

The Need for Precision Medicine in Active and Post COVID-19

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As a pathologist and virologist, and having incorporated oncology over the past 30 years in academia and industry, I find it puzzling that precision medicine has not been used extensively in this very complex and heterogeneous disease, COVID-19. If this were cancer, we would have abundant data identifying mutations that support certain therapeutics and other biomarkers that are incorporated into algorithms that determine prognosis or survival. The time has come to have these types of diagnostics and prognostics available for both improved patient care and resource utilization. I will discuss the state of current diagnostics, as well as what is coming, so we can better serve our patients and give our healthcare professionals the information they need to make decisions and better triage patients that are consuming our hospital space.

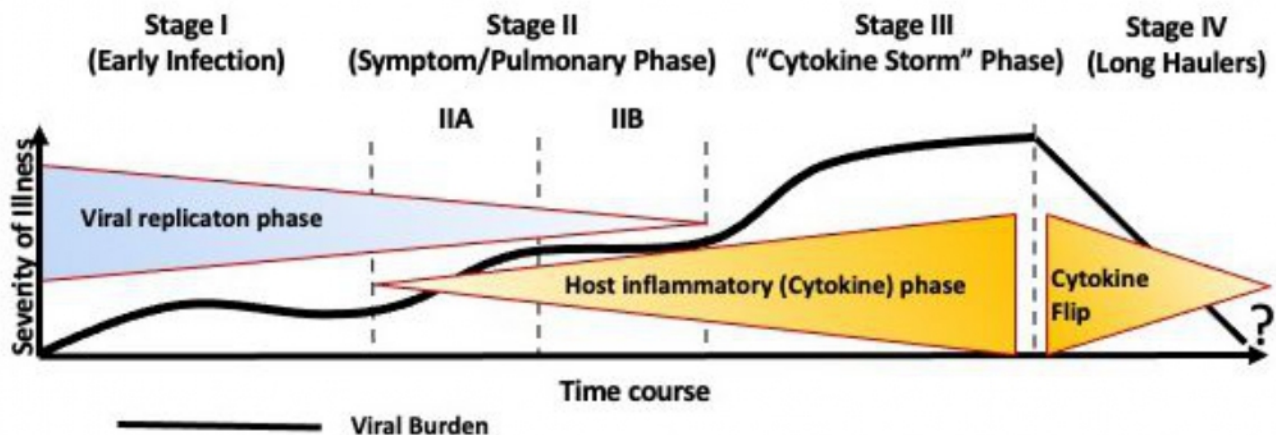
DIAGNOSTICS

At the beginning of the pandemic, I believed that there would be three phases of diagnostics necessary for providing the information needed to detect and triage infected individuals. A fourth phase for virus variant detection for surveillance is now becoming a necessity as well, but will not be discussed here. First, assays (presumably molecular) that would answer the question if one is infected, yes or no. Next, serologic assays that would answer the question "have you been infected?" and possibly determining whether the infection was recent (2-4 weeks) using IgM, or past (6-8 weeks) using IgG. In late January and early February 2020, IncellDx validated a panel of over 100 biomarkers, both cell-based and plasma-based, to elucidate the immunologic response to SARS-CoV-2, which had never been seen by the body's immune system. This work was necessary for what we believed was the third phase of critical diagnostics necessary in the fight against the pandemic - precision diagnostics to guide therapeutics and predict who will become severe.

As those of us trained in lab medicine are aware of and based on my past experience as the Director of Clinical Laboratories at Stanford Hospitals and Clinics, managing diagnostic test performance can be a challenge. The testing laboratories are inundated with tests reporting a certain sensitivity and specificity, and that is where laboratory medicine is critical. What is commonly reported is analytical sensitivity and specificity. What does that mean? Analytical sensitivity and specificity are numbers generated during product development using artificial models of a clinical sample. In other words, using different known quantities, an analyte (virus, in the case of SARS-CoV) is spiked into a buffer (e.g., normal blood or normal plasma) to establish how few copies can be detected (analytic sensitivity) and how analytically specific the assay is if other unrelated pathogens are spiked into the buffer. With PCR and other molecular technologies, these numbers tend to be very high (>90%). What is missing from this artificial system used in assay development is all the elements related to sampling a patient, including the type of swab, the type of sample, how long it takes for the sample to be transported to the lab (sample stability), sample processing once it reaches the lab, and any sample temperature dependence on the assay. All of these variables are incorporated into the most important test metric in real life: clinical sensitivity and clinical specificity. By taking the entire process into the performance, from the minute the swab hits the nose to the minute a patient gets results, healthcare providers get the most accurate test performance metrics.

Now that healthcare providers have clinical sensitivity and clinical specificity information, the most important test metric for management decisions can be generated - positive and negative predictive values (PPV/NPV). Essentially, these metrics mean if a

FIGURE 1.



test is positive, what are the chances that it is actually positive (i.e., false positive) or if a test is negative, what are the chances that it is actually negative" (i.e., false negative)? In COVID-19, a significant number of false positives and false negatives need adjudication, and the only way to adjudicate these errors has been repeat testing and quarantining, which has been a tremendous burden on the healthcare system.

