COVID-19 spread and severity. [**note: I think this will be the LAST BCG model I post. The vaccine is in trials of healthcare workers at several sites and that data should be good enough to solve this riddle.**

Most all of these papers are too naïve in their analyses and don't examine the epidemiology of the disease from countries that have gone through the pandemic.]
https://www.medrxiv.org/content/10.1101/2020.04.23.20077123v1

NEWLY REGISTERED CLINICAL TRIALS

- Here is a trial of a live biotherapeutic MRx4DP0004. It has been in trials for inflammation brought about by asthma. [**note: this is about all I have been able to find out about this product: MRx-4DP0004 is a commensal *Bifidobacte riumbreve* strain isolated from the microbiome of a healthy human infant.**] NCT04363372
- This is a prospective single-center registry with an embedded open-label single-arm clinical trial to determine the effects of standard of care treatment vs. standard of care plus AT-001 on cardiac structure and function and in-hospital survival in patients hospitalized for management of COVID-19 infection. Eligible subjects with COVID-19 infection will be identified at the time of hospital admission based on existing infection control surveillance protocols, and will have clinical data extracted from the electronic medical record to determine clinical characteristics associated with cardiac structure and function and in-hospital survival. A subset of patients with history of diabetes mellitus and/or acute hyperglycemia (any glucose measurement >126 mg/dl) and evidence of acute or chronic heart disease will be treated in an open-label fashion to receive an investigational aldose reductase inhibitor, AT-001 plus standard of care. **[note: this one comes from NYU where several of my loyal readers went to med school!**] NCT04365699
- This is a randomized, double-blind, placebo-controlled, single-ascending dose study of AVM0703 administered as a single intravenous (IV) infusion to patients with COVID-19. The study is designed to evaluate the safety, tolerability, and pharmacokinetics of single-ascending dosing of AVM0703 in patients with COVID-19. [**note: according to the protocol this is a super high dose of dexamethasone**] NCT04366115
- Another ACE inhibitor study, this time it is ramipril. Study will look at reduced ICU admission and need for mechanical ventilator. NCT04366050
- Seriously????????????? This is a clinical trial of a cash transfer on symptoms consistent with COVID-19 and adherence to physical distancing guidance. [**note: paging Andrew Yang!!! I love cash transfers but you have to be in Toronto for this study.**] NCT04359264
- Low doses of Naltrexone, a drug approved for treating alcoholism and opiate addiction, as well as Ketamine, a drug approved as an anesthetic, may be able to interrupt the inflammation that causes the worst COVID-19 symptoms and prove an effective new treatment. This study will investigate their effectiveness in a randomized, blinded trial versus standard treatment plus placebo. NCT04365985
- This is a multicenter randomized trial to evaluate the efficacy of local budesonide (nasal irrigation) in the management of persistent hyposmia in COVID-19 patients. NCT04361474
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CLINICAL TRIAL RESULTS

- The observational data keeps coming in!!!! Here is a nice paper from a British-Chinese group looking at the role of renin-angiotensin-aldosterone system drugs in a large cohort study. Medical editorials have suggested that angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) should not be given to people with arterial hypertension during the coronavirus disease 2019 (COVID-19) pandemic because of a potential increased risk of worse clinical outcomes and that calcium channel blockers (CCBs) should be used as an alternative. Methods Using a cohort of 610 COVID-19 cases and 48,667 population-based controls from Zheijang, China we have tested the role of usage of ACEIs, ARBs, CCBs and other medications on risk and severity of COVID 19. Analyses were adjusted for age, sex and BMI and for presence of relevant comorbidities. Findings: Higher BMI, diabetes and cardio/ cerebrovascular disease as independent risk factors for the development of COVID-19. Individuals with hypertension taking CCBs had significantly increased risk [odds ratio (OR)= 1.67 (95% CI 1.2-2.9)) of manifesting symptoms of COVID-19 whereas those taking ARBs and diuretics had significantly lower disease risk (OR=0.24; 95%CI 0.17-0.34 and OR=0.32; 95%CI 0.19-0.57 respectively). Other antihypertensive drugs were not associated with increased risk of severe or critical form of the infection. Use of glucocorticoids was significantly associated with a severe/critical form of COVID-19 (OR= 7.56; 95%CI 1.17-48.93). Interpretation: we found no evidence to alter ARBs or ACEIs therapy in the context of the pandemic*. Patients on corticosteroids with COVID-19 are at higher risk of developing a severe form of COVID-19and therefore should be monitored closely.* [**note to self: when will I ever be able to use Flonase?**] https://www.medrxiv.org/content/10.1101/2020.04.24.20077875v1
- This is a very small cluster of patients from the Tibetan plateau area. Since the outbreak of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan, a series of confirmed cases of COVID-19 were found on the Qinghai-Tibet plateau. We aimed to describe the epidemiological, clinical characteristics, and outcomes of all confirmed cases in Qinghai, a province at high altitude. With efficient measures to stop the spread of coronavirus, no new cases were found in Qinghai Province for 60 consecutive days between Feb 6 and April 6, 2020. Of all 18 patients with confirmed SARS-CoV-2 infection, 15 patients comprising 4 transmission clusters were identified. Three patients were infected by direct contact without travel history to Wuhan. Seven patients were asymptomatic on admission. Of 18 patients, 10 patients showed bilateral pneumonia and 2 patients showed no abnormalities. Three patients with comorbidities such as hypertension, liver diseases or diabetes developed severe illness. High C-reactive protein levels and elevations of both ALT and AST were observed in 3 severely ill patients on admission. All 18 patients were eventually discharged, including the 3 severe patients who recovered after treatment with non-invasive mechanical ventilation, convalescent plasma and other therapies. Our findings confirmed human-to-human transmission of SARS-CoV-2 in clusters. The strategies of early diagnosis, early isolation, and early treatment are important to prevent the spread of COVID-19 and improve the cure rate. Patients with comorbidities are more likely to develop severe illness and could benefit from convalescent plasma transfusion. [**note: The full paper makes for interesting reading. The clinicians treated everyone with antiviral therapy of lopinavir/ritonavir and interferon-α2b and some received oseltamivir and ribavirin. Surprisingly hydroxychloroquine was not used!** 😊 **Everyone survived which was good but**

trying to make sense of the pharmaceutical effect is hopeless.]
https://www.medrxiv.org/content/10.1101/2020.04.23.20077644v1

- Yet more information on the renin-angiotensin-aldosterone system inhibitors from the large New Jersey outbreak. Hypertension is the most common pre-existing condition amongst COVID-19 patients. Upregulation of the renin-angiotensin-aldosterone system (RAAS) is common in hypertensive patients and may promote inflammation and ensuing cytokine storm in COVID-19. It is unknown whether RAAS inhibition with ACE1 inhibitors or angiotensin-receptor blockers (ARB) can be harmful or beneficial. Methods: Within Hackensack Meridian Health network, the largest healthcare provider in New Jersey, we performed a retrospective, multicenter, convenience sampling study of hospitalized COVID-19 patients. Demographics, clinical characteristics, treatments, and outcomes were manually abstracted. Fishers exact tests, and logistic regression were performed. Results: Among 3017 hospitalized COVID-19 patients, 1584 (52.5%) carried a diagnosis of hypertension. In the discharged or deceased cohort, the overall mortality was significantly increased at 35% vs 13% among COVID-19 patients with hypertension. However, when adjusted for age, the effect of hypertension on mortality was greatly diminished, with a reduction in odds-ratio by over half; and completely disappeared when adjusted for other major covariates. The mortality rates were lower for hypertensive patients prescribed ACE1 (27%, p=0.001) or ARBs (33%, p=0.12) compared to other anti-hypertensive agents (39%) in the unadjusted analyses. RAAS inhibitor therapy appeared protective compared to other anti-hypertensive agents (p=0.001). Conclusions: While our results are limited by the retrospective nature of our study and by potential confounders, our data argue against a harmful effect of RAAS inhibition and support the HFSA/AHA/ACC joint statement recommending continuing ACE1 and ARB therapy in hypertensive COVID-19 patients. https://www.medrxiv.org/content/10.1101/2020.04.24.20077388v1
- High rates of concurrent gastrointestinal manifestations have been noted in patients with COVID- 19, however the association between these digestive manifestations and need for hospitalization has not been established. Methods: Following expedited approval from our Institutional Review Board, we analyzed retrospectively collected data from consecutive patients with confirmed COVID-19 based on a positive polymerase chain reaction testing at our institution from March 03, 2020 to April 7, 2020. Baseline demographic, clinical, laboratory and patient-reported symptom data were collected at presentation in the emergency room. Multivariable logistic regression analyses were performed to evaluate the association between hospitalization and presence of gastrointestinal symptoms. Results: During this study period, we identified 207 consecutive patients with confirmed COVID-19. 34.5% noted concurrent gastrointestinal symptoms; of which 90% of gastrointestinal symptoms were mild. In a multivariate regression model controlled for demographics and disease severity, an increased risk for hospitalization was noted in patients with any gastrointestinal symptom (adjusted OR 4.84 95% CI: 1.68-13.94]. Diarrhea was associated with a seven-fold higher likelihood for hospitalization (adjusted OR=7.58, 95% CI: 2.49-20.02, P <0.001) and nausea or vomiting had a four times higher odds (adjusted OR 4.39, 95% CI: 1.61-11.4, P = 0.005). Conclusion: We demonstrate that a significant portion of COVID19 patients have concurrent mild gastrointestinal symptoms and that the presence of these digestive symptoms is associated with a need for hospitalization. With the current focus on streamlining triaging efforts, first responders and frontline providers should consider assessing for digestive symptoms in their

initial clinical evaluation and decision-making.
https://www.medrxiv.org/content/10.1101/2020.04.23.20076935v1

- From the hot spot in New Orleans information on Vitamin D insufficiency. Emerging health disparities data regarding African American and homeless populations suggest that vitamin D insufficiency (VDI) may be an underlying driver of COVID-19 severity. To better define the VDI-COVID-19 link, we determined the prevalence of VDI among our COVID-19 intensive care unit (ICU) patients. Methods: In an Institutional Review Board approved study performed at a single, tertiary care academic medical center, the medical records of COVID-19 patients were retrospectively reviewed. Subjects were included for whom serum 25-hydroxycholecalcifoerol (25OHD) levels were determined. COVID-19-relevant data were compiled and analyzed. We determined the frequency of VDI among COVID-19 patients to evaluate the likelihood of a VDI-COVID-19 relationship. Results: Twenty COVID-19 patients with serum 25OHD levels were identified; 65.0% required ICU admission.The VDI prevalence in ICU patients was 84.6%, vs. 57.1% in floor patients. Strikingly, 100% of ICU patients less than 75 years old had VDI. Coagulopathy was present in 62.5% of ICU COVID-19 patients, and 92.3% were lymphocytopenic. Conclusions: VDI is highly prevalent in severe COVID-19 patients. VDI and severe COVID-19 share numerous associations including hypertension, obesity, male sex, advanced age, concentration in northern climates, coagulopathy, and immune dysfunction. Thus, we suggest that prospective, randomized controlled studies of VDI in COVID-19 patients are warranted. https://www.medrxiv.org/content/10.1101/2020.04.24.20075838v1
- Here is a large study of hospitalized patients from the UK. Objective: To characterize the clinical features of patients with severe COVID-19 in the UK. Design: Prospective observational cohort study with rapid data gathering and near real-time analysis, using a pre-approved questionnaire adopted by the WHO. Setting: 166 UK hospitals between 6th February and 18th April 2020. Participants: 16,749 people with COVID-19. Interventions: No interventions were performed, but with consent samples were taken for research purposes. Many participants were co-enrolled in other interventional studies and clinical trials. Results: The median age was 72 years [IQR 57, 82; range 0, 104], the median duration of symptoms before admission was 4 days [IQR 1,8] and the median duration of hospital stay was 7 days [IQR 4,12]. The commonest comorbidities were chronic cardiac disease (29%), uncomplicated diabetes (19%), non-asthmatic chronic pulmonary disease (19%) and asthma (14%); 47% had no documented reported comorbidity. Increased age and comorbidities including obesity were associated with a higher probability of mortality. Distinct clusters of symptoms were found: 1. respiratory (cough, sputum, sore throat, runny nose, ear pain, wheeze, and chest pain); 2. systemic (myalgia, joint pain and fatigue); 3. enteric (abdominal pain, vomiting and diarrhoea). Overall, 49% of patients were discharged alive, 33% have died and 17% continued to receive care at date of reporting. 17% required admission to High Dependency or Intensive Care Units; of these, 31% were discharged alive, 45% died and 24% continued to receive care at the reporting date. Of those receiving mechanical ventilation, 20% were discharged alive, 53% died and 27% remained in hospital. Conclusions: We present the largest detailed description of COVID-19 in Europe, demonstrating the importance of pandemic preparedness and the need to maintain readiness to launch research studies in response to outbreaks. Trial documentation: Available at https://isaric4c.net/protocols .
https://www.medrxiv.org/content/10.1101/2020.04.23.20076042v1

