

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 seminar participants related to the following panel at the R3 Seminar *Understanding Neurological Manifestations of PASC and Cerebral Vascular Injury* held on February 13, 2024:

- ***Cerebrospinal Fluid in PASC: A Window into the COVID Mind***
Serena Spudich, MD, MA
- ***Pathogenic Mechanisms of Neuro-PASC in Older Adults***
Igor J. Koralnik, MD
- ***Imaging Brain Neuro-Glial Dysfunction in People with Post-COVID-19 Neurological Symptoms***
Shibani S. Mukerji, MD, PhD
- **Discussant: Jeymohan Joseph, PhD**

* Responses may have been edited for clarity.

All Presenters: Questions and Responses

Q. Dr. Spudich's study showed no evidence of viral persistence in PASC patients in cerebrospinal fluid (CSF). But there have been some studies from other investigators that show that viral RNA persists, for example, in colorectal tissue for up to 2 years. Viral protein has been identified in plasma for up to 16 months. Do you think these persistent RNA or proteins in peripheral tissue could have some impact on central nervous system (CNS) pathophysiology?

Response:

Dr. Spudich: I'd like to just make a statement that so far, we have a small study. Looking at CSF antigen is just one marker of persistence in the nervous system. I think that the clinical data from acute COVID, though, when people

have tons and tons of virus in their nasopharynx and in their lungs, very few studies have convincingly shown detection of SARS-CoV-2 virus in the brain.

I think the presence of antigen in [the Swedish study](#) has been interesting. It was readily detected in the CSF and acute COVID, so that was one measure we thought we might find as positive in post-neuro-PASC. But I don't think we've proven definitively that there's no viral persistence in the brain. We just used a single measure in the spinal fluid that was positive in acute COVID. Even the fact that there may be viral particles, or potentially virus, deep in the brain tissues—we know from other infections, sometimes things are sequestered. So, I don't think we've totally ruled that out, but we don't see evidence for it.

I also think that you're bringing up this really interesting finding of persistent virus or virus particles, for example, in the gut. We know from other infections, including HIV, that gut inflammation can lead to systemic inflammation and can influence the brain. And so, I think there's a real potential connection between what's happening in the gut and the brain. On the other hand, from just a small panel of markers, we haven't detected changes in immune responses, such as inflammatory markers in the brain. So, the most natural connection I would think is that viral persistence would incite inflammation, which would then affect the brain.

I think even the detection of spike antigen in the blood that's being reported was not actually associated with Long COVID. That was simply found in people some months after COVID, but there wasn't necessarily a connection. So, even the persistence of that antigen in the blood detected in that study, I don't know whether there's any correlation at all to symptoms. So, I think the viral persistence question is really important.

Typically, with viral persistence, though, you see a hallmark of that, meaning ongoing inflammation or some other result or ongoing neuronal injury. So far, we're not seeing that. But we have not laid the question to rest, and we have other markers we're looking at.

Q. Dr. Koralnik, you showed that there are important differences between neuro-PASC patients who have previously been hospitalized for COVID pneumonia and those who had mild COVID disease and did not need hospitalization for pneumonia or hypoxemia. What are the implications for future research in neuro-PASC, as well as treatment studies?

Response:

Dr. Koralnik: As you saw today, all the presenters paid great attention to what control group they were using for those studies. By the same token, we know that those two groups of patients are very different. Those patients who have been intubated for severe COVID pneumonia may have incurred permanent brain damage, and then they survive and they still complain of brain fog. But they are different than those who were previously healthy, younger, no comorbidities, have a mild case of COVID, and then develop cognitive dysfunction and brain fog.

So, I think it's very important to distinguish them. We have to study both of them, but we have to analyze the results separately. Because I don't think anybody can argue that somebody who had anoxic brain damage will benefit from a short term of Paxlovid, for example. Whereas those patients who were previously healthy, who may have viral persistence, may benefit actually from Paxlovid—so they should be analyzed separately. As we go along in the pandemic, there are fewer and fewer patients being hospitalized, fortunately. Ninety percent of the patients coming to the clinic are those who are never hospitalized, so these are the patients that are going to be seen in the future. But we still have patients who go to the hospital, they come out and they have the same problems, and we need to care for them just the same.

