FLOWCELL

Biomarkers as comprehensive glue for COVID-19 research

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1 Introduction

1.1 COVID-19 induces immunopathology

In severe cases COVID-19 results in hyper-immune response called the cytokine storm, which leads to micro-thrombosis, invasion of immune cells, and damage to multiple organs, especially the lungs. Immunopathology can have a delayed onset of symptoms, such as exhaustion and depletion of lymphocytes, circulatory problems among other effects, which vary with individual immune system. Remarkably, most people infected with SARS-CoV-2 experience nothing more than a common cold. The impact of individual biology on susceptibility to severe COVID-19 disease is unfortunately not reflected in the majority of ongoing research that follows traditional route of drug development – without the biomarkers necessary for determination of disease status and precision treatment.

1.2 Viral supercomputer

The virus acts as a distributed supercomputer, conducting a massive optimization search in the sequence space. Physical search is continuously carried out by billions of virions with unique mutations within an individual, and are amplified over time and with number of infected. The good news about this is that the virus adopts to the new host. However, this is a bilateral co-evolution of host and pathogen. The best hope appears to be for the virus to rapidly adopt to humans to reduce it's immunopathology. Given the specific pathways involved, this could be a multi-year process, with possibility of additional waves driven by mutations in different regions circling the globe. Even as there are positive results coming from Europe and USA that the pathology of recent SARS-CoV-2 strains is reduced compared with earlier infections, the number of cases in India and Latin America is rapidly climbing.

1.3 Tools available

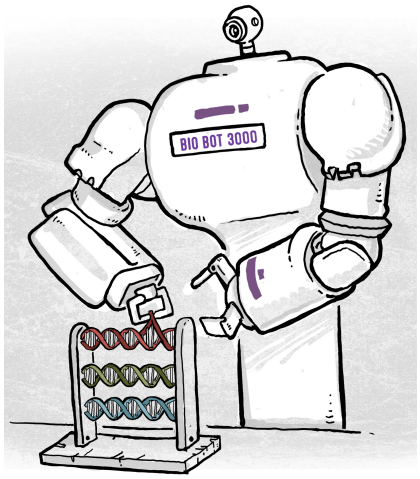

Figure 1: Current lab automation

The scientists working on SARS-CoV-2 have biological knowledge, but lack efficient tools that are effective on the time-scale of viral evolution. There is a cooperation on tracking ARS-CoV-2 mutations, however, the implications of these for cross-immunity with infection by prior strains is completely unknown. There are cases where antibody titer (concentration) in serum of recovered individuals increases, suggesting potential re-infection. Even more concerning are cases where symptoms persists for weeks, or even months. Our ability to track **functional** state of the viral mutation is low. Such testing requires the ability to test infections by different strains, or with unique antibodies. These experiments require manipulation of microscopic systems that simulate physiology of key tissues, like lung and gut. Such systems are just starting to appear. In addition, minimal model systems require miniaturized biology models to include 3D tissues, immune cells and viral infection. These types of tools would have capabilities to efficiently carry out "biocomputation", in analogy with integrated circuits, with the output of high-throughput biological insights.

Tools that exist are old. Traditional multi-well plates with stationary media are unsuitable for the challenge. Lab automation that increases the through-put of these tools is not addressing the key limitation, but masking it, like a robot using an abacus Figure 1. Discovery of natural enzymes with special properties like polymerase from theromophylic bacteria and CRISPR are utilized in cumbersome pipette transfer of fluids between miniature cups. This approach is incapable of increasing the volume of testing as required (my over a million-fold), or of delivering physiologically relevant data, as 3D cultures cannot survive in stationary media. Certainly for a complex immunopathology like COVID-19 the invasion of immune cells into lungs is an important pathology, which *cannot be effectively studied outside of humans*.