DRUG DEVELOPMENT

- WOW! Here is something I knew nothing about and I do know a little about a lot of different things (one of my alternative mantras)! Novaferon, a novel protein drug approved for the treatment of chronic hepatitis B in China, exhibits potent antiviral activities. We aimed to determine the anti-SARS-CoV-2 effects of Novaferon in vitro, and conducted a randomized, open-label, parallel group study to explore the antiviral effects of Novaferon for COVID-19. Methods In laboratory, the inhibition of Novaferon on viral replication in cells infected with SARS-CoV-2, and on SARS-CoV-2 entry into healthy cells was determined. Antiviral effects of Novaferon were evaluated in COVID-19 patients with treatment of Novaferon, Novaferon plus Lopinavir/Ritonavir, or Lopinavir/Ritonavir. The primary endpoint was the SARS-CoV-2 clearance rates on day 6 of treatment, and the secondary endpoint was the time to the SARS-CoV-2 clearance in COVID-19 patients Results Novaferon inhibited the viral replication in infected cells (EC50=1.02 ng/ml), and protected healthy cells from SARS-CoV-2 infection (EC50=0.1 ng/ml). Results from the 89 enrolled COVID-19 patients showed that both Novaferon and Novaferon plus Lopinavir/Ritonavir groups had significantly higher SARS-CoV-2 clearance rates on day 6 than the Lopinavir/Ritonavir group (50.0% vs.24.1%, p = 0.0400, and 60.0% vs.24.1%, p = 0.0053). Median time to SARS-CoV-2 clearance were 6 days, 6 days, and 9 days for three groups respectively, suggesting a 3-dayreduction of time to SARS-CoV-2 clearance in both Novaferon and Novaferon plus Lopinavir/Ritonavir groups compared with Lopinavir/Ritonavir group. Conclusions Novaferon exhibited anti-SARS-CoV-2 effects in vitro and in COVID-19 patients. These data justified the further evaluation of Novaferon. [**note: this is apparently an interferon variant created through gene shuffling. The patent reference is HERE. It is assigned to a patent holding company headquartered in the Cayman Islands. I have no idea whether the drug is approved outside of China**.]
  https://www.medrxiv.org/content/10.1101/2020.04.24.20077735v1
- More interesting work on heparin from an Italo/British group. The dependence of the host on the interaction of hundreds of extracellular proteins with the cell surface glycosaminoglycan heparan sulphate (HS) for the regulation of homeostasis is exploited by many microbial pathogens as a means of adherence and invasion. The closely related polysaccharide heparin, the widely used anticoagulant drug, which is structurally similar to HS and is a common experimental proxy, can be expected to mimic the properties of HS. Heparin prevents infection by a range of viruses if added exogenously, including S-associated coronavirus strain HSR1 and here, we show that the addition of heparin (100 μg.ml-1) to Vero cells inhibits invasion by SARS-CoV-2 by 70%. We also demonstrate that heparin binds to the Spike (S1) protein receptor binding domain and induces a conformational change, illustrated by surface plasmon resonance and circular dichroism spectroscopy studies. The structural features of heparin on which this interaction depends were investigated using a library of heparin derivatives and size-defined fragments. Binding is more strongly dependent on the presence of 2-O or 6-O sulphation, and the consequent conformational consequences in the heparin structure, than on N-sulphation. A hexasaccharide is required for conformational changes to be induced in the secondary structure that are comparable to those that arise from heparin binding. Enoxaparin, a low molecular weight clinical anticoagulant, also binds the S1 RBD protein and induces conformational change.

These findings have implications for the rapid development of a first-line therapeutic by repurposing heparin as well as for next-generation, tailor-made, GAG-based antiviral agents against SARS-CoV-2 and other members of the Coronaviridae. [**I wonder if muco-polysaccharides have any binding affinity to SARS-CoV-2. For those of us with persistent nasal symptoms, could this be protective? I'm happy to donate a sample or two for *in vitro* testing (of course this will be after I file a patent application for a novel SARS-CoV-2 treatment!**] https://www.biorxiv.org/content/10.1101/2020.04.28.066761v1

- **Cautionary Note Time:** It is essential to investigate the clinical characteristics of COVID-19 and uncover potential risk factors for severe disease to reduce the overall mortality rate of COVID-19. Methods Sixty-one critical COVID-19 patients admitted to the intensive care unit (ICU) and 93 severe non-ICU patients at Huoshenshan Hospital (Wuhan, China) were included in this study. Medical records, including demographic, platelet counts, heparin-involved treatments, heparin-induced thrombocytopenia-(HIT) related laboratory tests, and fatal outcomes of COVID-19 patients were analyzed and compared between survivors and nonsurvivors. Findings Sixty-one critical COVID-19 patients treated in ICU included 15 survivors and 46 nonsurvivors. Forty-one percent of them (25/61) had severe thrombocytopenia, with a platelet count (PLT) less than 50x109/L, of whom 76% (19/25) had a platelet decrease of >50% compared to baseline; 96% of these patients (24/25) had a fatal outcome. Among the 46 nonsurvivors, 52.2% (24/46) had severe thrombocytopenia, compared to 6.7% (1/15) among survivors. Moreover, continuous renal replacement therapy (CRRT) could induce a significant decrease in PLT in 81.3% of critical CRRT patients (13/16), resulting in a fatal outcome. In addition, a high level of anti-heparin-PF4 antibodies, a marker of HIT, was observed in most ICU patients. Surprisingly, HIT occurred not only in patients with heparin exposure, such as CRRT, but also in heparin-naive patients, suggesting that spontaneous HIT may occur in COVID-19. Interpretation Anti-heparin-PF4 antibodies are induced in critical COVID-19 patients, resulting in a progressive platelet decrease. *Exposure to a high dose of heparin may trigger further severe thrombocytopenia with a fatal outcome. An alternative anticoagulant other than heparin should be used to treat COVID-19 patients in critical condition*. [**note: further proof that there is too much confounding information about the severe illness cohort.**] https://www.medrxiv.org/content/10.1101/2020.04.23.20076851v1
- Chloroquine/hydroxychloroquine has recently been the subject of intense debate in regard to its potential antiviral activity against SARS-Cov-2, the etiological agent of COVID-19. Some report possible curative effects, others do not. In order to shed some light on this rather controversial topic, we used mathematical modelling to simulate possible scenarios of response to hydroxychloroquine in COVID-19 patients. Our computer-aided simulations show that hydroxychloroquine may have an impact on the amplitude of the viral load peak but that viral clearance is not significantly accelerated if the drug is not administered early enough (i.e. when viral loads range from 1 to 1,000 copies/mL). Although some authors had used the trough plasma concentrations or the theoretical drug distribution in the lung to model the effect of chloroquine/hydroxychloroquine on COVID-19, the theoretical drug response based on the trough whole blood concentrations of the drug agreed well with the results of the clinical trials so far reported. Moreover, the effects of chloroquine/hydroxychloroquine could be fully explained when taking into account also the capacity of this drug to raise cell-mediated responses against the productively SARS-Cov-2-infected cells. On the whole, the present study

suggests that chloroquine/hydroxychloroquine has a narrow therapeutic window, which overlaps with the highest tolerated doses. These considerations may have implications for development of anti-COVID-19 combination therapies and prevention strategies. [**note: I find some of this information confusing in light of the long serum half-life of hydroxychloroquine. Perhaps it is a tissue distribution issue and once it is bound to something in the serum it is no longer available. I really would like to understand this better than I do.**]
https://www.medrxiv.org/content/10.1101/2020.04.23.20076471v1

DIAGNOSTIC DEVELOPMENT

- Here is a very thorough Dutch paper comparing several ELISA and rapid lateral flow diagnostic test kits. The world is entering a new era of the COVID-19 pandemic in which there is an increasing call for reliable antibody testing. To support decision making on the deployment of serology for either population screening or diagnostics, we present a comprehensive comparison of serological COVID-19 assays. We show that the assay detecting total immunoglobulins against the receptor binding domain of SARS CoV-2, had optimal characteristics for antibody detection in different stages of disease. [**note: they don't say so in the abstract but the quick lateral flow tests were not robust enough for anything other than rough population antibody screening.**]
https://www.medrxiv.org/content/10.1101/2020.04.23.20077156v1
- Here is a new antibody test approach that 'might' substitute for RT-PCR testing of nasal swabs. COVID-19 Ag Respi-Strip, an immunochromatographic (ICT) assay for the rapid detection of SARS-CoV-2 antigen on nasopharyngeal specimen, has been developed to identify positive COVID-19 patients allowing prompt clinical and quarantine decisions. In this Original Research article, we describe the conception, the analytical and clinical performances as well as the risk management of implementing the COVID-19 Ag Respi-Strip in a diagnostic decision algorithm. Materials and Methods: Development of the COVID-19 Ag Respi-Strip resulted in a ready-to-use ICT assay based on a membrane technology with colloidal gold nanoparticles using monoclonal antibodies directed against the SARS-CoV and SARS-CoV-2 highly conserved nucleoprotein antigen. Four hundred observations were recorded for the analytical performance study and thirty tests were analysed for the cross-reactivity study. The clinical performance study was performed in a retrospective multi-centric evaluation on aliquots of 328 nasopharyngeal samples. COVID-19 Ag Respi-Strip results were compared with qRT-PCR as golden standard for COVID-19 diagnostics. Results: In the analytical performance study, the reproducibility showed a between-observer disagreement of 1.7%, a robustness of 98%, an overall satisfying user friendliness and no cross-reactivity with other virus-infected nasopharyngeal samples. In the clinical performance study performed in three different clinical laboratories we found an overall sensitivity and specificity of 57.6% and 99.5% respectively with an accuracy of 82.6%. The cut-off of the assay was found at Ct<22. User-friendliness analysis and risk management assessment through Ishikawa diagram demonstrate that COVID-19 Ag Respi-Strip may be implemented in clinical laboratories according to biosafety recommendations. Conclusion: The COVID-19 Ag Respi-Strip represents a promising rapid SARS-CoV-2 antigen assay for the first-line diagnosis of COVID-19 in 15 minutes. Its role in the proposed diagnostic algorithm is complementary to the

currently-used molecular techniques.
https://www.medrxiv.org/content/10.1101/2020.04.24.20077776v1

- Here is a large UCSF group who looked at a number of different serological tests, both ELISA and Lateral Flow. We conducted an evaluation of 10 lateral flow assays (LFAs) and two ELISAs to detect anti-SARS-CoV-2 antibodies. The specimen set comprised 130 plasma or serum samples from 80 symptomatic SARS-CoV-2 RT-PCR-positive individuals; 108 pre-COVID-19 negative controls; and 52 recent samples from individuals who underwent respiratory viral testing but were not diagnosed with Coronavirus Disease 2019 (COVID-19). Samples were blinded and LFA results were interpreted by two independent readers, using a standardized intensity scoring system. Results: Among specimens from SARS-CoV-2 RT-PCR-positive individuals, the percent seropositive increased with time interval, peaking at 81.8-100.0% in samples taken >20 days after symptom onset. Test specificity ranged from 84.3-100.0% in pre-COVID-19 specimens. Specificity was higher when weak LFA bands were considered negative, but this decreased sensitivity. IgM detection was more variable than IgG, and detection was highest when IgM and IgG results were combined. Agreement between ELISAs and LFAs ranged from 75.8-94.8%. No consistent cross-reactivity was observed. Conclusion: Our evaluation showed heterogeneous assay performance. Reader training is key to reliable LFA performance, and can be tailored for survey goals. Informed use of serology will require evaluations covering the full spectrum of SARS-CoV-2 infections, from asymptomatic and mild infection to severe disease, and later convalescence. Well-designed studies to elucidate the mechanisms and serological correlates of protective immunity will be crucial to guide rational clinical and public health policies. [**note: very solid research here**] https://www.medrxiv.org/content/10.1101/2020.04.25.20074856v1
- Good work from Indian researchers who want to figure out ways to extend testing while minimizing reagent use. There is an urgent need to find economical and scalable ways to test more people. We present Tapestry, a novel quantitative nonadaptive pooling scheme to test many samples using only a few tests. The underlying molecular diagnostic test is any real-time RT-PCR diagnostic panel approved for the detection of the SARS-CoV-2 virus. In cases where most samples are negative for the virus, Tapestry accurately identifies the status of each individual sample with a single round of testing in fewer tests than simple two-round pooling. We also present a companion Android application BYOM Smart Testing which guides users through the pipetting steps required to perform the combinatorial pooling. The results of the pooled tests can be fed into the application to recover the status and estimated viral load for each individual sample. https://www.medrxiv.org/content/10.1101/2020.04.23.20077727v1

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2020-04-30

How have I ignored Chuck Berry??? I need to correct this STAT! Here is Chuck, live at the Roxy, singing Memphis: https://www.youtube.com/watch?v=B7fyNnoZ7IA It's an understated performance compared to those of his younger days but arresting nonetheless. **Contest Alert: first person to correctly provide the answer as to the classical composer featured in one of Chuck's greatest hits will win a nice prize! Hint: the song was covered by a number of well-known bands including Paul Shaffer and The World's Most Dangerous Band (this should really help you solve this one).**

I need to give a big shout out to Tyler Cowan over at George Mason for getting a COVID-19 fast grant process off the ground in what must be record time. The Washington Post has a nice article on the project and it's good to see some big donors chip in the money to fund projects that are going to make a difference.

Ed Jong of The Atlantic is one fine science writer. Here he is discussing why the Coronavirus is so Confusing. Well worth reading!

And a good article from The New Yorker discusses many of the clinical mysteries of SARS-CoV-2 and also on article on the what the virus reveals about American medicine (hint: it is not flattering). Money quote from the 2nd article, "*Finally, we need to acknowledge that our E.M.R. systems are worse than an infuriating time sink; in times of crisis, they actively obstruct patient care. We should reimagine the continuous medical record as its founders first envisaged it: as an open, searchable library of a patient's medical life. Think of it as a kind of intranet: flexible, programmable, easy to use. Right now, its potential as a resource is blocked, not least by the owners of the proprietary software, who maintain it as a closed system, and by complex rules and regulations designed to protect patient privacy. It should be a simple task to encrypt or remove a patient's identifying details while enlisting his or her medical information for the common good. A storm-forecasting system that warns us* after *the storm has passed is useless. What we want is an E.M.R. system that's versatile enough to serve as a tool for everyday use but also as a research application during a crisis, identifying techniques that improve medical outcomes, and disseminating that information to physicians across the country in real time*." OMOP and OHDSI have had to struggle with this for a long time as the system is first and foremost about billing.