Q. What specific advantage does translocator protein (TSPO) or positron emission tomography (PET) imaging offer in assessing treatment efficacy and predicting patient outcomes in neurology-related clinical trials? Are there concerns regarding reproducibility and standardization of TSPO or PET imaging across different sites or populations?

Response:

Dr. Mukerji: That is an excellent, sophisticated question. One of the things that sites do is standardize protocols. This is not unique to TSPO. This would be something that would have to be done for magnetic resonance imaging (MRI) as well. So, protocols are standardized across the sites. Ensuring that you're using the same radioligand and the same protocols for cyclotron are operational logistical items. If we're going to think about a multi-site center that's going to use something that's going to use imaging, it's going to have to be standardized in its protocol. Then, the concept of variability holds true for almost any of our imaging modalities as well. There's going to be day-to-day variability for an individual, as well as participant-to-participant variability. The important question is how you're going to design that study trial. Statistical design of these study trials, whether you're going to use two time points, pre and post treatment, is one study design. It could be another type of study design. The group is using TSPO PET right now for a dietary interventional trial in a syndrome that also has fatigue, as well as diminished cognitive focus. Those design trials had preliminary data to inform how they're going to look at regions of interest and things like that. So, the answer is yes, it is appropriate to be concerned about variability. I think study designs will have to be thoughtful when you're using an imaging endpoint. To be very clear, our endpoint should also be improvement in a participant-related relevant outcome, not just an imaging endpoint. As I think it's going to be valuable for Long COVID, it's valuable for other diseases as well.

Q. In your findings of endothelial dysfunction in Long COVID patients, is that localized to the brain, or could it be present in other organs? Also, do you see temporal dissolution of endothelial dysfunction?

Response:

Dr. Spudich: To the first question, we measured these markers in blood, where we're taking blood from the arm. In fact, these markers are abnormal generally in the body. We actually don't know that they're even specifically abnormal in the brain. But what we're seeing is that many, many people have abnormal vascular function throughout the body during the acute COVID.

Some studies have begun to look at abnormal vascular dysfunction, which affects all organs—kidneys, heart, lungs, brain—and could be one of the unifying explanations for some people having multiple types of symptoms in Long COVID. In fact, we're now doing imaging studies that are specifically looking at the blood vessel walls in the brain. That will give us more information about whether the brain is specifically affected. But, in fact, what we're seeing is actually a marker of general vascular dysfunction.

The other thing I would say, in terms of whether this gets better over time, is that we know many of these markers are severely abnormal during acute COVID. What we're seeing now is that they also seem to be somewhat abnormal for people with PASC versus people who have had COVID and don't have PASC. Our study is going to do longitudinal assessments at 1 year and 2 years, to try to answer that question of whether it resolves, but we haven't gotten there yet. That was a great question.

Q. How do you affect cerebral small vessel disease?

Response:

Dr. Mukerji: One of our interests is whether or not there's going to be vascular dysfunction in people who have Long COVID, and trying to understand whether or not the concept of perfusion is going to be altered in people with Long COVID. I think MRI imaging would be one of the great ways to really detect that. It sounds like other individuals, including Dr. Spudich, are doing MRI imaging modalities.

One of the things that we use is arterial spin labeling in the COVID BRAIN Consortia. That allows us to not require contrast, and so we can look at cerebral blood flow at least, and that is one marker of vascular disease that we've looked at, or people have looked at for small vessel disease and cerebral vascular disease in general.

Q. Has there been evidence that previous infections such as viral meningitis and shingles and injuries such as spinal punctures indicate a persisting viral load and severity of PASC? Is PASC known to cause bilateral shingles outbreak?

Response:

Dr. Spudich: There's not yet evidence about the effect of prior infections, but it's an important area for future research. PASC isn't known to cause shingles outbreak.

Q. Have you looked at gender?

Response:

Dr. Spudich: Sex/gender is a key question, and we will analyze sex effects in the Yale study, but the RECOVER Observational Consortium Study will be able to answer this much better with thousands of patients.

Q. If you don't study people who had COVID-19 but recovered fully, how can you distinguish what is from the virus vs. what's from the immune response to the virus?

