COVID-19 Risk: Clinical Tools for Assessing and Personalizing Immunity

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Abstract

Researchers and clinicians all over the world are struggling to bring the spread of SARS-CoV-2 under control. Whether we refer to COVID-19 as a *pandemic* (a widely used word) or a *syndemic* (a new emerging term), we now know that specific immunotypes have been linked to both risk to infection and presentation of this disease. Application of assessment tools that support a fuller understanding of individual immune system status is next-level care that providers must prepare for and deliver. Commonly used biometric devices already gather data that can be relevant and useful to a phenotypic evaluation of immune function. These variables include pulse rate, blood oxygenation, sleep cycles, respiration rate, heart rate variability, continuous blood glucose monitoring, and ambulatory blood pressure. When coupled with traditional blood analytes and measurements of nutrient status, a more complete picture of immunological function may be revealed. Innovative questionnaires and algorithms can also be helpful additions to a clinician's toolkit. In a therapeutic relationship between provider and patient, this approach may lead to options for personalized immune intervention using diet, medical nutrition, and lifestyle medicine.

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Have we all been thinking about COVID-19 the wrong way? Medicine is a field that depends heavily on categories, and within those categories, there is a hierarchy of words and phrases that clinicians use to contextualize information. Think back to the first time you heard about COVID-19. Since the earliest days of media coverage, this virus has been intrinsically linked to one word: *pandemic*. The depth of the COVID-19 problem—its persistence, its impact, all of the controversies that surround it—has led some health leaders to view the situation through a new lens. Richard Horton, Editor-in-Chief of *The Lancet*, is one of them. In an editorial he published in the fall of 2020, Mr. Horton suggested that COVID-19 is not a *pandemic*, but rather a *syndemic*. *Syndemic*, he explained, is a descriptor that leaves behind the narrow approach of managing an outbreak by cutting the lines of viral transmission and better reflects the complexity of SAR-CoV-2. This is a worldwide health crisis that represents two categories of disease that are simultaneously and synergistically interacting: an infectious virus plus an array of non-communicable diseases. In specific populations, we are seeing this combination have a deleterious impact on immune function and a catastrophic outcome on lives.[1]

By now, we are all familiar with the pre-existing immune risk factors in the non-communicable disease category. They include hypertension, age, gender, diabetes or insulin resistance, cardiovascular disease, and respiratory disorders. Additional variables that can result in alterations in immune function and an increased risk to infection with SARS-CoV-2 are the use of immune suppressant medications, exposure to environmental xenobiotics, and well-known social determinants of disease such as stress, racism, poverty, and poor quality diets. These factors can also play a significant role in the severity and duration of COVID-19 symptoms.[2,3]

Mechanistically, this constellation of risk factors has been associated with the unique genetics of the SARS-CoV-2 spike proteins, which are the structures that allow the virus to attach to the mucosal surfaces of the host and gain access to the mucosal cell's physiology.[4] Immunological research that pre-dates the emergence of COVID-19 demonstrated that alterations in immune function associated with these factors can have a significant adverse impact on response to vaccination.[5] As I write this article, the first shipments of COVID-19 vaccines are being administered all around the world. Many researchers—myself included—will be watching the data about vaccine effectiveness as it becomes available in the months ahead.

What Can Clinicians Do Right Now?

Our understanding of the many factors that can adversely influence immune function signals a need for clinical tools that support the accurate assessment of patients, and guide us in personalizing interventions to optimize immune vigilance. Recently, it has been found that data collected by certain types of wearable biometric devices can provide insights into changes in immune system function and perhaps lead to early detection of COVID-19.[6] These devices have evolved to be quite sophisticated. Metrics such as increases in respiration rate, sleep disturbances, percent oxygen saturation, heart rate, nighttime body temperature, fitness levels, and alterations in heart rate variability have all been found to contribute to an early warning assessment of COVID-19. How? During the initial stages of a viral infection, activation of the immune response has been associated with a higher respiration rate during sleep, lower blood oxygen saturation, an accelerated pulse rate, an increased body temperature at night, and decreased heart rate variability. All of these parameters have a mechanistic connection to alterations in immune system function. This is research that a team at the Scripps Translational Institute is taking an intense interest in through their work on the Digital Engagement and Tracking for Early Control and Treatment (DETECT) study. Lead investigator, Giorgio Quer, PhD, offered the following quote in a December 2020 interview: "DETECT could play an important role in alerting individuals that they may have contracted the virus."[7]