Here is the 21st century way of doing public health tracking for this virus. It can maintain weeks of contact events and has appropriate encryption. One of the project leaders was on the team that wrote the popular RSA encryption algorithm. Good outside the box thinking!!!

There was a press announcement yesterday that remdesivir was successful in the first controlled trial run by NIAID. Hospital stays were shortened by four days. There is no conclusive data whether the drug affects progression of illness or mortality. Also problematic are kidney and blood pressure side effects from drug administration as these are also observed in viral illness. I don't know when the study will be published and all we have to go by are the press reports.

MODELING

- This Irish group conducted a literature search to infer the duration of infectious period. Our objective was to review the literature on the inferred duration of the infectious period of COVID-19, caused by SARS-COV-2 virus, and provide an overview of the variation depending on the methodological approach. Design: Rapid scoping review. Literature review with fixed search terms, up to 1st April 2020. Central tendency and variation of the parameter estimates for infectious period in (a) asymptomatic (b) symptomatic cases from (i) virological studies (repeated testing), (ii) tracing studies (iii) modelling studies were gathered. Narrative review of viral dynamics. Information sources: Search strategies developed and the following searched: PubMed, Google Scholar, MedRxiv, BioRxiv. Additionally, the Health Information Quality

Authority (Ireland) viral load synthesis was utilised, which screened literature from PubMed, Embase, ScienceDirect, NHS evidence, Cochrane, medRxiv and bioRxiv, HRB open databases. Results: There was substantial variation in the estimates, and how infectious period was inferred. One study provided approximate median infectious period for asymptomatic cases of 6.5-9.5 days. Median pre-symptomatic infectious period across studies varied over <1-4 days. Estimated mean time from symptom onset to two negative RT-PCR tests was 13.4 days (95%CI: 10.9-15.8), but was shorter when studies included children or less severe cases. Estimated mean duration from symptom onset to hospital discharge or death (potential maximal infectious period) was 18.1 days (95%CI: 15.1-21.0); time to discharge was on average 4 days shorter than time-to-death. Viral dynamic data and model infectious parameters were often shorter than repeated diagnostic data. Conclusions: There are limitations of inferring infectiousness from repeated diagnosis, viral loads, and viral replication data alone, and also potential patient recall bias relevant to estimating exposure and symptom onset times. Despite this, available data provides a preliminary evidence base to inform models of central tendency for key parameters, and variation for exploring parameter space and sensitivity analysis. Some current models may be underestimating infectious period. [**note: we still need better information here.**]
https://www.medrxiv.org/content/10.1101/2020.04.25.20079889v1

- Molecular epidemiology from Germany. We whole-genome sequenced 55 SARS-CoV-2 isolates from Western Germany and investigated the genetic structure of SARS-CoV-2 outbreaks in the Heinsberg district and Düsseldorf. While the genetic structure of the Heinsberg outbreak indicates a clonal origin, reflective of superspreading dynamics during the carnival season, distinct viral strains are circulating in Düsseldorf, reflecting the city's international links. Limited detection of Heinsberg strains in the Düsseldorf area despite geographical proximity may reflect efficient containment and contact tracing efforts.
https://www.medrxiv.org/content/10.1101/2020.04.25.20079517v1

NEWLY REGISTERED CLINICAL TRIALS

- I believe I posted something about estrogen/progesterone as possible approaches to treatment. Here is the UCLA trial on progesterone. NCT04365127
- Here is a UK trial to see if additional lung surfactant can help lung function. The hypothesis behind the proposed trial of surfactant therapy for COVID-19 infected patients requiring ventilator support is that endogenous surfactant is dysfunctional. This could be due to decreased concentration of surfactant phospholipid and protein, altered surfactant phospholipid composition, surfactant protein proteolysis and/or oedema protein inhibition of surfactant surface tension function and/or oxidative inactivation of surfactant proteins. Variations of these dysfunctional mechanisms have been reported in a range of lung diseases, including cystic fibrosis and severe asthma, and in child and adult patients with ARDS. Our studies of surfactant metabolism in adult ARDS patients showed altered percentage composition of surfactant PC, with decreased DPPC and increased surface tension-inactive unsaturated species, and decreased concentrations of both total PC and phosphatidylglycerol (PG) NCT04362059

- Here is a Spanish trial looking at dexmedetomidine. A continuous infusion of Dexmedetomidine (DEX) will be administered to 80 patients admitted to Critical Care because of signs of Respiratory Insufficiency requiring non-invasive ventilation. Measurements of respiratory performance and quantification of cellular and molecular inflammatory mediators. The primary outcome will be the avoidance of mechanical ventilation with secondary outcomes duration of mechanical ventilation, avoidance of delirium after sedation and association of mediators of inflammation to outcomes. Outcomes will be compared to a matched historical control (no DEX) series NCT04358627
- Systemic medical ozone has proved to help in several viral diseases, chronic obstructive pulmonary disease and chronic inflammation process. The investigators are sure that its application to COVID-19 patients, as an adjuvant therapy, will improve the health status of these individuals. [**note: who knew ozone might have therapeutic effects? I was going to suggest doing a study in Los Angeles, but there are hardly any cars on the road and ozone levels are too low to observe anything.**] NCT04359303

CLINICAL TRIAL RESULTS

- Here is a mainly UK study on the use of a mobile app to come up with key predictors of hospitalization. Community survey Setting: The COVID Symptom Tracker mobile application co-developed by physicians and scientists at Kings College London, Massachusetts General Hospital, Boston and Zoe Global Limited was launched in the UK and US on 24th and 29th March 2020 respectively. It captured self-reported information related to COVID-19 symptoms and testing. Participants: 2,618,948 users of the COVID Symptom Tracker App. UK (95.7%) and US (4.3%) population. Data cut-off for this analysis was 21st April 2020. Main outcome measures: Visit to hospital and for those who attended hospital, the need for respiratory support in three subgroups (i) self-reported COVID-19 infection with classical symptoms (SR-COVID-19), (ii) self-reported positive COVID-19 test results (T-COVID-19), and (iii) imputed/predicted COVID-19 infection based on symptomatology (I-COVID-19). Multivariate logistic regressions for each outcome and each subgroup were adjusted for age and gender, with sensitivity analyses adjusted for comorbidities. Classical symptoms were defined as high fever and persistent cough for several days. Results: Older age and all comorbidities tested were found to be associated with increased odds of requiring hospital care for COVID-19. Obesity (BMI >30) predicted hospital care in all models, with odds ratios (OR) varying from 1.20 [1.11; 1.31] to 1.40 [1.23; 1.60] across population groups. Pre-existing lung disease and diabetes were consistently found to be associated with hospital visit with a maximum OR of 1.79 [1.64,1.95] and 1.72 [1.27; 2.31]) respectively. Findings were similar when assessing the need for respiratory support, for which age and male gender played an additional role. Conclusions: Being older, obese, diabetic or suffering from pre-existing lung, heart or renal disease placed participants at increased risk of visiting hospital with COVID-19. It is of utmost importance for governments and the scientific and medical communities to work together to find evidence-based means of protecting those deemed most vulnerable from COVID-19.
  https://www.medrxiv.org/content/10.1101/2020.04.25.20079251v1
- Here is a small Chinese study on the effect of lopiinivir/ritonavir ± umifnovir (Arbidol). In this retrospective observational study, COVID-19 hospitalized patients were identified and divided

into two groups based on the antiviral agents used during their hospitalization. Group-LR patients were treated with single antiviral drug of lopinavir-ritonavir. Group-LR+Ar patients were treated with lopinavir-ritonavir combined with arbidol for antiviral therapy at least 3 days. Patients were assessed for different clinical outcomes. Results: A total of 34 and 39 patients were identified for Group-LR and Group-LR+Ar, respectively. Treatment with lopinavir-ritonavir alone was not difference from lopinavir-ritonavir combined with arbidol in overall cure rate of COVID-19 hospitalized patients (92.3% and 97.1%, respectively). In a modified intention-to-treat analysis, lopinavir-ritonavir combined with abidol led to a median time of hospital stay that was shorter by 1.5 days than group-LR (12.5 days vs. 14 days). The percentages of COVID-19 RNA clearance was 92.3 in group-LR and 97.1 in group-LR+Ar. The mean time of virus turning negative was 11.5 plus-or-minus sign 9.0 days in group-LR+Ar that were longer than group-LR. Treatment of lopinavir-ritonavir combined with arbidol did not significantly accelerate main symptoms improvement and promote the image absorption of pulmonary inflammation. Conclusion: No benefit was observed in the anti-virus effect of lopinavir-ritonavir combined with arbidol compared with lopinavir-ritonavir alone in the hospitalized patients with COVID-19. More clinical observations in COVID-19 patients may help to confirm or exclude the effect of antiviral agents. https://www.medrxiv.org/content/10.1101/2020.04.25.20079079v1

- I think this is the first study from Vietnam to make the newsletter! We conducted a prospective study at a quarantine centre for COVID-19 in Ho Chi Minh City, Vietnam. We enrolled quarantined people with RT-PCR-confirmed SARS-CoV-2 infection, collecting clinical data, travel and contact history, and saliva at enrolment and daily nasopharyngeal throat swabs (NTS) for RT-PCR testing. We compared the natural history and transmission potential of asymptomatic and symptomatic individuals. Results: Between March 10th and April 4th, 2020, 14,000 quarantined people were tested for SARS-CoV-2; 49 were positive. Of these, 30 participated in the study: 13(43%) never had symptoms and 17(57%) were symptomatic. 17(57%) participants acquired their infection outside Vietnam. Compared with symptomatic individuals, asymptomatic people were less likely to have detectable SARS-CoV-2 in NTS samples collected at enrolment (8/13 (62%) vs. 17/17 (100%) P=0.02). SARS-CoV-2 RNA was detected in 20/27 (74%) available saliva; 7/11 (64%) in the asymptomatic and 13/16 (81%) in the symptomatic group (P=0.56). Analysis of the probability of RT-PCR positivity showed asymptomatic participants had faster viral clearance than symptomatic participants (P<0.001 for difference over first 19 days). This difference was most pronounced during the first week of follow-up. Two of the asymptomatic individuals appeared to transmit the infection to up to four contacts. Conclusions: Asymptomatic SARS-CoV-2 infection is common and can be detected by analysis of saliva or NTS. NTS viral loads fall faster in asymptomatic individuals, but they appear able to transmit the virus to others. [**note: I don't know whether anyone is doing a comprehensive look at viral clearance in asymptomatic vs. symptomatic patients.**] https://www.medrxiv.org/content/10.1101/2020.04.27.20082347v1
- Although generally safe and well tolerated they are potentially lethal in overdose. These two drugs are now candidates for the prevention and treatment of COVID19. In vitro data suggest that high concentrations and thus high doses will be needed if they are to be of benefit, but as yet there is no convincing evidence they are clinically effective. Nevertheless they are already being used very widely and fatal accidental overdoses have been reported. Methods: Individual data from prospectively studied French patients who had taken intentional chloroquine

overdoses and were managed in the national toxicology intensive care unit in Paris were pooled. Bayesian logistic regression was used to estimate a concentration-fatality curve. The probabilities of fatal iatrogenic toxicity with the chloroquine regimens currently being trialled for the treatment of COVID19 were estimated from a combined pharmacokinetic-pharmacodynamic model. Findings: In total, 258 patients were studied of whom 26 died (10%). There was a steep sigmoid relationship between admission whole blood chloroquine concentrations and death. Concentrations above 13umol/L (95% credible interval (C.I.), 10 to 16) were associated with greater than 1% mortality. Based on peak concentrations, absolute fatality ratios in the high dose arm (chloroquine base equivalent adult dose of 600mg given twice daily for ten days) of a recently terminated trial were estimated between 0.06% (90kg adult, 95%C.I. 0 to 0.3%) and 4.8% (40kg adult, 95% C.I. 1.9 to 9.7%). This regimen results in peak concentrations above 10umol/L in more than 60% of adults weighing 70kg. The other high dose regimens trialled currently for COVID19 result in peak concentrations above 10umol/L in only 0.2% of adults weighting 70kg. Interpretation:} High-dose chloroquine treatment regimens which result in whole blood chloroquine concentrations below 10umol/L for the majority of patients should not result in life-threatening cardiovascular toxicity. [**note: no big surprise. High doses of chloroquine may be problematic.**]
https://www.medrxiv.org/content/10.1101/2020.04.24.20078303v1

DRUG DEVELOPMENT

- It has not been registered as a trial in the US but Pfizer and German firm, BioNTech, are in trials with their mRNA SARS-CoV-2 vaccine. This is the same technology that Moderna are using. In an interview, Pfizer indicated they could have millions of doses ready by this fall if testing is accelerated. A US trial may begin next week.
- I'm pretty sure I already referenced the preprint of this work. Here is the citation to the published paper showing β-D-N4-hydroxycytidine-5' -isopropyl ester is a potent inhibitor of SARS-CoV-2 at a reasonable concentration.
https://stm.sciencemag.org/content/scitransmed/early/2020/04/03/scitranslmed.abb5883.full.pdf
- Some good work from Singapore on the enrichment of SARS-CoV-2 specific T cells. The feasibility of rapid clinical-grade manufacturing of virus-specific T cells from convalescent donors has not been demonstrated for this or prior pandemics. Methods One unit of whole blood was collected from each convalescent donor following standard blood bank practices. After the plasma was separated and stored separately, the leukocytes were stimulated using overlapping peptides of SARS-CoV-2, covering the immunodominant sequence domains of the S protein and the complete sequence of the N and M proteins. Thereafter, functionally reactive cells were enriched overnight using an automated device capturing IFNγ-secreting cells. Findings From 1x10[9] leukocytes, 0.56 to 1.16x10[6] IFNγ+ T cells were produced from each of the first two donors. Most of the T cells (64% to 71%) were IFNγ+, with preferential enrichment of CD56+ T cells, effector memory T cells, and effector memory RA+ T cells. TCRVβ spectratyping revealed oligoclonal distribution, with over-representation of subfamilies including Vβ3, Vβ16 and Vβ17. With just two donors, the probability that a recipient in the same ethnic group would share at least one donor HLA allele or one haplotype could be as high as >90% and >30%, respectively.

