

# Transcript

## Claire Quiner:

Welcome to the RECOVER Research Review or R3 Seminar. My name is Claire Quiner and I'm an Epidemiologist with the RECOVER Administrative Coordinating Center and the Moderator of today's seminar. The goal of this seminar series is to catalyze a shared understanding of the research within the RECOVER Consortium. I want to start by thanking everyone who submitted questions in advance. Please submit any additional questions that arise during today's presentation using the Q&A feature in Zoom. After the presentation, we'll answer as many questions as possible. A Q&A document will be posted with the recording of the seminar on [recoverCOVID.org](https://recoverCOVID.org). It will include the answers for submitted questions relevant to today's presentation. Questions about other scientific topics will be addressed in future seminars, and answers to broader questions about RECOVER will be available on the FAQs at the [recoverCOVID.org](https://recoverCOVID.org) website. As a reminder, we cannot answer individual questions about clinical care. Slide, please.

Our presenters today are Dr. Serena Spudich, Dr. Igor Koralnik, Dr. Shibani Mukerji, and our discussant is Dr. Jeymohan Joseph. Oops. Next slide, please. Thank you. Dr. Serena Spudich is a Gilbert H. Glaser Professor of Neurology at Yale University. Her clinical and translational research explores the effects of HIV and SARS-CoV-2 infection in the nervous system, focusing on effects of acute infection, antiviral and immune treatments, and interventional strategies on viral pathogenesis and persistence in the central nervous system. She currently serves as a Steering Committee Chair for the NIH RECOVER Study, and she clinically cares for patients with viral infections and neurological disorders at Yale.

Dr. Igor Koralnik is the Archibald Church Professor of Neurology at Northwestern University in Chicago. As a physician scientist, his laboratory investigates how viruses affect the nervous system. In May 2020, Dr. Koralnik created one of the first neuro COVID-19 clinics in the country, where he and his team investigate, diagnose and manage neurological symptoms of past patients. He currently serves as Co-director of the Northwestern Medical and Comprehensive COVID Clinic, which now includes 13 specialties dedicated to the total care of patients with PASC.

Dr. Shibani Mukerji is the Associate Director of the Neurology Infectious Diseases Unit at Massachusetts General Hospital. Dr. Mukerji brings a clinical focus on improving neurological outcomes, enhancing quality treatment, and reducing costs for people with HIV. She is the PI for the SOOTHE Study, an NIMH-funded randomized phase-two trial to determine the role of pregnenolone on brain function and inflammation in people living with HIV and depression. Dr. Mukerji is also a Co-PI for an observational study on the H1 reservoir in the central nervous system.

Finally, for our discussant, Dr. Jeymohan Joseph, currently serves as Chief of HIV Neuropathogenesis, Genetics, and Therapeutics Branch at the Division of AIDS Research at the National Institute of Mental Health, which supports integrated research programs to include those that work to understand the pathogenic mechanisms and genetic factors involved in the pathophysiology of HIV-induced central nervous system dysfunction, as well as research on the mechanisms of the establishment of persistence of HIV-1 viral reservoir in the central nervous system. Next slide, please.

The topic of today's seminar is understanding the neurological manifestations of PASC and cerebral vascular injury. Today's speakers will share our current understanding, the gaps in our knowledge, and how RECOVER will contribute to filling these knowledge gaps. With that, I'd like to hand it over to Dr. Spudich.

## Dr. Serena Spudich:

Thank you very much. Before I share my slides, I just wanted to make a comment. I have two hats. One of my hats is as the Steering Committee Co-Chair for the Observational Cohort of RECOVER. That is separate from me having this NOSI Award and our funding there. These were considered by separate entities, and so this is, hopefully, not a conflict of interest. Also, in that role, I wanted to provide just a little bit of explanation of the three talks that are happening today.

All three of these talks are generously funded by the RECOVER Consortium that were actually given as supplements to individual grants that each of us held. These are called NOSIs, which was a Notice of Scientific Interest for a supplement. The number of RECOVER NOSIs were funded in the first round, that one was actually, I

think, in 2022 when these were funded. Actually, really excitingly for me and for my co-neurologist on this call, a great number of the NOSIs actually focus on nervous system complications. You're just hearing from three of us today that are focusing on cognitive and other effects of Long COVID, but this is part of a really exciting group of studies that I think are going to add a lot to our understanding of Long COVID, in particular in the nervous system. We're a branch of RECOVER, though not part of the actual RECOVER longitudinal study.