In recent years, emerging research has demonstrated the existence of different immunotypes. In 2020, it was quickly recognized that identifying the immunotypes of patients with COVID-19 infection could result in personalization of their therapy.[8] Last fall, a particularly compelling article was published in *Science* with the following title: "Deep Immune Profiling of COVID-19 Patients Reveals Distinct Immunotypes with Therapeutic Implications." The authors of this piece state that individual immunological function can vary not only due to genetics, but—to a larger extent—as a result of environmental perturbations, which would include factors that influence microbiome composition and gastrointestinal immune system function.[9]

My research group has recently developed a simple questionnaire to evaluate the clinical history of an individual's immunophenotype and correlate this information with four different immuno-identities (see Figure 1). Our algorithm was developed from testing several hundred individuals, and we opted to use descriptive words to represent the immunophenotypes: Angry, Confused, Sensitive, and Withdrawn. Each of these immuno-identities is associated with a specific imbalance of the innate and/or adaptive immune systems. It is our belief that this questionnaire provides a useful first-level interrogation of individual immune system function. It represents a qualitative evaluation of immuno-identity that can guide further evaluation of immune system function using the additional biomarkers and epigenetic assessment tools that follow.

Figure 1. Immuno-Identity™ Questionnaire

1. I suffer from symptoms related to autoimmune disease (these are conditions where the immune systems attacks the body).

Not At All	A Little Bit	Sometimes	Quite A Bit	Very Much
☐	☐	☐	☐	☐

2. I am concerned that I could have autoimmune issues.

Not At All	A Little Bit	Somewhat	Quite A Bit	Very Much
☐	☐	☐	☐	☐

3. I have issues with itchy, watery eyes.

Not At All	A Little Bit	Somewhat	Quite A Bit	Very Much
☐	☐	☐	☐	☐

4. I have issues with itchy or otherwise irritated skin.

Not At All	A Little Bit	Somewhat	Quite A Bit	Very Much
☐	☐	☐	☐	☐

5. I have issues with sneezing, congestion or a runny nose.

Not At All	A Little Bit	Somewhat	Quite A Bit	Very Much
☐	☐	☐	☐	☐

6. I have issues with low mood.

Not At All	A Little Bit	Somewhat	Quite A Bit	Very Much
☐	☐	☐	☐	☐

7. I am concerned that I feel or look older than other people my age.

Not At All	A Little Bit	Somewhat	Quite A Bit	Very Much
☐	☐	☐	☐	☐

8. I feel that I am under chronic stress.

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

9. I have GI issues like pain, bloating, cramps, diarrhea or constipation.

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

10. Do you or your healthcare provider have concerns about your blood pressure?

Not At All	A Little Bit	Somewhat	Quite A Bit	Very Much
☐	☐	☐	☐	☐

11. Do you or your healthcare provider have concerns about your blood sugar?

Not At All	A Little Bit	Somewhat	Quite A Bit	Very Much
☐	☐	☐	☐	☐

12. Do you or your healthcare provider have concerns that you are overweight?

Not At All	A Little Bit	Somewhat	Quite A Bit	Very Much
☐	☐	☐	☐	☐

13. Do you or your healthcare provider have concerns about your thinking or memory?

Not At All	A Little Bit	Somewhat	Quite A Bit	Very Much
☐	☐	☐	☐	☐

14. Are you or your healthcare provider concerned that you get frequent or prolonged infections?

Not At All	A Little Bit	Somewhat	Quite A Bit	Very Much
☐	☐	☐	☐	☐

Using Biomarkers to Assess Immune Function

Tools used to assess aspects of immune status can be divided into two categories: surrogate markers of immune-related parameters and biomarkers of specific immune functions. The function of an individual's immune system is, in part, dependent upon the quality of their diet.[10] Nutritional status, therefore, plays a very important role in an individual's defense against SARS-CoV-2 infection.[11] An expert on this topic, Dr. Philip Calder, published an excellent review article in 2020 in which he discussed our need for specific nutrients that are required to support immune cell function increases during times of immune system stress. This would include vitamins A, C, D, and E, as well as the B vitamins, zinc, selenium, and omega-3 fatty acids.[12] Assessing these nutrient levels is an essential step in evaluating the functional status of the immune system. This valuable information would be considered a surrogate marker of immune cell function.

Vitamin D status is a topic that warrants additional attention due to its particular importance in supporting both innate and adaptive immune function.[13] It can be evaluated using the 25-hydroxyvitamin D3 serology test. This test represents a surrogate biomarker for determining vitamin D status because the active hormonal form of vitamin D is 1,25-dihydroxy vitamin D3 calcitriol, which is produced in the kidney from the 25-hydroxy form. The precursor—25-hydroxy vitamin D3, with a long half-life of 15 days—is measured due to the short 15-hour half-life of the 1,25 dihydroxy form.