Interpretations This study is limited by small number of donors and absence of recipient data; however, crucial first proof-of-principle data are provided demonstrating the feasibility of clinical-grade production of SARS-CoV-2 specific T cells for urgent clinical use, conceivably with plasma therapy concurrently. Our data showing that virus-specific T cells can be detected easily after brief stimulation with SARS-CoV-2 specific peptides suggest that a parallel diagnostic assay can be developed alongside serology testing.
https://www.medrxiv.org/content/10.1101/2020.04.24.20077487v1

- This is pretty cool technology. Like other coronaviruses, this novel virus relies on the surface Spike glycoprotein to access the host cells, mainly through the interaction of its Receptor Binding Domain (RBD) with the human angiotensin-converting enzyme 2 (ACE2). Therefore, molecular entities able to interfere with binding of the SARS-CoV-2 Spike protein to ACE2 have a great potential to inhibit viral entry. Starting from the available structural data on the interaction between SARS-CoV-2 Spike protein and the host ACE2 receptor, we here engineered a mini-protein with the aim of creating a soluble and stable Spike interactor. This mini-protein, which was recombinantly produced in high yields, possesses a stable α helical conformation and is able to interact with the RBD of glycosylated Spike protein from SARS-CoV-2 with nanomolar affinity, as measured by microscale thermophoresis. By plugging the Spike protein, our mini-protein constitutes a valid tool for the development of treatments against different types of coronavirus. https://www.biorxiv.org/content/10.1101/2020.04.29.067728v1
- The dependence of the host on the interaction of hundreds of extracellular proteins with the cell surface glycosaminoglycan heparan sulphate (HS) for the regulation of homeostasis is exploited by many microbial pathogens as a means of adherence and invasion. The closely related polysaccharide heparin, the widely used anticoagulant drug, which is structurally similar to HS and is a common experimental proxy, can be expected to mimic the properties of HS. Heparin prevents infection by a range of viruses when added exogenously, including S-associated coronavirus strain HSR1 and inhibits cellular invasion by SARS-CoV-2. We have previously demonstrated that unfractionated heparin binds to the Spike (S1) protein receptor binding domain, induces a conformational change and have reported the structural features of heparin on which this interaction depends. Furthermore, we have demonstrated that enoxaparin, a low molecular weight clinical anticoagulant, also binds the S1 RBD protein and induces conformational change. Here we expand upon these studies, to a wide range of low molecular weight heparins and demonstrate that they induce a variety of conformational changes in the SARS-CoV-2 RBD. These findings may have further implications for the rapid development of a first-line therapeutic by repurposing low molecular weight heparins, as well as for next-generation, tailor-made, GAG-based antiviral agents, against SARS-CoV-2 and other members of the Coronaviridae. [**note: blocking binding is always a good approach; whether it works *in vivo* is the big question.**]
https://www.biorxiv.org/content/10.1101/2020.04.29.068486v1
- Here is an *in vitro* study of FDA-approved antivirals. The global pandemic of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 or 2019-nCoV) has prompted multiple clinical trials to jumpstart search for anti-SARS-CoV-2 therapies from existing drugs, including those with reported in vitro efficacies as well as those ones that are not known to inhibit SARS-CoV-2, such a Ritonavir/lopinavir and Favilavir. Here we report that after screening 19 antiviral drugs that are either in clinical trials or with proposed activity against SARS-CoV-2, remdesivir was the most

effective. Chloroquine only effectively protected virus-induced cytopathic effect at around 30 micromolar with a therapeutic index of 1.5. Our findings also show that velpatasvir, ledipasvir, litonavir, lopinavir, favilavir, sofosbuvir do not have direct antiviral effect. [**note: curiously, they did not test hydroxychloroquine in this study**]
https://www.biorxiv.org/content/10.1101/2020.04.29.067983v1

DIAGNOSTIC DEVELOPMENT

- A first from Ecuador!! Nasopharyngeal sampling protocols for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnosis guidelines only recommend synthetic fiber swabs. We show that simple and cheap cotton tipped plastic swabs do not inhibit PCR and have equivalent performance to rayon swabs. Cotton tipped plastic swabs are massively produced worldwide and would prevent swabs supplies shortage during current high SARS-CoV-2 testing demands, particularly on developing countries.
https://www.medrxiv.org/content/10.1101/2020.04.28.20079947v1

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2020-05-01

Happy May Day!!!

Let's move on to one of the great instrumentalists of our time. Yes, dear readers, it is time to listen to Ravi Shankar live from the 1967 Monterrey Pop festival (and before you ask, I was not there, nor was I at Woodstock either; in those days jazz was my thing): https://www.youtube.com/watch?v=lk60ObnbIOk Speaking of Monterrey, it is a shame that I cannot find a video anywhere of John Handy's 1965 Jazz Festival performance. I wore out my LP playing 'Spanish Lady' so much. I you are interested, the full recording is on YouTube and is one of the great jazz festival performances IMO.

We had a winner of yesterday's contest!!! The correct answer was Ludwig von Beethoven and the Chuck Berry song was 'Roll over Beethoven.' While Paul Shaffer's band did cover this song, The Beatles version is the most famous.

Derek Lowe weighs in on the remdesivir announcement. Clearly, we will need more clinical trial data to see whether this drug fulfills its promise, particularly in terms of reducing mortality.

CDC announced a national initiative to speed research into how the coronavirus was spreading around the country. New York Times story and CDC weblink. The calendar turned to May and this is just happening now????

Here is an interesting study of how a UK artificial intelligence company identified baricitinib as a potential SARS=CoV-2 treatment. Unlike a lot of other AI efforts I have read, this compound is in clinical trials right now!

While lots of new trials are announced every day, there is a lack of new compounds being tested.

MODELING

- This is a useful study *IF* a quick sensitive and specific serology test is available. In this paper, we examine how serological testing can reduce the risk of relaxing social distancing measures while also providing a way for test-positive individuals to return to more normal levels of activity. Methods: We use an SEIR-like compartmental model that accounts for serological test status to examine if widespread serological testing can reduce the adverse effects of relaxing social distancing measures, in terms of total deaths and health system burden. In our model, social distancing measures are relaxed to a greater extent for those who test positive compared to those who have not been tested or test negative, allowing a return to work and partial restoration of other social contacts to pre-pandemic levels. All in- dividuals preferentially interact with those who have tested positive, such that seropositive individuals act as immunological shields. We consider a range of potential testing capacities and the implications of an imperfect test for this strategy. Results: Although relaxing social distancing interventions increases total deaths, serologic testing as a part of this strategy can reduce population risk. If social distancing restrictions are relaxed by 50% in tandem with monthly serological testing of the general United States (US) population, 174,000 deaths would be averted and 67% of the US population would be released from social distancing after 1 year, as compared to a scenario without serological testing. Sustaining moderate levels of social distancing can help to flatten the epidemic curve, reducing health system burden below the US critical care capacity. Implications: Modeling studies suggest that serological testing can be used to relax social distancing measures preferentially for seropositive individuals, insofar as antibodies can be established as a correlate of protection against SARS-CoV-2 infection. Implementing a strategy of serological testing and shielding can reduce population risk while offsetting the severe social and economic costs of a sustained shutdown.
  https://www.medrxiv.org/content/10.1101/2020.04.24.20078576v1
- Paul Romer, whom I have referenced before, and colleagues weigh in on population scale testing. We propose an additional intervention that would contribute to the control of the COVID-19 pandemic, offer more protection for people working in essential jobs, and help guide an eventual reopening of society. The intervention is based on: (1) testing every individual (2) repeatedly, and (3) self-quarantine of infected individuals. Using a standard epidemiological model (SIR), we show here that by identification and isolation of the majority of infectious individuals, including those who may be asymptomatic, the reproduction number R0 of SARS-CoV-2 would be reduced well below 1.0, and the epidemic would collapse. We replicate these observations in a more complex stochastic dynamic model on a social network graph. We also find that the testing regime would be additive to other interventions, and be effective at any level of prevalence. If adopted as a policy, any industrial society could sustain the regime for as long as it takes to find a safe and effective cure or vaccine. Our model also indicates that unlike sampling-based tests, population-scale testing does not need to be very accurate: false negative rates up to 15% could be tolerated if 80% comply with testing every ten days, and false positives can be almost arbitrarily high when a high fraction of the population is already effectively quarantined. Testing at the required scale would be feasible if existing qPCR-based methods are scaled up and multiplexed. A mass produced, low throughput field test kit could also be carried out at home. Economic analysis also supports the feasibility of the approach: current reagent costs for tests are in the range of a dollar or less, and the estimated benefits for population-

scale testing are so large that the policy would be cost-effective even if the costs were larger by more than two orders of magnitude. To identify both active and previous infections, both viral RNA and antibodies could be tested. All technologies to build such test kits, and to produce them in the scale required to test the entire world's population exist already. Integrating them, scaling up production, and implementing the testing regime will require resources and planning, but at a scale that is very small compared to the effort that every nation would devote to defending itself against a more traditional foe.
https://www.medrxiv.org/content/10.1101/2020.04.27.20078329v1

- The experience in Italy argues for extensive testing. We examined data on the progression of COVID-19 epidemics in four regions in northern Italy. Lombardy, Emilia-Romagna, and Piedmont had an extremely steeper increase in mortality with increasing number of tests performed than Veneto, which applied a policy of broader swab testing. This suggests that the strategy adopted in Veneto, similar to that in South Korea, is effective in containing COVID-19 epidemics and should be applied in other regions of Italy and countries in Europe.
https://www.medrxiv.org/content/10.1101/2020.04.24.20078709v1
- Analysis of the Australian outbreak shows they did a lot of things right. As of 18 April 2020, there had been 6,533 confirmed cases of COVID-19 in Australia. Of these, 67 had died from the disease. The daily count of new confirmed cases was declining. This suggests that the collective actions of the Australian public and government authorities in response to COVID-19 were sufficiently early and assiduous to avert a public health crisis - for now. Analysing factors, such as the intensity and timing public health interventions, that contribute to individual country experiences of COVID-19 will assist in the next stage of response planning globally. Using data from the Australian national COVID-19 database, we describe how the epidemic and public health response unfolded in Australia up to 13 April 2020. We estimate that the effective reproduction number was likely below 1 (the threshold value for control) in each Australian state since mid-March and forecast that hospital ward and intensive care unit occupancy will remain below capacity thresholds over the next two weeks.
https://www.medrxiv.org/content/10.1101/2020.04.25.20080127v1

NEWLY REGISTERED CLINICAL TRIALS

- The overall objective is to evaluate the efficacy, tolerability, and safety of a single dose of RBT-9 (Stannous protoporphyrin) versus placebo in preventing the progression of coronavirus disease 2019 (COVID-19) infection in non-critically ill adults who are at high risk due to age or comorbid conditions. [**note: I have no clue what the rationale is for this trial. Information on the compound is rather sparse.**] NCT04364763

CLINICAL TRIAL RESULTS

- From Germany, interesting conjecture about the role autoimmunity may play in the lung. Since we observed clinical and histopathological similarities between COVID-19 and lung manifestations of connective tissue disease (CTD-ILD) in our clinical practice, aim of the present study is to analyze a possible role of autoimmunity in SARS-CoV-2-associated respiratory failure.