1.4 Top down approach

Although the effort to combat COVID-19 is occurring worldwide, there are concerns about splitting of the effort, such as a feuding of USA with China over origins of SARS-CoV-2 and lack of sharing on biological knowledge. After appearance of SARS-CoV-1 scientists proposed centralized studies to maximize accumulation of knowledge against an epidemic. Repurposing of drugs has been paid lip service, while large pharmaceuticals continue to push untested treatments like Remdesivir. Cooperation between pharamaceutical companies in terms of coordination of trials of similar drugs has not resulted in data that could be combined into unified databases. Development of biomarkers is a particular blind spot, possibly due to balance of power has traditionally favored drug development over diagnostics.

The major organizations handling COVID-19 have a poor track record. WHO organization was slow to react, has provided conflicting guidance about survival of SARS-CoV-2 on surfaces, and has been written off by US administration. Within US the track record of CDC, President and state governors leaves a lot of room for improvement. CDC has failed in it's early diagnostic tests, and had recently defended its failure by saying it wouldn't have made much difference. In fact, the only thing that could have made a difference was effective containment and good fortune which have prevented SARS-CoV-1 and MERS-CoV from also becoming pandemics.

US government is spending vast amounts of money to sustain the lock-down and minimize the spread of COVID-19, but its tools are blunt, where finesse is required. State-wide shutdowns treat New Your city and small towns with little sign of COVID-19 with the same approach. Guidance from the president about the length of lock-downs, or medicine like hydroxychloroquine has had the opposite of the desired effect. In stead of calming the public, it reduces confidence. The same could be said about Dr. Fauci, who has advocated Remdesivir becoming a *de facto* standard of care without clear evidence of efficacy. This is exactly what scientists have warned against after the first pandemic. The same can be said about large companies and even top-tier scientists, who pay lip service for use of repurposed drugs for COVID-19, but then push novel drugs like Remdesivir.

This approach is not working at any level: development of diagnostics, treatment, or even communication where policy of projecting confidence without data, and shifting narratives is self-defeating. The historical precedent form decades of drug development in general, or for SARS-CoV-1 for over 15 years is not being taken into account. Perhaps it is too difficult for those who have the power to admit they lack not only a solution, but even a good approach to develop one.

During the 2003-04 global outbreak of SARS-CoV, thousands of patients received treatments of uncertain efficacy and known toxicity such as ribavirin and corticosteroids. Despite this, no controlled clinical trials assessing the efficacy of these agents were conducted no sysematic data was accumulated on their efficacy, consequently clinicians had no controlled data on which to

base therapeutic decisions for SARS-CoV-2. Agents of unproven efficacy and definite toxicity are being used for COVID-19 without the accumulation of good data on their efficacy, and are again becoming “standard of care” in the absence of good evidence. The clinical trails have not implemented suggestions for large scale collaborative efforts, systematic prospective, controlled trials that were recommended after SARS-COV epidemic.

The problem with the current approach is that it is essentially working harder to apply inadequate approaches that have never succeeded on the required timescale. The pre-clinical models are also slow, and typically have "low translational value". The current approach in many ways skips pre-clinical work to accelerate clinical trials. This is very hazardous, especially for vaccines, where the phenomenon of antibody-dependent enhancement is a serious and underappreciated risk.

In COVID-19 both the WHO and CDC have faced their *raison d'être*, but their performance and candor was lacking. The most likely result of the current trajectory is lowering of public expectations about the time-frame for results, as well as risks of treatments and vaccines. Doctors commenting on the CDC's performance described it as: “They let us down”, “The C.D.C. is no longer the reliable go-to place", “Here is an agency that has been waiting its entire existence for this moment,” And then they flub it. It is very sad. That is what they were set up to do.”

This is likely to further weaken the shaken authority of healthcare organizations and pharmaceutical companies. This prognosis is based on prior history, not only with COVID. In *False Positive* a retired doctor Theodore Dalrymple describes the history of organizations like WHO and CDC as well as scientific journals reporting on the introduction of cholera to Haiti in 2010, and other healthcare topics. The same premier journals are also contributing to the confusion surrounding the COVID-19 treatments: both *Lancet* and *New England Journal of Medicine* have published articles critical of chloroquine (CQ) and hydroxychloroquine (HCQ) for SARS-COV-2, and the FDA has retracted its Emergency Authorization (EA) on June 15th citing lack of consistency and low efficacy in "large randomized controlled trial" of 150 patients who were treated with standard of care and hydroxychloroquine, but without use of any identifying biomarkers.