Zinc represents another very important nutrient for the support of immune function. The best surrogate measure for zinc status is the zinc tolerance test. In 1978, it was reported that zinc supplementation resulted in improved taste tolerance in aged individuals.[14] No specific mechanistic explanation was provided at that time as to how zinc influenced taste perception, and—40 years later—there is still uncertainty about the connection between zinc deficiency and reduction in bitter taste threshold to a dilute zinc sulfate solution challenge. It was reported in a clinical study that the reduced response to the "zinc taste test" composed of a 0.1% solution of zinc sulfate in water was correlated with reduced dietary zinc intake.[15] The clinical accuracy and precision of the zinc taste test in evaluating zinc status was challenged in a 2012 study, but was later improved with the introduction of a taste-intensity visual analog scaling protocol in 2015.[16,17]

Along with zinc, dietary intake of omega-3 fatty acids has been identified to be very important for improving immune defense against viral infection.[18] The Omega-3 Index blood spot test is one method that has been used to determine omega-3 fatty acid status.[19] This test utilizes a finger stick procedure to capture a drop of blood on a paper collection system for analysis of red cell membrane bound fatty acids. An omega-3 index above 7% has been found to be associated with reduced inflammatory immune profiles.[20] Dr. Charles Serhan is a leading researcher who has reported that the conversion of omega-3 fatty acids by the immune system into specialized pro-resolving mediators (SPMs) is very important in regulating the inflammatory process and may also play a role in the immune response to viral infections.[21] Minimally processed cod liver oil has been found to contain increased levels of the precursors to the SPMs, 17-hydroxydocosapentaenoic acid and 14-hydroxydocosapentaenoic acid, as well as immune active forms of vitamin A and vitamin D. Studies are now underway to evaluate the immune-associated effects of specialized pro-resolving SPMs in the management of inflammation and SARS-CoV-2 infection.[22]

It is recognized that type 2 diabetes represents a significant risk factor for COVID-19 and that blood glucose control is important in reducing the risk to SARS-CoV-2 infection.[23] Insulin resistance associated with type 2 diabetes has been identified to result in altered immune function and a state of chronic inflammation.[24] It's clear that the control of post-prandial glycemia is important in normalizing immune system function through the application of personalized nutrition.[25] Personal glycemic response to various foods can now be measured utilizing continuous glucose monitoring wearable biometric devices such as the Abbott Freestyle Libre or the Dexcom G6 CGM systems.[26] In 2019, a systematic review and meta-analysis was published that indicated intervention with a plant-based, Mediterranean-style dietary pattern results in improved glycemic control, a reduction in inflammation, and normalization of immune biomarkers. Systemic immune biomarkers evaluated included high sensitivity C-reactive protein (hsCRP), fibrinogen, and total leukocyte count, all of which were lower in the plant-based dietary group.[27]

C-reactive protein (CRP) is an acute-phase protein that is produced in the liver through activation by interleukin-6 produced systemically. CRP interacts with Fc receptors on phagocytic cells and acts as an opsonin, thus the main biological function of CRP appears to be host defense against bacteria, viruses, and endotoxins.[28] Elevated CRP is often clinically associated with an increase in the neutrophil-to-lymphocyte ratio. In conditions of insulin resistance, metabolic syndrome, and hypertension associated with alteration in the immune system through activation of the NLR inflammasome, there is an associated increase in both high sensitivity CRP and the neutrophil-to-lymphocyte ratio.[29] Increased neutrophil-to-lymphocyte ratios have been found to represent a risk factor for COVID-19 in middle-aged individuals without other comorbidities.[30] The combination of an elevated level of hsCRP and an increase in the lymphocyte-to-neutrophil and platelet-to-lymphocyte ratios has been shown to predict the severity of COVID-19.[31,32] In one study of a representative older population, elevated neutrophil-to-lymphocyte ratio was found to be associated with increased abdominal obesity and poor eating habits, suggesting it is a potential biomarker for evaluating personal lifestyle and dietary determinants of immune dysfunction.[33]

Recently, work at the National Institutes of Health has indicated that individuals with metabolic syndrome have increased risk to both macro- and microvascular coagulopathies associated with alterations in immune function that result in poor prognoses.[34] The key prognostic biomarkers of immune dysfunction associated with this risk of coagulopathies include elevated plasma ferritin, D-dimer, and homocysteine levels.[35] In a healthy patient population with SARS-CoV-2 infection, an evaluation found that those who had elevated levels of fibrinogen associated with altered immune function early in the infection had much poorer outcomes.[36] It is well known that plasma fibrinogen is a surrogate marker for inflammation of the vascular system and is associated with the risk of major cardiovascular diseases.[37] A 2019 study showed that elevated plasma fibrinogen is associated with increases in hemoglobin A1c and immune dysfunction that results in the chronic inflammation of type 2 diabetes.[38]