Methods: In this prospective, single-center trial, we enrolled 22 consecutive patients with RT-PCR-confirmed SARS-CoV-2 infection hospitalized in March and April, 2020. We performed high-resolution computed tomography (HR-CT) and full laboratory testing including autoantibody (AAB) screening (anti-ANA, SS-B/La, Scl-70, Jo-1, CENP-B, PM-Scl). Transbronchial biopsies as well as post mortem tissue samples were obtained from 3 and 2 cases, respectively, and subsequent histopathologic analysis with special emphasis on characterization of interstitial lung disease was performed. Results: Twelve of 22 patients (54.5%) were male and median age was 69.0 (range: 28-88). 11 (50.0%) patients had to be undergo intensive care unit (ICU) treatment. Intubation with ventilation was required in 10/22 cases (46%). Median follow-up was 26 days. Clinical and serological parameters were comparable to previous reports. Radiological and histopathological findings were highly heterogeneous including patterns reminiscent of CTD-ILD. AAB titers ≥1:100 were detected in 10/11 (91.9%) COVID-19 patients who required ICU treatment, but in 4/11 (36.4%) patients with mild clinical course (p=0.024). Patients with AABs tended to require invasive ventilation and showed significantly more severe complications (64.3% vs. 12.5%, p=0.031). Overall COVID-19-related mortality was 18.2% among hospitalized patients at our institution. Conclusion: Our findings point out serological, radiological and histomorphological similarities between COVID-19-associated ARDS and acute exacerbation of CTD-ILD. While the exact mechanism is still unknown, we postulate that SARS-CoV-2 infection might trigger or simulate a form of organ-specific autoimmunity in predisposed patients. The detection of autoantibodies might identify patients who profit from immunosuppressive therapy to prevent the development of respiratory failure.
https://www.medrxiv.org/content/10.1101/2020.04.27.20077180v1

DRUG DEVELOPMENT

- This is an interesting biochemical finding. Molecular mimicry of host proteins is an evolutionary strategy adopted by viruses to evade immune surveillance and exploit host cell systems. We report that SARS-CoV-2 has evolved a unique S1/S2 cleavage site (RRARSVAS), absent in any previous coronavirus sequenced, that results in mimicry of an identical FURIN-cleavable peptide on the human epithelial sodium channel α-subunit (ENaC-α). Genetic truncation at this ENaC-α cleavage site causes aldosterone dysregulation in patients, highlighting the functional importance of the mimicked SARS-CoV-2 peptide. Single cell RNA-seq from 65 studies shows significant overlap between the expression of ENaC-α and ACE2, the putative receptor for the virus, in cell types linked to the cardiovascular-renal-pulmonary pathophysiology of COVID-19. Triangulating this cellular fingerprint with amino acid cleavage signatures of 178 human proteases shows the potential for tissue-specific proteolytic degeneracy wired into the SARS-CoV-2 lifecycle. We extrapolate that the evolution of SARS-CoV-2 into a global coronavirus pandemic may be in part due to its targeted mimicry of human ENaC and hijack of the associated host proteolytic network.
https://www.biorxiv.org/content/10.1101/2020.04.29.069476v1
- Many scientific questions about SARS-CoV-2 and COVID-19 were raised and urgently need to be answered, including the susceptibility of animals to SARS-CoV-2 infection. Here we tested whether tree shrew, an emerging experimental animal domesticated from wild animal, is susceptible to SARS-CoV-2 infection. No clinical signs were observed in SARS-CoV-2 inoculated

tree shrews during this experiment except the increasing body temperature (above 39 ℃) particular in female animals during infection. Low levels of virus shedding and replication in tissues occurred in all three age groups, each of which showed his own characteristics. Histopathological examine revealed that pulmonary abnormalities were mild but the main changes although slight lesions were also observed in other tissues. In summary, tree shrew is not susceptible to SARS-CoV-2 infection and may not be a suitable animal for COVID-19 related researches. [**note: I had no idea that there was an animal called the tree shrew. PETA will be relieved that it won't be raised for drug studies.**] https://www.biorxiv.org/content/10.1101/2020.04.30.029736v1

- Good stuff on chloroquine from Liverpool. Chloroquine has attracted intense attention as a potential clinical candidate for prevention and treatment of COVID-19 based on reports of in-vitro efficacy against SARS-CoV-2. While the pharmacokinetic-pharmacodynamic (PK-PD) relationship of chloroquine is well established for malaria, there is sparse information regarding its dose-effect relationship in the context of COVID-19. Here, we explore the PK-PD relationship of chloroquine for COVID-19 by modelling both achievable systemic and pulmonary drug concentrations. Our data indicate that the standard anti-malarial treatment dose of 25mg/kg over three days does not deliver sufficient systemic drug exposures for the inhibition of viral replication. In contrast, PK predictions of chloroquine in the lungs using in-vivo data or human physiologically-based PK models, suggest that doses as low as 3mg/kg/day for 3 days could deliver exposures that are significantly higher than reported antiviral-EC90s for up to a week. Moreover, if pulmonary exposure is a driver for prevention, simulations show that chronic daily dosing of chloroquine may be unnecessary for prophylaxis purposes. Instead, once weekly doses of 5mg/kg would be sufficient to achieve a continuous cover of therapeutically active pulmonary exposures. These findings reveal a highly compartmentalised distribution of chloroquine in man that may significantly affect its therapeutic potential against COVID-19. The systemic circulation is shown as one site where chloroquine exposure is insufficient to inhibit SARS-CoV-2 replication. However, if therapeutic activity is driven by pulmonary exposure, it should be possible to reduce the chloroquine dose to safe levels. Carefully designed randomized controlled trials are urgently required to address these outstanding issues. [**note: I assume the same story might hold for hydroxychloroquine. Perhaps everyone is being dosed way to high based on PK/PD data.**] https://www.medrxiv.org/content/10.1101/2020.04.24.20078741v1

DIAGNOSTIC DEVELOPMENT

- Here is another model for the optimal size of pool testing. This paper presents an analytical formulation for determining optimal pool size in the initial pooling stage and the subsequent retests for COVID-19. A generalized constant compaction approach confirms the efficiency of halving targeted population between retest stages. An analytical gain formula is derived to aid future test designs. It is observed that optimal gain relies on the proper choice of the initial pool size. This optimal compaction scheme could outperform the conventional algorithms in most cases and may provide a mathematically-native road map for us to operate beyond the standard super-even-number-based (64, 32, 16, 8, 1) group testing algorithms. https://www.medrxiv.org/content/10.1101/2020.04.26.20076265v1

- A Swiss group has a proposal for using imperfect serological tests. With the imperfect tests that are currently available to test for past SARS-CoV-2 infection, the fraction of seropositive individuals in serosurveys is a biased estimator of seroprevalence and is usually corrected post-hoc to account for the sensitivity and specificity. Here we introduce a likelihood-based inference method for the estimation of the seroprevalence that does not require to define cutoffs by integrating the quantitative test measures directly into the statistical inference procedure. The likelihood-based method outperforms the methods based on cutoffs and post-hoc corrections leading to less variation in point-estimates of the seroprevalence and its temporal trend. We show how the likelihood-based method can be used to optimize the design of serosurveys with imperfect serological tests. We also provide guidance on the number of control and case sera that are required to quantify the test's ambiguity sufficiently to enable the reliable estimation of the seroprevalence. An R-package with the likelihood and power analysis functions is provided. Our study opens an avenue to using serological tests without cutoffs, especially if they are used to determine parameters characterizing populations rather than individuals. This approach circumvents some of the shortcomings of cutoff-based methods with post-hoc correction at exactly the low seroprevalence levels and test accuracies that we are currently facing in COVID-19 serosurveys. https://www.biorxiv.org/content/10.1101/2020.04.29.068999v1

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2020-05-02

Lots of popular songs get taken up by jazz musicians who give the a special twist. I presented one example, 'Someday my Prince Will Come,' which came from a very popular movie. Sometimes, the song comes from a movie that is all but forgotten today. This is the case with 'Green Dolphin Street' from the film of the same name. Lyricist Ned Washington was best known for 'When You Wish Upon a Star' from Pinocchio. It was one of MGM's hits in 1947-48; it occasionally pops up on Turner Classic Movies (I have to stay on the lookout for this one). Here is the great John Coltrane with the sidemen who played with the Miles Davis Quintet. For some reason Miles, who was first to record this song, sat out this Düsseldorf performance: https://www.youtube.com/watch?v=ePScRElDHOY

Well this was pretty fast! FDA issued an Emergency Use Authorization for remdesivir to treat seriously ill adults and infants with SARS-CoV-2. The emergency use authorization allows for remdesivir to be distributed in the U.S. and administered intravenously by health care providers, as appropriate, to treat suspected or laboratory-confirmed COVID-19 in adults and children hospitalized with severe disease. Severe disease is defined as patients with low blood oxygen levels or needing oxygen therapy or more intensive breathing support such as a mechanical ventilator. Here is a twist; the primary endpoint for the remdesivir trial was changed while it was in progress. Survival was the initial primary endpoint but in mid-April it was changed to time to recovery. I wonder what the reaction would be if this were an industry sponsored cancer trial and the endpoint were changed midstream?

Here is a sobering report from some top epidemiologists. It's based on findings from past influenza outbreaks.

More on the controversy about human challenge studies of a SARS-CoV-2 vaccine can be found HERE. Those advocating letting the virus run its course so that herd immunity takes effect might want to reconsider this in light of what we currently know.

I was wondering when Moderna, manufacturer of the first mRNA vaccine to enter clinical trials, would sign a manufacturing agreement. They have just done this with Lonza. One billion shots a year!!!

For those wanting a clearly written article on drug repurposing, this one from The New York Times fills the bill! A number of the studies that I have posted over the past six weeks are discussed. There is mention of an experimental cancer drug that has much more activity than remdesivir and one clinician commented about the cytotoxicity of cancer drugs. I think this misses the mark as presumably a SARS-CoV-2 drug would only be given for a short duration relative to traditional cancer chemotherapy.

Sobering news from Montgomery County MD department of health (it's where I live). Two thirds of the fatalities from SARS-CoV-2 have been those living in congregate facility settings – nursing homes, assisted living facilities and similar group residences. There were another 20 "probable" COVID-19 deaths that were not verified by lab tests. Over ½ the deaths statewide have come from "congregate" facilities.

I try to annotate the most interesting abstracts, but today's deluge was too heavy for me to identify all of them. I urge my readers to scan them and you can always find a link to the complete preprint at the website of the abstract. Most interesting is a small observational study from Wuhan showing **the benefit of hydroxychloroquine in seriously ill patients**. They link this to control of cytokine storm. I also do not want to add new categories so you will find things that don't quite fit under the headings.

MODELING

- A multi-national group of investigators posits that individual variation in susceptibility or exposure to SARS-CoV-2 lowers the herd immunity threshold. As severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spreads, the susceptible subpopulation is depleted causing the rate at which new cases occur to decline. Variation in individual susceptibility or exposure to infection exacerbates this effect. Individuals that are frailer, and therefore more susceptible or more exposed, have higher probabilities of being infected, depleting the susceptible subpopulation of those who are at higher risk of infection, and thus intensifying the deceleration in occurrence of new cases. Eventually, susceptible numbers become low enough to prevent epidemic growth or, in other words, herd immunity is attained. Although estimates vary, it is currently believed that herd immunity to SARS-CoV-2 requires 60-70% of the population to be immune. Here we show that variation in susceptibility or exposure to infection can reduce these estimates. Achieving accurate estimates of heterogeneity for SARS-CoV-2 is therefore of paramount importance in controlling the COVID-19 pandemic. [**note: perhaps this may be the case but I'm not sure what the impact on mortality will be given the large over 70 years of age death rate, many of whom are living in 'congregate' facilities. However, that might be mitigated by more focus on disease control among the staff of these facilities (this should be done in any event).**]
  https://www.medrxiv.org/content/10.1101/2020.04.27.20081893v1

- I didn't have a real good category for this one which is an interesting finding. SARS-CoV-2 has a zoonotic origin and was transmitted to humans via an undetermined intermediate host, leading to widespread infections in humans and reports of infections in other mammals. To enter host cells, the viral spike protein binds to its receptor, angiotensin-converting enzyme 2 (ACE2), and is processed by a protease, transmembrane protease serine 2 (TMPRSS2). Whilst receptor binding contributes to the viral host range, changes in energy of the spike protein:ACE2 complexes in other animals have not been widely explored. Here, we analyse interactions between the spike protein and orthologues of ACE2 and TMPRSS2 from a broad range of 215 vertebrate species. Structures of these orthologues have not been determined, so we predicted them. Using models of the spike protein:ACE2 orthologue complexes, we calculated their changes in energy, and correlated these to COVID-19 severities in mammals. Across vertebrate orthologues, mutations are predicted to be more disruptive to the structure of ACE2 than TMPRSS2. Finally, we provide phylogenetic evidence that SARS-CoV-2 has recently transmitted from humans to animals. Our results suggest that SARS-CoV-2 can infect a broad range of mammals--but not fish, birds or reptiles--which could serve as reservoirs of the virus, necessitating careful ongoing animal management and surveillance. [**note: I would feel a lot better had they tested some insect species. The last thing we need is arbor viral transmission!]** https://www.biorxiv.org/content/10.1101/2020.05.01.072371v1
- Has it really come down to this? And the researchers are from a distinguished Ivy League school of public health. Social distancing has been one of the primary mitigation strategies in the United States to control the spread of novel coronavirus disease (COVID-19) and can be viewed as a multi-faceted public health measure. Using Twitter data, we aim to (1) define and quantify the prevalence and evolution of facets of social distancing during the COVID-19 pandemic in the US in a spatiotemporal context and (2) examine the most amplified tweets among social distancing facets. We analyzed a total of 259,529 unique tweets containing "coronavirus" from 115,485 unique users between January 23, 2020 and March 24, 2020 that were identified by the Twitter API as English and U.S.-based. Tweets containing specified keywords (determined a priori) were grouped into six social distancing facets: implementation, purpose, social disruption, adaptation, positive emotions, and negative emotions. Tweets about social disruptiveness were most retweeted, and implementation tweets were most favorited. Social distancing tweets became overall more prevalent in the U.S. from late January to March but were not geographically uniform. In January and February, facets of social distancing appeared in Los Angeles, San Francisco, and Seattle, which were among the first cities impacted by the COVID-19 outbreak. Tweets related to the "implementation" and "negative emotions" facets of social distancing largely dominated in combination with topics of "social disruption" and "adaptation", albeit to a lesser degree. Social distancing can be defined in terms of facets that respond and represent certain moments and events in a pandemic, including travel restrictions and rising COVID-19 case counts. For example, in February, Miami, FL had a low volume of social distancing tweets but grew in March which corresponded with the rise of COVID-19 cases in the city. This suggests that overall volume of social distancing tweets can reflect the relative case count in respective locations. [**note: this will still not affect my decision never to sign up for a Twitter account!**] https://www.medrxiv.org/content/10.1101/2020.04.26.20080937v1
- In the present study, the exposure to virus during soccer matches is calculated. Tracking data from 14 elite matches was used. One player in each match was carrying a virus. The exposure

score (measured in seconds) was calculated as time spent closer than 1.5m from the infected player or time spent in an exponentially declining zone where the infected player was positioned earlier. The results reveal that, on average, each player was exposed for 87.8s per match. [**note: since Euro soccer is the ONLY sport I watch this model is of special interest! Most of the leagues in Europe are canceling their seasons and allowing the results to stand. This is controversial in The Netherlands as two teams (including my beloved Ajax) are tied on points at the top of the table. Litigation over which team gets the Champions League spot for next season is on the horizon.**] https://www.medrxiv.org/content/10.1101/2020.04.26.20080614v1