There are also awards that were given for individual pathogenesis projects that were actually awarded to people who are part of RECOVER Consortium. Those are called ROAs and actually, there's a ROA announcement out right now to solicit new applications. We can have more discussion of that afterwards, but I was asked to make a couple of comments about these funding mechanisms. Again, we're doing our individual work at our individual sites, but we're all also part of the RECOVER program.

I will share my screen, and what I'm excited to talk to you about today is our RECOVER project. Now, I'm going to ask everyone, is this the right view that you're seeing? Correct?

**Dr. Jeymohan Joseph:**

Yes, that's correct.

**Dr. Shibani Mukerji:**

That's correct.

**Dr. Serena Spudich:**

Okay. Thank you. I just want to make sure I'm not showing my slides. We were very fortunate to get very interested in cerebrospinal fluid and neurologic aspects of COVID-19 very early in the pandemic and turn this into a long-term project, looking at long-term complications in people with Long COVID.

Our study is entitled Cerebrospinal Fluid in PASC, that's our grant title. But really, what our study is about is a window into the brain in people with Long COVID. You'll see in a moment that we actually have a multidisciplinary approach, and I'm going to be talking to you about that. It's not just cerebrospinal fluid. This is our very, very large study team, and you can see it's mostly based at Yale, but also involving other investigators and other sites.

In order to give an introduction to our study findings, I'm going to actually briefly review some of what we know about neuropathogenesis in Acute COVID, because this has actually developed our questions in Long COVID. And then, I'm going to tell you about our study and some of our preliminary findings.

Like Dr. Korallnik, we started early in the pandemic to really focus on understanding the nervous system complications of COVID. And during the first very large acute wave of COVID-19 in the Northeast, we actually had set up an inpatient consult service to see anyone who had COVID-19 and neurological problems. We learned a great deal by just seeing these patients trying to participate in their care, and also trying to figure out how what they have is COVID-specific versus a complication that we just see generally in hospitalized patients.

What we saw is really reflective of what's being reported in other publications, a very diverse panoply of syndromes ranging from stroke to encephalopathy, which was either confusion or coma, and even some other types of complications. Most of these were what we would consider immune-mediated conditions, which make us think of autoimmunity, but some of these also were clearly cerebrovascular injury.

What do we know about what's actually happening in the nervous system in these patients at this time? Again, I'm telling you about Acute COVID-19. This is a beautiful study by Dr. Mukerji at MGH, where they looked at people who were hospitalized with neurological symptoms during their Acute COVID-19. And they did a number of different tests focused on cerebrospinal fluid.

To remind everyone, cerebrospinal fluid is the liquid that surrounds the brain and the spinal cord. It's not exactly the same as the brain, but it can serve as a drain of things happening in the brain. So, we can often detect infections or other types of damage or inflammation by looking directly at the cerebrospinal fluid, which we can readily access by putting a needle in a patient's back.

In this case, they collected cerebrospinal fluid from people with Acute COVID, and these are shown in the graphs in the red dots, and compared it to people who did not have COVID and were healthy, which is gray controlled, as

well as a number of other types of groups that we know usually have neuro-inflammation. They looked, first of all, for presence of the virus, and they found no virus in 27 people who were tested, but they did find elevation of inflammatory markers in those with COVID-19.

That and other studies have supported the idea that there's generalized inflammation, for example, reflected by cytokines in the cerebrospinal fluid, suggesting inflammation in the brain during Acute COVID. This is another study that was much smaller, but run by my colleague here at Yale, where she also looked for the presence of virus, could not find it by many different methods, This is during Acute COVID, and also found elevated cytokines. But what's really interesting is she was also interested in autoimmunity and antibodies. She not only found that there were anti-SARS-CoV-2 antibodies, so antiviral antibodies found in the spinal fluid that were actually, in some, cases different than the ones found in the blood, but actually some of these antibodies turned out to be reacting against brain tissue.

So, sometimes antibodies can make a mistake, and instead of attacking, for example, a virus, they can make an error and instead attack your own cells. And in this case, some of these antibodies were attacking the cells of the brain. So, it's possible that some of the syndromes that we saw in Acute COVID were related to autoimmunity.