In summary, it can be said that the trial results, as well as most of others are mis-interpreted due to a demonstrably false assumption of possibility of a universal treatment. Specifically, it is not correct to conclude as the FDA did that "it is no longer reasonable to believe that oral formulations of HCQ and CQ may be effective in treating COVID-19". In fact, its logic fails to recognize the highly individual pathology and is misinterpreting bad, blind use of a treatment for a bad drug. Plenty of observations have been made, including those use a the basis for the original authorization to suggest CQ and HCQ have utility for *some* patients.

In stead of changing the approach – introducing biomarkers, and honest communication about the likely time-frame, the regulators and companies are pursuing treatments blindly, attempting to find a one-size fits all treatment, and express undue confidence for the sake of appearances, adjusting expectations later. The downgrading of expectation is likely to further weaken the shaken authority. It is already starting to happen with vaccines: vaccines that do not prevent infection but merely reduce chances of "severe" disease are being sold to the public as a useful solution, while they are likely to pose as much risk of causing severe disease by mechanism of antibody-dependent enhancement.

In short, the traditional approaches have not worked, and are going to continue to fail. Furthermore, continued obfuscation of the real difficulties of the situation: lack of tools and a logical approach are not likely to be acknowledged by the governments, regulators, and large pharmaceutical companies.

1.5 Bottom-up approach

There are many researchers who have been organically building up the tools for combat COVID-19. It might be up to these "grassroots" efforts to form effective coalitions of scientists to build tools, which would be able to keep up with this virus, and provide the world a measure of control for future pandemics. We cannot solve a problem at the same level of thinking at which we created it. There is a need to elevate our approach. The following sections describe several missing pieces in biology and technology.

What terminology is need to overcome our current challenges and to come up with improved technology? It has to do with extracting actionable information from biological measurements, in a word – bio-computation.

2 Bio-computation

2.1 Importance of biomarkers

Imbalanced immune response triggered by SARS-CoV-2 in severe infections leads to cytokine storm. This is a common feature in other recent coronaviruses SARS-CoV-1 and MERS-CoV. One interesting observation about COVID-19 in particular is the sporadic nature of severe infections. This important observation has not founds reflection in the clinical trials. Generally speaking besides diagnostic or observational trails clinical testing in humans is not accompanied by measurements of biomarkers.

Biomarkers are essential to characterize the state of individual immune system, state of disease, prediction of its progression and efficacious treatment. Biomarkers measured during the course of clinical trails can allow retrospective analysis to correlate response. In absence of this information, the study reports average response, which is not efficacious. Biomarkers are the glue that helps to bind multiple studies together. Biomarkers have tripled approval rate of treatments that have a companion diagnostic compared to those applied to all patients.

Critical biomarkers for SARS-CoV-2 cytokine storm include signaling molecules, including some interferons, interleukins, acute phase proteins and cells. There are additional biomarkers important for survival that pertain to blood coagulation, which is induced by the cytokine storm, as well as risk factors including diabetes, and metabolic state.

Majority of existing biomarkers are late predictors, which give short lead time before onset of severe symptoms. Although some efforts to develop biomarkers claim predictive potential, reports of individual clinical experiences generally suggest such biomarkers merely predict mortality, but do not provide effective guidance on treatment. Additional essential biomarkers include immune haplotype, which determines capability of the individual to recognize and combat different viruses, and has been linked to susceptibility to SARS-CoV-2.