- This New Zealand modeling of intravitreal anti-VEGF injections is of interest to those who need this treatment for macular degeneration. Background: Clinical ophthalmological guidelines encourage the assessment of potential benefits and harms when deciding whether to perform elective ophthalmology procedures during the COVID-19 pandemic, in order to minimize the risk of disease transmission. Method: We performed probability calculations to estimate COVID-19 infection status and likelihood of disease transmission among neovascular age-related macular degeneration patients and health care workers during anti-VEGF procedures, at various community prevalence levels of COVID-19. We then applied the expected burden of COVID-19 illness and death expressed through health-adjusted life-years (HALYs) lost. We compared these results to the expected disease burden of severe visual impairment if sight protecting anti-VEGF injections were not performed. Results: Our calculations estimate for a single treatment, where the background rate of COVID-19 in the community is 1000 active cases per million population, and full personal protective equipment (PPE) is available, that the benefits of treatment are greater than the expected harms to the patient and immediate health care team, provided the probability of severe visual impairment without treatment is >0.001%. Without effective PPE, and with a COVID-19 prevalence of 200,000 per million, an 8.5% chance of severe visual impairment could still justify monthly injections for six months. Conclusion: In most cases analysed, the reduced disease burden from avoiding visual impairment outweighs the expected HALYs lost from COVID-19 transmission. This finding is driven by the fact that HALYs lost when someone suffers severe visual impairment for 5 years are equivalent to nearly 400 moderate cases of infectious disease lasting 2 weeks each.
https://www.medrxiv.org/content/10.1101/2020.04.27.20075085v1
- Widespread testing and tracing efforts are employed in many countries in order to contain and mitigate this pandemic. Recent data has indicated that fecal shedding of SARS-CoV-2 is common, and that the virus can be detected in wastewater. This indicates that wastewater monitoring is a potentially efficient tool for epidemiological surveillance of SARS-CoV-2 infection in large populations at relevant scales. Collecting raw sewage data, representing specific districts, and crosslinking this data with the number of infected people from each location, will enable us to derive and provide quantitative surveillance tools. In particular, this will provide important means to (i) estimate the extent of outbreaks and their spatial distributions, based primarily on in-sewer measurements (ii) manage the early-warning system quantitatively and efficiently (and similarly, verify disease elimination). Here we report the development of a virus concentration method using PEG or alum, providing an important a tool for detection of SARS-CoV-2 RNA in sewage and relating it to the local populations and geographic information. This will provide a proof of concept for the use of sewage associated virus data as a reliable epidemiological tool. [**note: lots of us have had experience collecting small stool samples for fecal occult blood**

testing. Maybe we should expand this to SARS-CoV-2 testing.]
https://www.medrxiv.org/content/10.1101/2020.04.26.20073569v1

- OBJECTIVE: To forecast the death toll of COVID-19 in the whole world by fitting the time series of reported deaths with a parametric equation (integrated Gaussian equation) related to Farr s law. DATA: The time series of cumulative deaths due to COVID-19 produced by John Hopkins University and stored in a github repository. RESULTS: The projected total death toll will be 261680 (392520 to 183176) which represents the 0.003 % of world population. This number amounts to 0.054 deaths per 1000, while the mean in the world (all causes) is 7.7. The daily peak of deaths (7270 (+/-500)) happened the 15 (+/- 3) of April, meaning that we are in descending curve of the pandemic. The outbreak will end completely the 23th (+/-3) of June. However, already on 9th (+/- 3) of May, 2 sigma; (95.45%) of the deaths will have be occured. The projected death toll is much lower (5-10 times) than those forecasted by the Imperial College Group (ICG) even considering the best scenario of total suppression of virus transmission. Using actual mortality rates it is possible to back calculate which number of infected individuals would produce such mortality. The death toll arises from a number of infected individuals between 53 (worst case) and 3.3 million. The calculated number of infected individuals is significantly lower than that calculated by ICG (227.5 millions) with suppression. [**note: I really want this projection to come true. The reason for posting this one despite my aversion to most models is that it fills me with optimism (that may be misplaced).**]
https://www.medrxiv.org/content/10.1101/2020.04.26.20074377v1

NEWLY REGISTERED CLINICAL TRIALS

- I don't have a trial registration number for this but here is a proposed protocol for nebulized heparin. COVID 19 is associated with the development of ARDS displaying the typical features of diffuse alveolar damage with extensive pulmonary coagulation activation resulting in fibrin deposition in the microvasculature and formation of hyaline membranes in the air sacs. The anticoagulant actions of nebulised heparin limit fibrin deposition and progression of lung injury. Serendipitously, unfractionated heparin also inactivates the SARS CoV 2 virus and prevents its entry into mammalian cells. Nebulisation of heparin may therefore limit both fibrin mediated lung injury and inhibit pulmonary infection by SARS CoV 2. For these reasons we have initiated a multicentre international trial of nebulised heparin in patients with COVID 19. Methods and intervention: Mechanically ventilated patients with confirmed or strongly suspected SARS CoV 2 infection, hypoxaemia and an acute pulmonary opacity in at least one lung quadrant on chest Xray, will be randomised to nebulised heparin 25,000 Units every 6 hours or standard care for up to 10 days while mechanically ventilated. The primary outcome is the time to separation from invasive ventilation to day 28, where non survivors to day 28 are treated as though not separated from invasive ventilation. Ethics and dissemination: The study protocol has been submitted to the human research and ethics committee of St Vincents Hospital, Melbourne, Australia. Submission is pending in other jurisdictions. Results of this study will be published in scientific journals and presented at scientific meetings.
https://www.medrxiv.org/content/10.1101/2020.04.28.20082552v1

CLINICAL TRIAL RESULTS

- French study supporting ongoing treatment of blood pressure using ACE inhibitors and ATI receptor blockers. We hypothesised that if patients treated with ACE inhibitors (ACEI) or AT1 receptor blockers (ARB) were more prone to SARS-CoV2 infection and had a worse prognosis than untreated patients, the prevalence of consumption of these drugs would be higher in patients with COVID-19 compared to the general population. Methods and results: We used a clinical epidemiology approach based on the estimation of standardised prevalence ratio (SPR) of consumption of ACEI and ARB in four groups of patients (including 187 COVID-19 positive) with increasing severity referred to the University hospital of Lille and in three French reference samples (the exhaustive North population (n=1,569,968), a representative sample of the French population (n=414,046), a random sample of Lille area (n=1,584)). The SPRs of ACEI and ARB did not differ as the severity of the COVID-19 patients increased, being similar to the regular consumption of these drugs in the North of France population with the same non-significant increase for both treatment (1.17 [0.83-1.67]). A statistically significant increase in the SPR of ARB (1.56 [1.02-2.39]) was observed in intensive care unit patients only. After stratification on obesity, this increase was limited to the high risk subgroup of obese patients. Conclusions: Our results strongly support the recommendation that ACEI and ARB should be continued in the population and in COVID-19 positive patients, reinforcing the position of several scientific societies. https://www.medrxiv.org/content/10.1101/2020.04.28.20078071v1
- Interesting cohort study on plasma Angiotensin II and gender differences from Wuhan. Angiotensin-converting enzyme 2 (ACE2) is identified as an important functional receptor for SARS−Cov−2. ACE2 and ACE are homologues with inverse functions in the renin−angiotensin system. ACE converts angiotensin I into a vital vasoactive peptide called angiotensin II(AngII), whereas ACE2 hydrolyzes AngII into a series of vasodilators. There were few reports illustrated the expression of AngII in COVID−19. This study aimed to demonstrate the expression of angiotensin II in COVID−19 and how it correlated to the disease. Methods: We enrolled 55 patients with COVID−19 admitted to renmin Hospital of Wuhan University from January 21st to February 21st, 2020. Demographic data were collected upon admission. COVID−19 nuclear acid, plasma AngII, Renin and aldosterone in the lying position without sodium restriction, and other laboratory indicators were together measured by the laboratory department of our hospital. Findings: Of the 55 patients with COVID−19, 34(61.8%) had an increased level of AngII. The severity of COVID−19 and male is positively related with the level of AngII. The level of blood lymphocyte, PCT, ALT, and AST were remarkably severe with those of normal level of AngII ($P < 0.05$). CD4/CD8 cells ratio was significantly higher whereas CD3+CD8+ cells amount, CD3+CD8+ cells proportion, CD56+CD16+CD3- cells amount and CD19+CD3- cells amount were considerably lower than those of normal level of AngII ($P < 0.05$). Abnormal rates of blood lymphocyte and PCT were significantly higher in Patients with elevated AngII level. The results of binary logistic regression analysis showed that the severity of COVID−19 (OR=4.123) and CD4/CD8 ratio(OR=4.050) were the co-directional impact factor while female(OR=0.146) was inverse impact factor of elevated AngII level. Interpretation: High rate of increased level of AngII was detected in COVID−19 patients. Patients with elevated AngII level were more likely to be critically ill with COVID−19. Considering the gender differences in ACE2 expression and no gender differences in angiotensin expression, the gender differences in AngII level might indicate less loss of ACE2 in female patients. Elevated AngII level was correlated with CD4/CD8

ratio, suggesting it might involve in immune disorder. Keywords: 2019 Novel coronavirus disease(COVID−19), Angiotensin-converting enzyme 2 (ACE2), Angiotensin II(AngII), gender differences [**note: it's a small study, but suggestive of links to what others have been seeing.**] https://www.medrxiv.org/content/10.1101/2020.04.27.20080432v1

- From hard hit Italy. The clinical course of COVID-19 in patients undergoing chronic immunosuppressive therapy is yet poorly known. We performed a monocentric cross-sectional study describing the clinical course of COVID-19 in a cohort of patients from northern Italy treated with calcineurin-inhibitors for organ transplantation or rheumatic diseases. Data were collected by phone call and clinical chart review between March 27th- 31st 2020. COVID-19 related symptoms, rynopharingeal swab, therapeutic changes and outcome were assessed in 384 consecutive patients (57% males; median age 61 years, IQR 48-69). 331 patients (86%) received solid organ transplantation (kidney n=140, 36%, heart n=100, 26%, lung n=91, 24%) and 53 (14%) had a rheumatic disease. Calcineurin inhibitors were the only immunosuppressant administered in 46 patients (12%). 14 patients developed a confirmed COVID-19 (swab positivity) and 14 a clinical COVID-19 (only typical symptoms). Fever (75%) and diarrhoea (50%) were the most common symptoms. Fourteen patients were hospitalized and 11 have already been dismissed. No patient required start/changes of the O2 therapy or developed superinfection. Only one patient, with metastatic lung cancer, died. In conclusion, COVID-19 showed a mild course in our cohort, with low mortality. *Calcineurin inhibitor-based immunosuppressive regimens appear safe in this context and should not be discontinued*. https://www.medrxiv.org/content/10.1101/2020.04.26.20080663v1
- 500 patient cohort study from a London hospital. This retrospective cohort analysis, reports the demographic data and early outcome of the first 500 patients who were admitted to a District General Hospital in South West London, UK and tested positive to COVID-19. The patients were admitted between 10 January and 10 April 2020; with the first COVID-19 positive diagnosis on 6 March. A surge in admissions started around the 15 March and peaked at the beginning of April. 56.8% of the admissions were male and 43.2% were female. The average age of the 500 admissions was 69.32 years (SD 19.23 years, range 1 week to 99.21 years). By the morning of 14 April 2020, 199 patients had been discharged (Female 89, Male 111), 163 patients had died (female 61, male 102) and 131 remained as in-patients (female 66, male 71). Fewer than one in twenty deaths occurred in patients below the age of 50 years, in either gender. Mortality rose dramatically, for both genders, after the age of sixty with males being almost twice as vulnerable to dying, as females, during the 7th decade. Males older than their mid-fifties were more likely to die than leave hospital. The same applied to females beyond their mid seventies. We did not see any evidence of a poorer outcome associated with a lower decile for Index of Multiple Deprivation or convincing evidence that any Ethnic minority groups were more likely to die than the White subgroups. When compared to the equivalent medical conditions, normally treated in the early spring, COVID-19 has an increased mortality, adversely affecting more men and an older population. The mean duration from admission to discharge was 11.29 days (SD 11.50 days). For admission to death, the mean interval was 11.72 days (SD 11.05 days). 62 of the 500 admissions required ventilator support. Of this subgroup, 71% were male and 29% were female. By the morning of the 14 April, no female over the age of 60 had left the intensive care unit alive and no male over the age of 50 had left the intensive care unit alive. At this time-point, 1.2% of the 500 admitted patients had returned alive from the intensive care units, following a

period of ventilator support. This figure will rise if prolonged ventilator and renal support proves effective. While only providing a snapshot of a relatively small number of patients, reviewed over a short time period, from a small geographic area, the data supports the view that the younger members of society are less vulnerable to the adverse sequelae of COVID-19 infection and that any return to normal work and social activities should be considered initially for the individuals who are less than 40-50 years of age. There is an ongoing need for analyses on larger patient cohorts using both demographic and detailed clinical data. [**note: it sucks to be old!!**] https://www.medrxiv.org/content/10.1101/2020.04.28.20075119v1