Similarly, SARS-CoV-2 serology testing has also been variable with both false positive and false negative results. An in-house study by IncellDx of COVID-19 long hauler patients revealed that 27% of patients included in our immunologic study reported having no SARS-CoV-2 antibodies despite positive COVID-19 molecular testing.

THERAPEUTIC DETERMINATIONS AND MONITORING

This aspect of diagnostics is critical and demands the lessons and success seen in precision medicine for cancer. COVID-19 is an incredibly complex disease with many phases (Figure 1). The symptoms and presentation of patients have been used to develop the following criteria for different severity categories.

Mild

1. Fever, cough, sore throat, malaise, headache, myalgia, nausea, diarrhea, loss of taste and smell
2. No sign of pneumonia on chest imaging (chest X-ray or CT scan of chest)
3. No shortness of breath or dyspnea

Moderate

1. Radiological findings of pneumonia, fever, and respiratory symptoms
2. Saturation of oxygen (SpO₂) ≥94% on room air at sea level

Severe

1. Saturation of oxygen (SpO₂) <94% on room air at sea level
2. Arterial partial pressure of oxygen (PaO₂)/ fraction of inspired oxygen (FiO₂) <300mmHG
3. Lung infiltrate >50% within 24 to 48 hours
4. HR ≥125 bpm
5. Respiratory rate ≥30 breaths per minute

Critical

1. Respiratory failure and requiring mechanical ventilation, ECMO (extracorporeal membrane oxygenation), high-flow nasal cannula oxygen supplementation, noninvasive positive pressure ventilation (BiPAP, CPAP)
2. Septic shock, systolic blood pressure <90mmHg or diastolic blood pressure <60 mmHg or requiring vasopressors (levophed, vasopressin, epinephrine)
3. Multiple organ dysfunction (cardiac, hepatic, renal, CNS,

thrombotic disease)

Post-acute COVID-19 (Long COVID)

1. Extending beyond 3 weeks from the initial onset of first symptoms

Chronic COVID-19

1. Extending beyond 12 weeks from the initial onset of first symptoms

These disease status definitions, though helpful, do not provide any information on the status of the underlying pathology. Figure 1 attempts to define the continuum of active COVID-19, at least in a general way. As therapeutics are being developed, and starting to be approved, it becomes even more important to understand the underlying pathology in the context of drug targets. In other words, anti-viral drugs should be used when there is ongoing viral replication and immunomodulating drugs should be used when the immune system becomes chaotic and pathogenic. The classifications above certainly do not inform drug choice in a precise manner as laboratory data would. To that end, we have analyzed over 1000 samples from COVID-19 infected individuals that span the spectrum of disease demonstrated in Figure 1. We looked at both cellular immune profiling and plasma-based immune profiling to feed into our machine-learning algorithm, trying to find immune predictors of disease status and potentially predictors of disease progression. Ultimately, we would like to answer the following questions:

- What drugs would work at what point in the disease process?
- When to start therapy with a particular class of drugs?
- How long should a patient be treated?
- How is an active COVID-19 patient different from someone experiencing long COVID-19 symptoms?
- How do we treat long COVID-19?

To begin defining the immunopathology of the different phases of COVID-19 as shown in Figure 1, we performed machine learning on data sets from a panel of plasma cytokines and chemokines. These important inflammatory markers are essential elements of the innate and adaptive immune responses. As demonstrated in Figure 2, our first assessment of over 300 normal, mild-moderate, severe, and long hauler cytokines and chemokines yielded some fascinating results. We were able to make immunologic distinctions between normal individuals, severe active COVID-19, and long haulers. The mild-moderate group was a composite of normal-like, severe, and somewhere in between, therefore, immunologically heterogeneous. The severe group could be distinguished from mild-moderate by the severity score which is $(10 \cdot IL-10 + IL-6) - (IL-2 + IL-8)$. Using such a score could help triage patients who are beyond mild-moderate (by the symptomatic definitions above) and might require the initiation of immune therapy to prevent progression. Similarly, reduction of the score may help to determine when to stop, or taper, immune therapy. Of course, an assessment of viral replication or viral load would also help to determine the transitions between phases and the appropriate time to discontinue anti-viral therapy. As we previously published, plasma viral load is quantitative and is almost universally detected in severe/critical patients, which

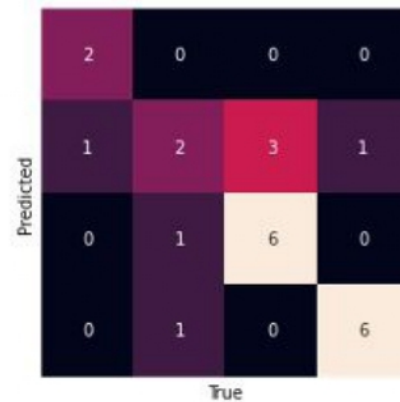
is when this information would be useful, especially with the widespread use of immunosuppressive drugs like dexamethasone.