Response:

Dr. Spudich: Absolutely. We have two control groups: one never-COVID for the initial studies, and one post-COVID no PASC. For the negative cytokine data, the control group is never-COVID; if that had been positive, we would have then compared to post-COVID, no PASC. For the positive vascular data, the control group is post-COVID, no PASC.

Q. Dr. Spudich, how did you determine that the control group never had COVID?

Response:

Dr. Spudich: Great question. Twenty-one of our never-COVID group were participants whose CSF, blood, and cognitive data were collected before 2020. Eight additional participants had no history of COVID-19 and had negative spike and nucleocapsid antibodies. This is not 100% proof but close.

Q. The case of refractory psychosis responding to intravenous immunoglobulin (IVIG) seems like an anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis. Was this confirmed?

Response:

Dr. Spudich: It was an autoimmune antibody, but a novel one (not anti-NMDAR).

Q. Are people that are mixed race more susceptible?

Response:

Dr. Spudich: The RECOVER study should be able to answer this question.

Q. What are the autoantibodies directed against? I am curious if G-protein coupled receptors are involved.

Response:

Dr. Spudich: Great question. We are working on this!

Q. Dr. Spudich, is there further research to identify the novel autoantibody that was found in the patient with abrupt onset psychosis? (I think it would potentially be useful to test for this in other patients who are presenting with autoimmune encephalitis symptoms, but the current panel of autoantibody testing offered by Mayo is coming up negative.)

Response:

Dr. Spudich: Yes, we are working on (in close collaboration with Sam Pleasure and Michael Wilson at UCSF) understanding more on the particular autoantibody in this case and assessing for autoantibodies in all of our participants.

Q. What significance do you attach to the autoantibodies given no evidence of ongoing neuronal damage?

Response:

Dr. Spudich: Great question. I agree, it's a paradox. I want to emphasize that the autoantibody data are very preliminary—just looked for/found in one participant so far. So, we don't yet have evidence that this is a frequent factor in neuro-PASC.

Q. Has anyone yet begun studying autoimmunity issues in relation to neuro-PASC?

Response:

Dr. Spudich: Yes, this is an important aspect of our project.

Q. Is there any published literature on IVIG treatment for patients with psychosis?

Response:

Dr. Spudich: Here is a link to the paper where we described our experience with the psychosis patient:

[Anti-SARS-CoV-2 and Autoantibody Profiling of a COVID-19 Patient With Subacute Psychosis Who Remitted After Treatment With Intravenous Immunoglobulin.](#)

McAlpine LS, et al. Biol Psychiatry. 2023 May 1;93(9):e25-e29. doi: 10.1016/j.biopsych.2022.09.007. Epub 2022 Dec 5. PMID: 36481066 Free PMC article.

Q. Is someone looking at the presence of other cofounders like Epstein-Barr virus (EBV), cytomegalovirus (CMV), or Herpes simplex virus (HSV) in people with neuro-PASC?

Response:

Dr. Spudich: We have the blood and CSF samples but haven't tested yet. We're planning to test.

Q. There may be no difference cognitively with controls, but there will be a difference within a single person. Is it important to look at the data on an individual basis?

Response:

Dr. Spudich: This is an absolutely critical point. Most studies don't have data on individual participants before acquiring COVID, which is extremely important, because as you state, cognitive 'change' doesn't necessarily meet thresholds for abnormality but can still cause significant symptoms. Fortunately, the very large RECOVER study has a subset of participants before and after COVID, in which this can be assessed.

Q. For Dr. Mukerji, did you control for different levels of binding in patients, i.e., rs6971 genotype binding?

Response:

Dr. Mukerji: Yes, we did control for genotype.

Q. Dr. Mukerji, can you explain the Z-statistic scale: PASC > controls and meaning of the colors regarding PASC vs controls?

Response:

Dr. Mukerji: Thank you for this question. Red and yellow mean that PASC has more TSPO signal than controls. Yellow means more significant, while red means less significant than yellow but still significant.

Q. Have you considered adding to your study, in addition to HIV patients, other post-viral onset disease/illness patients such as those with positive Epstein-Barr and/or those with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)?

Response:

Dr. Mukerji: Great question. The group along with Dr. Michael VanElzakker is interested in analyzing individuals with ME/CFS.

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- 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>