- I guess we should not give up on hydroxychloroquine just yet. Here is a retrospective cohort study from Wuhan. Importance: Coronavirus disease 2019 (COVID-19) is a pandemic with no specific drugs and high mortality. The most urgent thing is to find effective treatments. Objective: To determine whether hydroxychloroquine application may be associated with a decreased risk of death in critically ill COVID-19 patients and what is potential mechanism. Design, Setting and Patients: This retrospective study included all 568 critically ill COVID-19 patients who were confirmed by pathogen laboratory tests despite antiviral treatment and had severe acute respiratory distress syndrome, PAO2/FIO2 <300 with need of mechanical ventilation in Tongji Hospital, Wuhan, between February 1 of 2020 to April 8 of 2020. All 568 patients received comparable basic treatments including antiviral drugs and antibiotics, and 48 of them additionally received oral hydroxychloroquine (HCQ) treatment (200 mg twice a day for 7-10 days). Primary endpoint is mortality of patients, and inflammatory cytokines levels were compared between hydroxychloroquine and non-hydroxychloroquine (NHCQ) treatments. MAIN OUTCOMES AND MEASURES: In-hospital death and hospital stay time (day) were obtained, level of inflammatory cytokine (IL-6) was measured and compared between HCQ and NHCQ treatments. RESULTS: The median age of 568 critically ill patients is 68 (57, 76) years old with 37.0% being female. Mortalities are 18.8% (9/48) in HCQ group and 45.8% (238/520) in NHCQ group ($p<0.001$). The time of hospital stay before patient death is 15 (10-21) days and 8 (4 - 14) days for the HCQ and NHCQ groups, respectively ($p<0.05$). The level of inflammatory cytokine IL-6 was significantly lowered from 22.2 (8.3-118.9) pg/mL at the beginning of the treatment to 5.2 (3.0-23.4) pg/ml ($p<0.05$) at the end of the treatment in the HCQ group but there is no change in the NHCQ group. CONCLUSIONS AND RELEVANCE: Hydroxychloroquine treatment is significantly associated with a decreased mortality in critically ill patients with COVID-19 through attenuation of inflammatory cytokine storm. Therefore, hydroxychloroquine should be prescribed for treatment of critically ill COVID-19 patients to save lives. [**note: I'll leave it to the clinical trials people to pick over this paper. It is a small treatment arm and ongoing clinical trials with hydroxychloroquine should confirm this, or maybe they won't.**] https://www.medrxiv.org/content/10.1101/2020.04.27.20073379v1
- From NYU, confirmation of something we already knew. There is no known effective therapy for the disease. Initial reports suggesting the potential benefit of Hydroxychloroquine/Azithromycin (HY/AZ) have resulted in massive adoption of this combination worldwide. However, while the true efficacy of this regimen is unknown, initial reports have raised concerns regarding the potential risk of QT prolongation and induction of torsade de pointes (TdP). Methods: This is a multicenter retrospective study of 251 patients with COVID-19 treated with HY/AZ. We reviewed ECG tracings from baseline and until 3 days after completion of therapy to determine the progression of QTc and incidence of arrhythmia and mortality. Results: QTc prolonged in

parallel with increasing drug exposure and incompletely shortened after its completion. Extreme new QTc prolongation to > 500 ms, a known marker of high risk for TdP had developed in 15.9% of patients. One patient developed TdP requiring emergent cardioversion. Seven patients required premature termination of therapy. The baseline QTc of patients exhibiting QTc prolongation of > 60 ms was normal. Conclusion: The combination of HY/AZ significantly prolongs the QTc in patients with COVID-19. This prolongation may be responsible for life threating arrhythmia in the form of TdP. This risk mandates careful consideration of HY/AZ therapy in lights of its unproven efficacy. Strict QTc monitoring should be performed if the regimen is given. https://www.medrxiv.org/content/10.1101/2020.04.27.20074583v1

- I think this is the first cohort study of HIV infected patients on antiretroviral therapy. Data on people living with human immunodeficiency virus (PLWH) in the current SARS-CoV-2 pandemic is still scarce. This case series of 33 PLWH patients with COVID-19 reveals symptoms and outcome in this special population. Three out of 32 patients with documented outcome died (9%). However, 91% of the patients recovered and 76% have been classified as mild cases, indicating that there is no excess morbidity and mortality among PLWH with symptomatic COVID-19. All patients were on antiretroviral treatment, of them 22 on tenofovir-containing regimen, and 4 on the protease inhibitor darunavir. [**note: we need more data across a larger population to make any inference on whether these drugs are protective in any manner. It's reassuring that this small population did not markedly deviate from what has already be observed.** https://www.medrxiv.org/content/10.1101/2020.04.28.20073767v1

DRUG DEVELOPMENT

- One of the best drug development papers was just published in Nature. I linked to the preprint in one of the very early newsletters. In the paper they present *in vito* assay data done by colleagues in France and Mt. Sinai of some of the promising drugs. To address this, we cloned, tagged and expressed 26 of the 29 SARS-CoV-2 proteins in human cells and identified the human proteins physically associated with each using affinity-purification mass spectrometry (AP-MS), identifying 332 high-confidence SARS-CoV-2-human protein-protein interactions (PPIs). Among these, we identify 66 druggable human proteins or host factors targeted by 69 compounds (29 FDA-approved drugs, 12 drugs in clinical trials, and 28 preclinical compounds). Screening a subset of these in multiple viral assays identified two sets of pharmacological agents that displayed antiviral activity: inhibitors of mRNA translation and predicted regulators of the Sigma1 and Sigma2 receptors. Further studies of these host factor targeting agents, including their combination with drugs that directly target viral enzymes, could lead to a therapeutic regimen to treat COVID-19. [**note: on the website is a like to an Excel spreadsheet showing the results of the viral assays. There are a fair number of experimental drugs on the list. This is a paper well worth downloading and reading.**]
- Any port in a storm. Current treatment of COVID-19 is limited and mostly supportive. At present, there is no specific therapeutics against SARS-CoV-2. In this study, we discovered that protoporphyrin IX and verteporfin, two FDA-approved drugs for treatment of human diseases, had significant antiviral effect against SARS-CoV-2, with EC50 values for the reduction of viral RNA at nanomolar concentrations. Both drugs completely inhibited the cytopathic effect (CPE) produced by SARS-CoV-2 infection at lower drug concentrations than that of remdesivir. The

selection indices of protoporphyrin IX and verteporfin are 952.74 and 368.93, respectively, suggesting wide safety margins. Both drugs were able to prevent SARS-CoV-2 infection as well as suppress established SARS-CoV-2 infection. The compounds share a porphyrin ring structure. Molecular docking indicates that the compounds may interact with viral receptor ACE2 and could block the cell-cell fusion mediated by ACE2 and viral S protein. Our finding suggests that protoporphyrin IX and verteporfin might be potential antivirals against SARS-CoV-2 infection and also sheds new light on the development of a novel class of small compounds against SARS-CoV-2. [**note: I think this is the first time these drugs came up in a screening. It's weird that the two big US groups did not identify these.**]
https://www.biorxiv.org/content/10.1101/2020.04.30.071290v1

- Naproxen for everyone!!!! Forget about the possibility of ulcers and add some Pepcid AC for a double whammy against SARS-CoV-2. There is an urgent need for specific antiviral drugs directed against SARS-CoV-2 both to prevent the most severe forms of COVID-19 and to reduce viral excretion and subsequent virus dissemination; in the present pandemic context, drug repurposing is a priority. Targeting the nucleoprotein N of the SARS-CoV-2 coronavirus in order to inhibit its association with viral RNA could be a strategy to impeding viral replication and possibly other essential functions associated with viral N. The antiviral properties of naproxen, belonging to the NSAID family, previously demonstrated against Influenza A virus, were evaluated against SARS-CoV-2. Naproxen binding to the nucleoprotein of SARS-CoV2 was shown by molecular modeling. In VeroE6 cells and reconstituted human primary respiratory epithelium models of SARS-CoV-2 infection, naproxen inhibited viral replication and protected the bronchial epithelia against SARS-CoV-2 induced-damage. The benefit of naproxen addition to the standard of care is tested in an on-going clinical study. [**note: I interrupt the preparation of this newsletter to visit local CVS drugstores in an attempt to corner the market for Aleve.**]
https://www.biorxiv.org/content/10.1101/2020.04.30.069922v1

DIAGNOSTIC DEVELOPMENT

- A big shout out to the Beth Israel Deaconess hospital folks for figuring out how to produce needed reagents in house to support PCR testing!! The COVID-19 pandemic has severely disrupted worldwide supplies of viral transport media (VTM) due to widespread demand for SARS-CoV-2 RT-PCR testing. In response to this ongoing shortage, we began production of VTM in-house in support of diagnostic testing in our hospital network. As our diagnostic laboratory was not equipped for reagent production, we took advantage of space and personnel that became available due to closure of the research division of our medical center. We utilized a formulation of VTM described by the CDC that was simple to produce, did not require filtration for sterilization, and used reagents that were available from commercial suppliers. Performance of VTM was evaluated by several quality assurance measures. Based on Ct values of spiking experiments, we found that our VTM supported highly consistent amplification of the SARS-CoV-2 target (coefficient of variation = 2.95%) using the Abbott RealTime SARS-CoV-2 EUA assay on the Abbott m2000 platform. VTM was also found to be compatible with multiple swab types and, based on accelerated stability studies, able to maintain functionality for at least four months at room temperature. We further discuss how we met logistical challenges associated

with large-scale VTM production in a crisis setting including use of staged, assembly line for VTM transport tube production. https://www.medrxiv.org/content/10.1101/2020.04.29.20085514v1

- Here is a UK NHS group who also addressed the reagent shortage issue. In light of supply chain failures for reagents and consumables needed for purification of nucleic acid for detection of SARS-CoV-2 RNA by RT-PCR, we aim to verify the performance and utility of a non-extraction protocol for RT-PCR ("direct RT-PCR"). We report improved sensitivity compared to earlier reports of direct RT-PCR testing of swab samples, in particular at the lower limit of detection (sensitivity 93% overall; 100% for specimens with high to moderate viral titre, Ct <34; 81% for specimens with a low viral titre, Ct ≥34). Sensitivity is improved (from 90 to 93%) by testing in duplicate. We recommend swabs are re-suspended in water to minimise PCR inhibition. A cellular target is necessary to control for PCR inhibition and specimen quality. Direct RT-PCR is best suited to population level screening where results are not clinically actionable, however in the event of a critical supply chain failure direct RT-PCR is fit for purpose for the detection of SARS-CoV-2 infection. The results from our study offer front-line laboratories additional reagent options for performing extraction-free RT-PCR protocols.
https://www.medrxiv.org/content/10.1101/2020.04.29.20085910v1
- Polyester swabs are not a good substitute for collection of diagnostic specimens. As of April 15, 2020 FDA recommendations only allowed for the use of nasopharyngeal, flocked mid turbinate, or foam nasal swabs, all of which are in very low supply. Polyester swabs are more readily available and mass producible. We compare the performance of polyester and foam swabs stored in different transport media. Methods: Both polyester and foam nasal swabs were collected from convalescent COVID-19 patients at a single visit. Using the foam nasal swabs as the comparator, sensitivity of the polyester swabs in each media were calculated, three by three tables were constructed to measure concordance, and cycle threshold (Ct) values were compared. Findings: 126 visits had polyester and foam swabs stored in viral transport media (VTM), 51 had polyester and foam swabs stored in saline, and 63 had a foam swab in VTM and a polyester swab stored in a dry tube. Using nasal foam swabs as a comparator, polyester nasal swabs had a sensitivity of 86·5% when both samples were stored in VTM, 86·7% when both samples were stored in saline, and 72·4% when the polyester swab was stored dry and the foam swab was stored in VTM. Polyester and foam Ct values from the same visit were correlated, but polyester swabs showed decreased performance for cases with a viral load near the detection threshold and higher Ct values on average. Interpretation: Polyester nasal swabs showed a reduction in performance from foam nasal swabs, but may still provide a viable sample collection method given the current supply shortages and public health emergency.
https://www.medrxiv.org/content/10.1101/2020.04.28.20083055v1
- I'm running short of different categories!! Here is a serology study of asymptomatic healthcare professionals. We investigated the SARS-CoV-2 specific antibody titers in 133 asymptomatic healthcare providers working at the Department of Laboratory Medicine of our tertiary center. A commercial chemiluminescence immunoassay, validated according to the ISO15189 standard requirements, was used. All the enrolled healthcare professionals underwent, simultaneously to the blood sampling, a nasopharyngeal swab for molecular testing with quantitative reverse-transcriptase-based polymerase chain reaction (RT-PCR). An overall positiveness of 5.25% was found. We strongly promote a wide use of validated serologic assays in asymptomatic, healthy individuals, as a crucial information for epidemiological surveillance. [**note: this is a pretty well**

done paper as they did do validation of the serology test. They did not want to go out on a limb and extrapolate the 5.25% finding to the broader Italian population.]
https://www.medrxiv.org/content/10.1101/2020.04.27.20073858v1

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2020-05-03

Sunday is spiritual music day! Here is a very fine performance of Brahms A German Requiem : https://www.youtube.com/watch?v=A-1SishN_bc Despite taking the text from the scriptures, Brahms made this work non-liturgical and focused on humanism.

Derek Lowe on why clinical trials are so complicated.

It was a slow day for preprints. There is a good abstract on the Abbott serology test showing high sensitivity.