Finally, there's an important aspect of cerebrovascular injury in Acute COVID-19. This has been noted throughout the body, so not just cerebrovascular, but overall vascular inflammation. Endothelial activation, which is the lining of the cells that line the blood vessels, and this has been seen not only by blood tests during patients who have strokes during COVID-19, but also by really important studies that looked at autopsies of the brain with a high-field MRI scan and found areas of microvascular injury in the brain in people who died of COVID-19. This seemed to be an unusual feature of COVID.

It's also clear that there's actually acutely neuronal injury during COVID-19. This is a little bit frightening to hear, but this was actually found, and this was a set of hospitalized patients, neuro-asymptomatic, so they had no neurologic symptoms, and also neuro-symptomatic, who had neurologic symptoms. In the study, cerebrospinal neurofilament light chain was measured. This is a measure that indicates active breakdown of neurons in the brain. In comparison to healthy controls who did not have COVID, both those who had neuro symptoms and didn't had some elevations of this marker during Acute COVID, suggesting that there was some damage in the neurons, even in people without symptoms. Again, these are people who were hospitalized and so they tended to have moderate-to-severe disease.

Finally, the same group, these are colleagues in Sweden, also looked for the presence of a virus. And as I mentioned before, several studies have looked for the full virus by PCR and also by other methods. We, Dr. Mukerji, actually this group as well, have never found a virus by looking for the full virus, but this group later went back and looked at cerebrospinal fluid samples from people during Acute COVID, again, neuro-asymptomatic or symptomatic, and found actually that the antigen, the nucleocapsid antigen, was actually present in the spinal fluid during Acute COVID.

Interestingly, they did not really find spike antigen, but nucleocapsid antigen, which is an important feature of the SARS-CoV-2 virus, was detected during Acute COVID, suggesting that even if the whole virus is not entering the spinal fluid, there's some viral proteins that are still getting in and could be causing problems. This is a summary slide. I won't go through carefully, but this is a review that we wrote early on, January 2022, that I would direct you towards, that actually did come up with some hypotheses and a summary of what's happening during Acute COVID-19, and then ended with some questions about what could be happening after people survive.

Our study was then put together. It was a supplement to an NIMH grant that was looking at pathogenesis of HIV in the nervous system, and we decided to put together a lot of the tools that we've been using for a long time in HIV to study PASC. What we wanted to focus on specifically was nervous system PASC. We focused in this study on recruiting people who had either severe cognitive impairment, brain fog. Some people have severe headaches, and one or two people also have things like psychosis, a very, very severe psychiatric disease. What we decided was to look for the same types of things that we looked for, and others looked for, in Acute COVID; looking for evidence of immune alterations, inflammation and autoimmunity, and vascular dysfunction in people with neuro-PASC, looking for persistence of the virus in people with neuro-PASC, and then also looking at objective measurements of nervous system functions, such as MRI scans, neuronal injury proteins that I mentioned

earlier, and also cognitive and psychiatric measures. Trying to see if those were abnormal and also trying to tie those together with the possibilities of inflammation, vascular injury, or viral persistence.

This is the different, again, schematic of our study, and I'm going to show you this just as a snapshot to see how many collaborators we have, many of whom are top experts in their fields, who are working with us in the different branches of the study. Certainly, I myself am not an expert in all these areas, although I've worked with these tools for many years, and I also wanted to highlight my collaborator, Dr. Shelli Farhadian, who's really the co-PI on this supplement and this grant.

What's the approach of our study? I wanted to just be totally clear, we're studying people who have neurologic Long COVID from our Neuro COVID Clinic. Dr. McAlpine, one of my colleagues, runs a Neuro COVID Clinic, where she's seen thousands of patients. And these individuals are screened for how long they've had symptoms, what their symptoms are, and those people who have cognitive symptoms are referred, if they would like, to our study. And that's our neuro-PASC group.

We also have pre-pandemic control data from people who had volunteered as healthy controls before COVID started, and that's an interesting group in some ways because we know they're never COVID. But we also have been collecting contemporaneous community volunteers who had COVID, but do not have PASC. These numbers that I'm stating here are our target numbers for the grant, but we're actually finding we're actually getting more numbers. Again, we're getting symptom assessments, we're getting lumbar punctures and blood draws from people who consent to those procedures. The lumbar puncture is to collect spinal fluid. We're doing neuropsychological testing and brain MRI.