Lastly, evaluation of mucosal immunity through salivary IgA testing is an important analyte for the evaluation of immune function related to protection against SARS-CoV-2 infection. It has been reported recently that salivary anti-SARS-CoV-2 IgA is an accessible biomarker of mucosal immunity against COVID-19.[39] It is well recognized that mucosal immunity, including secretory IgA, plays an important role in host defense against respiratory pathogens. The non-invasive nature and ease of saliva collection presents the testing of salivary IgA as a valuable surrogate biomarker of mucosal immune function.

Mucosal immune defense is also related to the integrity of barrier functions of the mucosal surfaces in the respiratory and gastrointestinal systems.[40] There are a number of biomarkers used to assess gut mucosal integrity, including plasma zonulin and occludin levels. It has recently been reported that retinoic acid derived from vitamin A improves baseline mucosal barrier function and reduces TNF-alpha-induced barrier dysfunction in human bronchial cells (TNF-alpha is an inflammatory cytokine).[41] Vitamin A supplementation was found to improve the intestinal mucosal barrier and tight junction protein zonulin levels in rodents with diarrhea.[42] In humans, low-grade endotoxemia has been found to be associated with altered serum zonulin levels and increased coagulation factors in patients in the early phase of pneumonia.[43] In patients with type 2 diabetes, increased plasma levels of bacterial lipopolysaccharides associated with endotoxemia and elevated levels of serum zonulin signified alterations in immune system function associated with inflammation.[44]

Calprotectin is an analyte used to assess immune system imbalance and inflammation. Fecal levels of calprotectin have been found to reflect immune activation of the gastrointestinal immune system in patients with viral infections.[45] Elevation in fecal calprotectin due to gastrointestinal mucosal inflammation associated immune activation creates the systemic inflammation of inflammatory arthritis.[46] The studies cited here—as well as numerous others—point to the clinical importance of determining mucosal barrier status in immune function assessment and giving consideration to its association with diet and microbiome composition.

Epigenetic Biomarkers Associated with Immune Function

Epigenetic modifications that regulate the expression of genes are associated with immunophenotype and immune system function. Interestingly, telomere length of genes within the cells of the immune system is an indirect surrogate measure of immune competency.[47] Telomere length is most frequently measured as an average value in heterogeneous peripheral blood leukocytes. Reductions in peripheral blood leukocyte telomeres has been shown to be associated with immune system aging (or immunosenescence). It has been demonstrated that a personalized lifestyle intervention program of diet, exercise, and stress reduction can increase telomere length and improve immune function.[48]

Research has also demonstrated that specific methylation patterns of the immune cell genome are associated with immunosenescence and altered immune function.[49] Analysis of the methylation pattern of specific genes in immune cells provides information about the biological age of the immune system. The most interesting sites for altered genomic methylation within the immune system are those genes that regulate innate immunity and inflammation. Alterations in the methylation patterns in these regions of the immune genome produce the immunophenotype associated with immunosenescence, which is linked to reduced protection against infection and increased systemic inflammation (also known as inflammaging).[50] Inflammaging is an immune risk factor that is modifiable through the application of personalized diet and lifestyle intervention.[51,52] This emerging field of research is important to watch, and inclusion of an epigenetic analysis in the portfolio of clinical tools for assessing immune function represents forward thinking about the personalization of patient care.

Summary

The development of new assessment tools for the evaluation of immune system function has opened the door for personalizing immune intervention in individuals at risk to SARS-CoV-2 infection. The immune assessment tools discussed in this review include:

- Immuno-Identity™ Questionnaire
- Biometrics using wearable devices:
  - Pulse rate
  - Body temperature at night
  - Oxygen saturation
  - Sleep cycles
  - Respiration rate
  - Heart rate variability
  - Fitness level

- Vitamin D, zinc, and omega-3 fatty acid assessment
- Glycemic response assessment using continuous glucose monitoring
- High sensitivity C-reactive protein assessment
- Neutrophil-to-Lymphocyte ratio
- Plasma ferritin
- Salivary secretory IgA
- Plasma zonulin
- Fecal calprotectin
- Leukocyte telomere length
- Whole blood genome methylation age

Whether we refer to this complex time as the era of *pandemics* or *syndemics*, the application of these assessment tools to gain a better understanding of the immune status of the individual is next-level care that providers must prepare for and deliver. Personalized immune intervention using diet, medical nutrition therapy, and lifestyle medicine represents an approach that can shift patient outcomes, both now and in the future.

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