

Responses to Participants' Questions

The overarching goal of the R3 Seminars is to catalyze a shared understanding of the research being conducted by the scientific stakeholder community within the RECOVER Consortium. The R3 Seminars and the Q&As typically feature highly scientific material intended for researchers and clinicians. For other audiences interested in these topics, a link to the National Library of Medicine's MedlinePlus medical dictionary is provided at the end of the Q&As as a resource to help in understanding the scientific terminology.

This document provides responses* to questions raised by webinar participants related to the following presentations at the R3 Seminar *Understanding the Biomarkers of PASC* held on November 8, 2022:

- ***Presentation 1: Biomarkers for PASC: Why They Are Needed and Early Immunologic Findings***
Michael Peluso, MD
- ***Presentation 2: Ultrasensitive Viral Antigen Measurements in PASC Patients***
David Walt, PhD
- ***Presentation 3: Markers of Fungal Translocation Are Elevated During PASC and Induce NF- κ B Signaling***
Mohamed Abdel-Mohsen, PhD
- **Discussant: Grace McComsey, MD**

* Responses may have been edited for clarity.

All Presenters: Questions and Responses

Q. How would you explain that fungi but not bacteria are translocating?

Response:

Dr. Abdel-Mohsen: I don't think fungi but not bacteria translocated. I think we just didn't find evidence of the bacterial translocation. This could be due to the sensitivity or the kinetics of the marker we use to identify bacterial translocation compared with fungal translocation. For example, lipopolysaccharide binding protein (LBP) has been shown to be more sensitive to circadian rhythm when the samples were collected after meals, whereas the marker for fungal translocation is more stable. I don't think there's a possibility of a fungal translocation without a bacterial translocation. It's a classic case of lack of evidence is not evidence of lack. I think that both happen, but we need to find a better way to identify the bacterial translocation, which we're working on.

Q. Do you think your findings are specific to SARS-CoV-2 infection or if they may apply to other post-viral syndromes?

Response:

Dr. Abdel-Mohsen: PASC or Long COVID are great examples of post-acute syndromes associated with other viral infections. There's a great recent review by Akiko Iwasaki and her group that describes this ([Unexplained post-acute infection syndromes | Nature Medicine](#)). I think that microbial translocation and gut inflammation in general could contribute to the post-acute syndrome of multiple other viral infections. We know that it contributes to HIV, for example. That's a common theme, even after long-term suppressive therapy. It seems like it also happens with PASC, and I'd bet that it also happens with multiple other viral infections that lead to chronic inflammation.

Dr. McComsey: Could it be that you picked up more fungal [translocations] because you selected for people who are hospitalized? If they're hospitalized with COVID, they get steroids, they get the antibiotics, so you could see that they'll have more fungal translocation rather than bacterial. Was that the case with your population?

Dr. Abdel-Mohsen: No, because most of the individuals in the Long-term Impact of Infection with Novel Coronavirus (LIINC) study cohort were nonhospitalized during their acute COVID or PASC to avoid this potential bias. I think it has something to do with the kinetics of the marker, when we measured it, and how we measured it that may have caused us to miss the bacterial translocation more than the fibrillation itself. I think both are there, but that we didn't detect it yet. We're working on that.

Q. Do any of the speakers see any probability of overlap between some of the biomarkers; for example, the presence of persistent virus that might affect blood properties and produce microclotting?

Response:

Dr. Peluso: Yes, the schematic that I showed [in my presentation] was the most simplistic schematic we could make right now, where everything is in its own silo. But over the next year, what I think we're going to find, and I think that RECOVER certainly will contribute a great deal to our understanding, is that there are a million arrows connecting all those different mechanisms. These arrows might be different for different endotypes of PASC, but it's highly likely that many of these things are interrelated. We'll eventually figure that out.

Dr. Walt: I agree. One of the benefits of RECOVER is that because we're collecting large volumes of samples on many patients, we can run all these assays on the same patient cohort and do exactly that; make those correlations and really understand the relationships between the biomarkers. This can't be done right now simply because given the small sample aliquots obtained from a multitude of biobanks on different patients, there's no way to compare each patient with another.

Dr. Abdel-Mohsen: I'll add [that] most likely many of those markers are interconnected to each other. However, even if they're not interconnected, it's unlikely that one marker or a couple of markers can predict something as complicated as PASC or Long COVID. It's likely that both viral and host markers need to be combined and trained together to identify if they're not interconnected, although I do believe they are.