2.2 Impact on drug development

The alternative to using biomarkers to identify nuance of COVID-19 infection is to continue down the path of previously failed processes, applying a cudgel when a scalpel is required. This path has not been efficient for drug development in general, and has been ineffective for SARS-CoV-1 and MERS-CoV. For SARS-CoV-2 this approach lacking required nuance has started to bear expected results. World Health Organization (WHO) has called a halt to chroloquine and hydroxychloroquine testing. These drugs have shown promise in some studies, and toxicity in others. Similar results have started to be reported for Remdesivir. A Chinese study has shown no efficacy, while some US studies have lacked placebo controls for reasons of humanitarian

treatment. After the SARS-CoV-1 epidemic scientists have been observed that careful study design and scientific data collection are essential to make progress.

2.3 Impact on vaccine development

Vaccine development for SARS-CoV-1 and MERS-CoV has run into shortage of funding, with industry losing interest after the epidemics waned. In addition, many vaccines *induced* an immunopathology upon challenge with live virus. This critical barrier for safety and efficacy is not discussed. Recently, Moderna has announced the results of it's preliminary trail of mRNA vaccine, which produced antibody in vaccinated patients, and showed basic virus neutralization. This allowed Moderna to drive up its stock price so 30% with some executive immediately cashing out. This benchmark being reported is not very significant milestone. Majority of the vaccines under development were able to meet these benchmarks, and more that 85% resulted in some measure of immunopathology, which could be more dangerous than infection of naive, un-vaccinated hosts.

The immunopathology results strongly suggests that vaccination for a immune-impacting infections like SARS-CoV need to be done very carefully. This requires awareness of the state of the immune system, including the haplotype, which determines the ability of immune system to learn to recognize a particular pathogen through vaccination. Without such diagnostics is is very difficult to find an appropriate dose for an individual. If the dose is too large, there is a possibility of immediate adverse reaction, as has been seen in the first two vaccines for SARS-CoV-2 to be bested. Chinese first in-human clinical trail of vaccine based on adenovirus vector has revealed adverse reaction in almost half of the participants. This is not necessarily indicative of problems with vaccine – initial trails are meant to determine effective dose. A similar reaction was seen in a fraction of volunteers in the Moderna mRNA vaccine trail who received the highest dose.

An insufficient dose is also very dangerous, because the presence of sub-neutralizing antibody can lead to enhancement of infection through a rare viral mechanism of antibody-dependent enhancement (ADE). Coronaviriae appear to broadly share this phenomenon, which is best established in Dengue virus. Through ADE mechanism the viruses are able to infect immune B-cells, which do not have the typical angiotensin receptor (ACE2), but utilize antibody Fc receptor. This is a mechanism that directly attacks the immune system, and can lead to acute immunodeficiency, which in many ways is more hazardous than slow progression to chronic AIDS that follows HIV infection.

It has also been discovered that anbibodies against the spike proteins can stabilize the conformational change the the viral spike trimer undergoes as part of the entry mechanism. This conformational change mimics docking to ACE2 receptor, exposing the viral spike to the transmembrane protease, which enables the entry of the docked virus. This is a particular challenge with sub-neutralizing antibodies. As a consequence, vaccinated individuals can experience a range of potential problems from over-stimulated immune response, to under-stimuated one, both of which are a particular problem for recently emerged human coronaviruses (SARS, MERS).

3 Tools for COVID-19

3.1 Pre-clinical studies and research

Pre-clinical research needs to elevate the physiological relevance of its models, which should include minimal features of COVID-19 disease immunopathology. Specifically, there different types of affected organ, immune cells, inflammatory signals, and the virus itself. Platforms suitable for this research are coming into existence under the name of micro-physiological systems (MPS).

MPS consists of two elements: biological and technological. The biology uses 3-dimensional culture such as organoids, which mimic key functions of a specific organ, with multiple cell types, including stem cells. The technology is needed to provide microfluidic perfusion, which mimics blood flow, feeding these 3D cultures. Several organoid models for have been demonstrated for SARS-CoV-2. They need to be connected to clinical trials.