The reason that this is really realistic in our group is we've been using these tools for many, many years to study HIV. So, we have a lot of comfort with these procedures and a lot of infrastructure with them. We found great success and people consented to these studies.

This is the timeline for our study. We are looking at people after Acute COVID, usually about a year after they had Acute COVID, who have PASC. We're then going to see them a year later to see if symptoms persist, improve or change. And then, for those who fall within the window of our follow-up, we're going to also even see them two years after their baseline. I know I'm going through a lot quickly, but I really want to get to some of our data.

This is just the preliminary demographics and clinical parameters, in this case, looking at controls who did not have COVID. I'm showing you these because some of our data slides are those who did not have COVID. This is people with neuro-PASC, as I mentioned, and people who did not have COVID. You can see here that the gender breakdown is a little different in these groups and the racial breakdown is different in the groups, but the age is similar.

You can also see, very importantly, that of the COVID people, the days from COVID-19 symptom onset is 300 days. These people were first studied an average of about a year after they first had COVID, so we're not looking at lingering effects of Acute COVID. 80% of the people in our studies were only convalesced at home, so were never hospitalized and they only had mild disease.

This is really just initial findings on our neuropsychological performance. The control in the neuropsychological performance here is the dark line at zero, and what we're seeing is anything below zero is different than the controls. This is what we're seeing in the PASC participants, and I think mostly what this tells us is we are seeing people who are having some impairment. Again, this doesn't prove that this is all from COVID. Some of this could have been earlier, but we are seeing people with significant issues.

I know there was a question earlier that was submitted about what types of symptoms people have, and here you can see that some of the perceptual and being able to use words fluently, as well as some actually fine motor function are some of the things that we're seeing major deficits in. Also, psychiatric symptoms, and I know this is a very busy slide, but I wanted to emphasize that we're not just focused on neurologic outcomes. We're very, very focused also on helping people who are having persistent neuropsychiatric symptoms, and we're doing a very, very detailed assessment of that to try to assess what happens over time.

What's interesting here is that people are not necessarily having impairment in every area of mood. People are having specific deficits in different types of things, such as anxiety and cognition. Let's look at some of the initial data that we have found. Again, this is all fairly preliminary. We have a study that's actually ongoing for two years, and we have a lot more samples that we have collected and more data to come. But in our initial

studies, we just look at clinical markers in the spinal fluid and consistent with what both people have seen. If they go to the hospital or they go to the clinic with Long COVID, the regular clinical markers are normal. So, no elevation in protein or white blood cells.

What was also very surprising to us is, we wanted to look to see whether the cytokines in the cerebrospinal fluid were elevated in people with neuro-PASC. Again, the question here would be, is lingering generalized inflammation one of the explanations for neuro-PASC? And that's a very strong hypothesis. A lot of people think inflammation is one of the underlying key mechanisms of PASC. In our study to date, where we looked at 37 people who had neuro-PASC and 22 pre-pandemic never-COVID controls, which we thought were the best first comparison, because this is looking to see if there's any change in neuro-PASC, we found no significant differences in the spinal fluid or the plasma in a 15-Plex cytokine array.

We can get back to this later in the Q&A. Does this answer the question about inflammation? It doesn't. But in this first test, where we looked at many samples and we looked at many different markers, we did not find significant differences in inflammation in people who have neuro-PASC and who did not. We also wondered whether we were going to see evidence of ongoing neuronal injury. Are we seeing people who are having ongoing damage to the neurons while they're having these neuro-PASC symptoms? I can say in this case, again, this is preliminary data, this is not all of our samples, but I'm happy to say that we don't see any evidence of ongoing injury in the brain in people who have neuro-PASC. And this is in great contrast to something like people with HIV. People with HIV, for many, many years until they've been on treatment, have abnormally elevated levels of, for example, the neuronal injury markers chronically, and we can easily detect that. The fact that we're not detecting this in cerebrospinal fluid in past patients, we think, is pretty informative.