Using a similar machine learning approach, we modeled the immunologic abnormalities in COVID-19 long haulers. Our initial impression of these patients with symptoms long after their COVID-19 diagnosis was that their symptom complex was incredibly heterogeneous and it remains so to this day. Much to our surprise the computer modeling revealed an immunologic signature (long hauler index-LHI) that shows that long haulers are distinct immunologically, and we can use this information to treat in a precision medicine manner guided by quantitative, non-subjective, metrics. Follow up on studies of now over 300 long hauler patients has allowed us to link specific symptoms with a specific cytokine or cytokines, a term we call Symptom Specific Cytokines (SSC) as shown in Table 1. Similarly, we are analyzing treatment data in another analysis to determine what treatments reduced which cytokines so that, ultimately, we can support tailored therapy, based on the cytokine/chemokine profile, to restore normal immunity and recovering in these long-suffering patients.

In summary, Precision Medicine absolutely needs to be deployed in COVID-19 and, to date, there isn't much evidence that physicians are provided with all the necessary information to manage patients now and certainly in the near future when more therapeutics will become available. Furthermore, many unknowns still remain concerning the extent and length of protection conferred by vaccines. Again, this creates another opportunity for vaccine monitoring that will inform future versions. These include sequencing of new variants to see if mutations fall in the immunogenic domains, assessment of neutralizing antibody activity over time, and assessment of cell-mediated immunity duration. Some of these assays are complex but the progress of high complexity assays in cancer diagnostics gives hope that we can have a full complement of precision diagnostics to guide the future interventions in this pandemic.

1. Patterson BK, et al. CCR5 inhibition in critical COVID-19 patients decreases inflammatory cytokines, increases CD8 T-cells, and decreases SARS-CoV2 RNA in plasma by day 14. *Int J Infect Dis.* 2021;103:25-32.
2. Patterson BK, et al. Immune-Based Prediction of COVID-19 Severity and Chronicity Decoded Using Machine Learning. *bioRxiv.* 2020. doi: 10.1101/2020.12.16.423122
3. Turner JS, et al. SARS-CoV-2 viral RNA shedding for more than 87 din an individual with an impaired CD8+ T cell response. *Front Immunol.* 2021 Jan 8;11:618402. doi: 10.3389/

FIGURE 2.



Overview of metrics for Multi-class classifier

	precision	recall	f1-score
Long Haulers	1.00	0.67	0.80
M-M	0.29	0.50	0.36
Normals	0.86	0.67	0.75
Severe	0.86	0.86	0.86

TABLE 1.

	TNF-α	IL-4	IL-13	IL-2	GM-CSF	sCD40L	CCL5	CCL3	IL-6	IL-10	IFN-γ	VEGF	IL-8	CCL4
Fatigue	WNL	+	-	+	-	WNL	WNL	+	+	+	+	+	-	WNL
Myalgias	WNL	WNL	WNL	WNL	-	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL
Chills	WNL	WNL	WNL	+	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL
Brain Fog	WNL	+	WNL	+	-	WNL	WNL	+	+	+	+	+	WNL	-
Arthralgia	WNL	WNL	WNL	+	-	WNL	WNL	WNL	WNL	WNL	WNL	WNL	-	WNL
Burning Sensation	WNL	WNL	WNL	+	WNL	WNL	WNL	+	+	+	WNL	WNL	WNL	WNL
Chest Pain	WNL	WNL	WNL	+	WNL	+	WNL	+	+	+	+	+	WNL	WNL
Tachycardia	WNL	WNL	WNL	+	WNL	WNL	WNL	WNL	+	WNL	WNL	WNL	WNL	WNL
Cough	-	+	WNL	+	-	WNL	WNL	+	+	+	+	WNL	WNL	WNL
SOB	WNL	WNL	WNL	WNL	-	WNL	WNL	+	+	+	+	+	WNL	WNL
Neuropathy	WNL	WNL	WNL	+	-	WNL	WNL	WNL	WNL	+	+	+	WNL	-
Rash	WNL	WNL	WNL	+	-	WNL	WNL	WNL	WNL	+	+	WNL	WNL	-
GI	WNL	WNL	WNL	+	WNL	WNL	WNL	+	+	+	+	+	WNL	-
Insomnia	WNL	+	WNL	+	-	WNL	WNL	WNL	WNL	+	+	WNL	WNL	-
Loss of Taste or Smell	WNL	WNL	WNL	+	-	WNL	WNL	+	+	+	+	WNL	WNL	-
Headache	WNL	+	WNL	+	WNL	WNL	WNL	+	+	+	+	+	WNL	WNL