3.2 Clinical studies

Clinical trials for COVID-19 are following traditional approaches, seeking to find a universal silver bullet. The few intervention clinical trails which include biological measurements are very restrictive, typically basing administration of a specific drug on a level of a single biomarker. These measurements are inadequate to describe the state of immune disease, or the cytokine storm induced by COVID-19. Immune response is specific to the individual, and broadly applicable solution to the immunopathology may be completely unrealistic. There is a real possibility that none of the drugs being tested can be effective without regard to individual's biological state. Biomarkers would reduce risk for the study by providing options for further differentiation of responsive population by describing the disease state.

The equipment necessary for measurement of biomarkers is not available for clinical trails, which typically involve dozens of sites, primarily hospitals, in order to meet patient enrollment requirements. This distributed nature of the clinical trials makes it difficult to have access to certain technologies, such as flow cytometry (FC) at all sites. FC has been the gold-standard for cell-based analysis for research for over 30 years, but its high price, large size, complexity of maintenance and operation, and well as lack of standards and inter-operability have made it virtually inaccessible for clinical trials.

Miniaturization of FC technologies, and new reagents such as quantum dots can address this challenge. These solutions are not yet available, however, technology for integrated microfluidics and optics has seen enough development to place these applications within reach. These systems could be called Bio-Meter, and they provide measurable for bio-computation – measurement of biological state with biomarkers. Biomarkers validated in clinical context would predict effective treatment.

3.3 Data integration

When SARS-CoV-2 first appeared, a group of Chinese scientists carried out a search of available data in existing databases for recent emerge nCoVs. Despite the pressing need and thorough search, the authors were forced to draw the conclusion that there was a "very low quality of evidence and wide heterogeneity of interventions and indications" as a results of which they "could not draw a clear conclusion for the recommendation of potential therapies".

Biomarkers are the factor that could allow integration of small data sets. For this purpose, the number of biomarkers should not simply be a minimally required set for area-under-the-curve analysis. Analytical models seek to account for that accounts for the complexity and variability observed the data, so redundant factors are eliminated. For the integration of data sets from research publications or clinical trials, a superset of biomarkers is required. This will allow reconciliation of datasets when either one of the the reduntant factors is utilized. Acquisition of more than a minimal set of biomarkers worthwhile effort, as many as possible should be measured.

4 Conclusions

COVID-19 leads to a highly individual response, characterized by cytokine storm hyperinflammation, which depends on a series of individual factors, but these biomarkers of risk and disease progression are not adequately tracked, especially in clinical trails. Traditional pre-clinical models lack physiological relevance to model immunopathology, and are also very slow. Clinical trials are largely approaching treatment without biomarkers – blindly. There is an urgent need to improve both processes.

We have described the unifying role of biomarkers for connection of pre-clinical research and clinical trials, and the two types of systems are needed to accelerate the pace of bio-computation. MPS platforms can accelerate basic research, and allow thorough pre-clincial testing before human trials, while developing actionable biomarkers. These biomarkers can be validated in clinical trails producing companion diagnostics for COVID-19 that would prescribe treatment based on objectively measured pathological state.

The emphasis on speed in the recently announced USA "Warp Speed Project" for development of vaccine for COVID-19, aiming to provide bold political leadership to address COVID-19 could lead to unmet expectations through underestimation of underlying challenges and risks. The under-developed treatments, especially vaccines, going into large-scale clinical trials without biomarkers risk not only high-visibility failure, but inability to meaningfully combine results of different efforts, and to identify responder populations. Without biomarkers they might yield little to no useful results. In addition to risk to patients, this would impact credibility of the pharmaceutical industry and its regulators.

Top-down approach appears ill-suited to rapid change of approach that is required in development of drugs, or communication of the challenges. It is up to research organizations and pharmaceutical companies, or more likely, maverick scientists there, to drive for adoption of technologies to acquire longitudinal biological measurements to identify biomarkers that predict effectiveness of treatment. The general public can support this effort by demanding more transparency of risks, more effective approaches and accountability. The pressure to produce results quickly need to change to focus on safety and efficacy, and correct approach.