MODELING

- Here is a paper from Chinese scientists comparting SARS-CoV-2 outbreaks in 25 countries. We evaluate the effectiveness of COVID-19 control strategies of 25 countries which have endured more than four weeks of community infections. With an extended SEIR model that allows infections in both the exposed and infected states, the key epidemic parameters are estimated from each country's data, which facilitate the evaluation and cross-country comparison. It is found quicker control measures significantly reduce the average reproduction numbers and shorten the time length to infection peaks. If the swift control measures of Korea and China were implemented, average reductions of 88% in the confirmed cases and 80% in deaths would had been attained for the other 23 countries from start to April 10. Effects of earlier or delayed interventions in the US and the UK are experimented which show at least 75% (29%) less infections and deaths can be attained for the US (the UK) under a Five-Day Earlier experiment. The impacts of two removal regimes (Korea and Italy) on the total infection and death tolls on the other countries are compared with the naturally forecast ones, which suggest there are still ample opportunity for countries to reduce the final death numbers by improving the removal process. [**note: interesting that they do not do any calculations on Israel who seem to have an extremely low mortality rate: https://www.timesofisrael.com/its-not-over-and-uncertainty-abounds-but-israels-covid-19-stats-are-stunning/**]
https://www.medrxiv.org/content/10.1101/2020.04.27.20081232v1
- Wow, a lot of people on this manuscript. They did close contact tracing of nine individuals. As part of initial response activities in the United States, enhanced contact investigations were conducted to enable early identification and isolation of additional cases and to learn more about risk factors for transmission. Methods Close contacts of nine early travel-related cases in the United States were identified. Close contacts meeting criteria for active monitoring were followed, and selected individuals were targeted for collection of additional exposure details and respiratory samples. Respiratory samples were tested for SARS-CoV-2 by real-time reverse transcription polymerase chain reaction (RT-PCR) at the Centers for Disease Control and Prevention. Results There were 404 close contacts who underwent active monitoring in the

response jurisdictions; 338 had at least basic exposure data, of whom 159 had at least 1 set of respiratory samples collected and tested. Across all known close contacts under monitoring, two additional cases were identified; both secondary cases were in spouses of travel-associated case patients. The secondary attack rate among household members, all of whom had at least 1 respiratory sample tested, was 13% (95% CI: 4 - 38%). Conclusions The enhanced contact tracing investigations undertaken around nine early travel-related cases of COVID-19 in the United States identified two cases of secondary transmission, both spouses. Rapid detection and isolation of the travel-associated case patients, enabled by public awareness of COVID-19 among travelers from China, may have mitigated transmission risk among close contacts of these cases. [**note: only two additional case of viral infection! I would have thought there might be more. Maybe this virus is quite as infectious as we think.**]
https://www.medrxiv.org/content/10.1101/2020.04.27.20081901v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow as Sunday is usually a slow day for new trial registration.

CLINICAL TRIAL RESULTS

- More on the linkage to loss of olfactory loss and SARS-CoV-2 diagnoses. In this cross-sectional controlled cohort study, 500 patients who presented with symptoms of a common cold to a corona testing center and fulfilled corona testing criteria, completed a standardized diagnostic questionnaire which included the patients main symptoms, time course and an additional self-assessment of the patients current smell, taste function and nasal breathing compared to the level before onset of symptoms. Results: Out of the 500 patients, 69 presented with olfactory loss. Twenty-two of them subsequently tested positive for SARS-CoV-2. Only twelve out of the patients without olfactory loss tested positive, resulting in a frequency of 64.7% for the symptom sudden smell loss in COVID-19 patients. Compared to COVID-19 patients without smell loss, they were significantly younger and less severely affected. Changes in nasal airflow were significantly more pronounced in SARS-CoV-2 negative patients with olfactory complaints compared to the patients with smell loss who were tested positive for SARS-CoV-2. By excluding patients with a blocked nose, the symptom sudden smell loss can be attested a high specificity (97%) and a sensitivity of 65% with a PPV of 63% and NPV of 97% for COVID-19. Conclusion: Considering the high frequency of smell loss in non-hospitalized COVID-19 patients, acute olfactory impairment should be included in the WHO symptoms list and should be recognized as an early symptom of the disease. In contrast to other acute viral smell impairment, COVID-19 associated smell loss seems to be only rarely accompanied by a severely blocked nose.
https://www.medrxiv.org/content/10.1101/2020.04.27.20081356v1
- A cohort study of outcomes in a live registry of heart failure patients across an integrated health care system. we sought to characterize the prevalence and outcomes of COVID-19 in a live registry of heart failure patients across an integrated health care system in Connecticut. Methods: In this retrospective analysis, the Yale Heart Failure Registry (NCT04237701) that includes 26,703 patients with heart failure across a 6-hospital integrated health care system in

Connecticut, was queried on April 16th, 2020 for all patients tested for COVID-19. Sociodemographic and geospatial data as well as, clinical management, respiratory failure, and patient mortality were obtained via the real-time registry. Data on COVID-19 specific care was extracted by retrospective chart review. Results: COVID-19 testing was performed on 900 symptomatic patients, comprising 3.4% of the Yale Heart Failure Registry (N=26,703). Overall, 206 (23%) were COVID-19+. As compared to COVID-19-, these patients were more likely to be older, black, have hypertension, coronary artery disease, and were less likely to be on renin angiotensin blockers (P<0.05, all). COVID-19- patients tended to be more diffusely spread across the state whereas COVID-19+ were largely clustered around urban centers. 20% of COVID-19+ patients died, and age was associated with increased risk of death [OR 1.92 95% CI (1.33-2.78); P<0.001]. Among COVID-19+ patients who were ≥85 years of age rates of hospitalization were 87%, rates of death 36%, and continuing hospitalization 62% at time of manuscript preparation. Conclusions: In this real-world snapshot of COVID-19 infection among a large cohort of heart failure patients, we found that a small proportion had undergone testing. Patients found to be COVID-19+ tended to be black with multiple comorbidities and clustered around lower socioeconomic status communities. Elderly COVID-19+ patients were very likely to be admitted to the hospital and experience high rates of mortality. [**note: another study showing the usefulness of observational studies in well maintained databases.**]
https://www.medrxiv.org/content/10.1101/2020.04.27.20082016v1

- From Emory, critically ill SARS-CoV-2 patients display lupus-like hallmarks of B cell activation. Wide heterogeneity of disease course ranging from asymptomatic spread to respiratory failure and death has become a hallmark of the SARS-CoV-2 pandemic. While this clinical spectrum is well documented, its immunologic underpinnings are less clear. We have therefore, initiated studies of the B cell responses as they would participate in both early effector responses and in the initiation of memory formation. In terms of effector responses, we were particularly interested in the engagement and clinical correlates of the extra-follicular pathway (EF), we recently described in flaring SLE. In this systemic autoimmune disease, the EF pathway is initiated by newly activated naive B cell (aN) leading to large expansion of autoantibody-producing antibody-secreting cells through the generation of an epigenetically primed B cell precursor which are double negative (DN) for naive (IgD) and memory markers (CD27) and lacking expression of CXCR5 and CD21 (DN2). These highly activated D2 cells are also distinguished by high expression of CD11c and T-bet and are TLR7-driven. Both, TLR7-stimulation which is triggered by ssRNA and the central role played by their murine counterparts (typically characterized as Age-Associated B cells), in viral clearance, strongly supported the hypothesis that DN2 cells and the global EF pathway could be prominently engaged in COVID-19 patients. Also of note, EF B cell activation is particularly prominent in SLE patients of African-American ancestry, a population disproportionately represented in severe COVID-19. In this study we find that critically-ill patients with COVID-19 robustly upregulate constituents of the extrafollicular pathway, produce enormous numbers of antibody secreting cells, and lose unique transitional B cell populations that correlate with positive prognosis. This patient cluster associates tightly with biomarkers of poor outcomes and exhibits high rates of mortality. Thus, this B cell phenotype might serve as an immunological marker of severe COVID infection at early stages and could therefore identify a patient subset likely to benefit from targeted

immunomodulatory therapy aimed at alleviating disease burden. https://www.medrxiv.org/content/10.1101/2020.04.29.20083717v1

- More on the loss of smell from a German group. **Objectives:** Coronaviruses (CoVs) have a neuroinvasive propensity, and the frequently reported symptoms of smelling and taste dysfunction in many COVID-19 patients may be related to the respective capability of SARS-CoV2, the cause of the current pandemic. In this study we objecti-fied and quantified the magnitude and underreporting of the smelling dysfunction caused by COVID-19 using a standardized test. **Methods:** We conducted a prospective cross-sectional study comparing the proportion of anos-mia using Sniffin-sticks in those reporting a loss of smell, in those who did not as well as in unin-fected controls. The outcome of anosmic versus not anosmic patients were recorded during hospital stay and at day 15 on a six-category ordinal scale. The study was approved by the insti-tutional review board, all participants consented to the study. **Results:** 40% of 45 consecutive hospitalized COVID-19 patients and 0% of 45 uninfected con-trols consenting were diagnosed with anosmia. 44% of anosmic and 50% of hyposmic patients did not report having smelling problems. Anosmia or hyposmia was not predictive of a severe COVID-19 manifestation. **Conclusions:** The majority of COVID-19 patients have an objective anosmia and hyposmia, which often occurs unnoticed. These symptoms may be related to the neuroinvasive propensity of SARS-COV-2 and the unusual presentation of COVID-19 disease manifestations. https://www.medrxiv.org/content/10.1101/2020.04.28.20083311v1
- More confusing data on hydroxychloroquine. To assess the efficacy of HCQ in increasing SARS-CoV-2 viral clearance Design: Retrospective observational study Setting: Cleveland Clinic Abu Dhabi Participants: Hospitalized adult patients with confirmed SARS-CoV-2 infection Intervention: None Measurements: The primary outcome was the time from a confirmed positive nasopharyngeal swab to turn negative. A negative nasopharyngeal swab conversion was defined as a confirmed SARS-CoV-2 case followed by two negative results using RT-PCR assay with samples obtained 24 hours apart Results: 34 confirmed COVID-19 patients were included. Nineteen (55.9%) patients presented with symptoms, and 14 (41.2%) had pneumonia. Only 21 (61.8%) patients received HCQ. The time to SARS-CoV-2 negativity nasopharyngeal test was significantly longer in patients who received HCQ compared to those who did not receive HCQ (17 [13-21] vs. 10 [4-13] days, p=0.023). HCQ was independently associated with time to negativity test after adjustment for potential confounders (symptoms, pneumonia or oxygen therapy) in multivariable linear regression analysis. On day 14, 47.8% (14/23) patients tested negative in the HCQ group compared to 90.9% (10/11) patients who did not receive HCQ (p=0.016). Limitations: Small sample size and retrospective design with a potential risk of selection bias Conclusion: HCQ was associated with a slower viral clearance in COVID-19 patients with mild to moderate disease. Data from ongoing randomized clinical trials with HCQ should provide a definitive answer regarding the efficacy and safety of this treatment. [**note: is it even worth reporting out such small patient populations?**] https://www.medrxiv.org/content/10.1101/2020.04.27.20082180v1
- From Shenzhen researchers, a study of viral clearance and antibody markers in asymptomatic carriers. Background Asymptomatic carriers contribute to the spread of Coronavirus Disease 2019 (COVID-19), but their clinical characteristics, viral kinetics, and antibody responses remain unclear. Methods A total of 56 COVID-19 patients without symptoms at admission and 19 age-matched symptomatic patients were enrolled. RNA of SARS-CoV-2 was tested using

transcriptase quantitative PCR, and the total antibodies (Ab), IgG, IgA and IgM against the SARS-CoV-2 were tested using Chemiluminescence Microparticle Immuno Assay. Results Among 56 patients without symptoms at admission, 33 cases displayed symptoms and 23 remained asymptomatic throughout the follow-up period. 43.8% of the asymptomatic carriers were children and none of the asymptomatic cases had recognizable changes in C-reactive protein or interleukin-6, except one 64-year-old patient. The initial threshold cycle value of nasopharyngeal SARS-CoV-2 in asymptomatic carriers was similar to that in pre-symptomatic and symptomatic patients, but the communicable period of asymptomatic carriers (9.63 days) was shorter than pre-symptomatic patients (13.6 days). There was no obvious differences of the seropositive conversion rate of total Ab, IgG, and IgA among the three groups, though the rates of IgM varied largely. The average peak IgG and IgM COI of asymptomatic cases was 3.5 and 0.8, respectively, which is also lower than those in symptomatic patients with peaked IgG and IgM COI of 4.5 and 2.4 ($p < 0.05$). Conclusion Young COVID-19 patients seem to be asymptomatic cases with early clearance of SARS-CoV-2 and low levels of IgM generation but high total Ab, IgG and IgA. Our findings provide empirical information for viral clearance and antibody kinetics of asymptomatic COVID-19 patients. https://www.medrxiv.org/content/10.1101/2020.04.28.20083139v1

DRUG DEVELOPMENT

- Nothing to Report

DIAGNOSTIC DEVELOPMENT

- Good news!! The Abbott serological test performs with high accuracy. The rollout of diagnostic testing in the United States was slow, leading to numerous cases that were not tested for SARS-CoV-2 in February and March 2020, necessitating the use of serological testing to determine past infections. Here, we evaluated the Abbott SARS-CoV-2 IgG test for detection of anti-SARS-CoV-2 IgG antibodies by testing 3 distinct patient populations. We tested 1,020 serum specimens collected prior to SARS-CoV-2 circulation in the United States and found one false positive, indicating a specificity of 99.90%. We tested 125 patients who tested RT-PCR positive for SARS-CoV-2 for which 689 excess serum specimens were available and found sensitivity reached 100% at day 17 after symptom onset and day 13 after PCR positivity. Alternative index value thresholds for positivity resulted in 100% sensitivity and 100% specificity. We then tested 4,856 individuals from Boise, Idaho collected over one week in April 2020 as part of the Crush the Curve initiative and detected 87 positives for a positivity rate of 1.79%. These data demonstrate excellent analytical performance of the Abbott SARS-CoV-2 IgG test as well as the limited circulation of the virus on the West Coast. We expect the availability of high-quality serological testing will be a key tool in the fight against SARS-CoV-2. [**note: hard to do better than 100%**] https://www.medrxiv.org/content/10.1101/2020.04.27.20082362v1