Q. Many Long COVID studies use record of positive COVID-19 test as an inclusion criterion or to determine past infection, but this excludes many people who did not get tested. Has there been progress made on markers to indicate or confirm prior COVID-19 infection?

Response:

Dr. Walt: The presence of anti-nucleocapsid is a signature for prior infection. This marker is easy to test.

Dr. Peluso: One of the challenges with anti-nucleocapsid antibodies, however, is that they can wane over time. This really depends on the specific platform used to make the measurement. It is possible that someone who had COVID 6 months or a year ago could have a negative anti-nucleocapsid test now. We've shown antibody dynamics in the Long-term Impact of Infection with Novel Coronavirus (LIINC) study in another analysis that compared 12 different platforms ([SARS-CoV-2 antibody magnitude and detectability are driven by disease severity, timing, and assay | Science Advances](#)). It's also important to note that the vaccine produces anti-spike antibodies; consequently, this test is not useful in ascertaining prior infection in a person who's been vaccinated.

Q. Is it possible that ongoing SARS CoV2 replication in the gut could result in both increased permeability and contribute to circulating spike protein, uniting some of these findings?

Response:

Dr. Abdel-Mohsen: Yes, it is possible.

Dr. Walt: Absolutely. We also see circulating spike protein in the blood of children with Multisystem Inflammatory Syndrome in Children (MIS-C) caused by leakage from the gut. See the following article for more detail: [JCI - Multisystem inflammatory syndrome in children is driven by zonulin-dependent loss of gut mucosal barrier](#)

Q. Have any biomarkers been shown to be correlated with a Long COVID symptom flare? If so, would an elevation of these biomarkers suggest a need for any specific treatment, such as corticosteroids for patients whose Long COVID symptoms seem to be primarily autoimmune/inflammatory in nature?

Response:

Dr. Peluso: No specific markers have been shown to be related specifically to symptomatic flares. Currently, these biomarkers are only useful for understanding disease processes, but they don't have diagnostic or treatment value.

As of December 2022, laboratory measurements are primarily used to check on overall health and rule out other conditions, but not to guide treatment for Long COVID.

Q. Are there any specific autoantibodies that should be checked in blood and/or cerebrospinal fluid in cases where post-infectious autoimmune/inflammatory encephalopathy is suspected?

Response:

Dr. Peluso: The Centers for Disease Control and Prevention recommends consideration of specific autoimmune tests in certain cases of Long COVID. Up-to-date guidance can be found at <https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/post-covid-conditions.html#table-1a>.

Q. What other specific testing may be indicated in Long COVID patients with neurological symptoms?

Response:

Dr. Peluso: As I mentioned before, the CDC has issued some recommendations: <https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/post-covid-conditions.html#table-1a>. Depending on the specific neurological symptoms—such as headache, neuropathy, or concentration problems—different testing might be warranted. There’s currently no standard approach to clinical testing.

Q. Are there any specific biomarkers associated with peripheral neuropathy, involuntary movements, fatigue, and/or sleep-wake disorders in Long COVID?

Response:

Dr. Peluso: I’m not aware of specific biomarkers associated with these conditions.

Q. Which autoantibodies are you seeing most in PASC?

Response:

Dr. Peluso: Some studies of PASC have detected antinuclear antibodies. However, these occur in some proportion of people who are without any medical problems, so the significance is unclear. We haven’t found a high level of autoimmunity in our cohort.

Q. Are elevated pro-inflammatory cytokines a biomarker for PASC?

Response:

Dr. Walt: Extensive work in this area has been conducted. Multiple cytokines have been identified in some studies; however, they need to be validated.

Q. Are these biomarkers still likely to be present in a [COVID] long-hauler who is nearly 3 years out? Does that answer change if they're more or less symptomatic?

Response:

Dr. Peluso: I'm not aware of any studies of Long COVID at 3 years, but I'm sure that we'll soon have data.

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To Learn More

- Information about RECOVER research and to volunteer for studies: <https://recovercovid.org/research>
- Frequently Asked Questions about RECOVER and PASC: <https://recovercovid.org/faqs>
- CDC information: Information for the general public and for healthcare providers about post-Covid conditions: <https://www.cdc.gov/coronavirus/2019-ncov/long-term-effects/>
- For medical/scientific terminology: <https://medlineplus.gov/healthtopics.html>