What we have found is that there are anti-SARS-CoV-2 antibodies in both the blood and the CSF. You may remember that spike can be positive after vaccination, but nucleocapsid antibody should not be positive after vaccination. In fact, many of these measures were taken before people had been vaccinated. All of the pink were pre-vaccination, and you can see that both spike and nucleocapsid is detectable in the spinal fluid in many of these people, even a year after SARS-CoV-2.

What does that mean? Does that mean there's still antigen in the spinal fluid and we're bringing in these anti-SARS-CoV-2 antibodies? To our data so far, that's not true. So, what we did is we used the same assay, SARS nucleocapsid antigen, that had been found to be positive in Acute COVID-19. We looked at a few people with Acute COVID and found it to be positive in CSF in blood in a couple of people. But then we looked at people who had had neuro-PASC, people who were post-COVID but asymptomatic and, of course, are pre-pandemic controls, and found no evidence of SARS-CoV-2 antigen in any of these groups to date. Does this prove that there is no persistence of virus in the nervous system? We can discuss that. I don't think so, but it does mean that a readily measurable viral protein is not there, where it was there in Acute COVID.

I'll end by just a couple of final thoughts. We actually have measured plasma markers of vascular and endothelial dysfunction in 40 people with neuro-PASC and 20 people who had COVID and no PASC symptoms. We were very surprised to find that we actually found positive measures that are actually still positive when they're corrected from multiple comparisons in almost every marker that we measure that is associated with endothelial and vascular inflammation and dysfunction. For example, L-selectin is a very important marker that allows cells to traverse the blood-brain barrier, and you can see that and many other of these markers were very different from people who had PASC but no neuro symptoms. So, we're hoping this may be our first clue into some of the pathogenesis of neuro-PASC.

We also had some very interesting findings on one specific patient that we're, of course, following up with many more studies of a gentleman who had psychosis after COVID-19. This was a refractory to any kind of anti-psychotic treatment. He was failing every medication that was tried. He had no history of psychosis. We ended up giving him IVIG, just out of the possibility that this could be autoimmune psychosis, and his psychosis resolved immediately and permanently. And then, later, we took his spinal fluid and we found that he had autoantibodies in his spinal fluid that was reactive to brain tissue. It's possible that some of these post-COVID conditions also may have autoimmunity underlying them, and that's a really important part of our project. I don't think I need to go through this in detail, but we have the gray as some of the things that I've already showed you in terms of the data that we have produced, but the black are things that we still are working on in the project, so there's a lot more for us to learn from this terrific COVID supplement.

What are the underlying biological mechanisms? I think we've learned some things. I would just talk about our preliminary data, but none of these are completely definitive. So far, we have no evidence of generalized inflammation. We also have no evidence of active ongoing neuronal injury. And finally, based on just looking for viral antigen, we do not see any evidence of viral persistence in the spinal fluid. We do see some evidence of vascular dysfunction, and so we have a lot more experiments we're doing to follow that up, including neuroimaging. And it's possible we still are really interested in studying autoantibodies. We do see anti-SARS-CoV-2 antibodies in the CSF.

I wanted to end with just a couple of critical questions that will be needed to be answered by long-term studies. We're talking about Framingham-type of studies, but I think things that we can't answer the next couple of years, but the field will have to answer. We have many millions of people worldwide who are experiencing Long COVID. Will we be at risk, will these people be at risk for long-term cognitive or psychiatric consequences? So, we need to really follow people long-term.

There's a big concern in the field whether the generalized neuro-inflammation, microglial activation that happens in Acute COVID could trigger neurodegenerative disease, either unmask that or worsen neurodegenerative disease or autoimmune disease? And some of those studies have already been emerging. Finally, are there long-term neurologic consequences of COVID-19 in people, such as young children or young people, who would be at long-term risk after living a long lifetime? Also, in particular, populations that are close to my heart, such as people living with HIV?

We'll end there with our acknowledgements. I thank very much both the NIMH, which is the funder of our parent grant, and the RECOVER Study for funding the supplement work, and I'm excited to answer questions, which I think we'll mostly have time for in the Q&A. Thanks very much for your attention.

### **Claire Quiner:**

Wonderful. Thank you, Dr. Spudich. Next up, we have Dr. Igor Koralnik.

### **Dr. Igor Koralnik:**

Thank you very much. It's always a pleasure to present our data and also to speak after Dr. Spudich, who has set the stage so nicely about all the important aspects of neuro-PASC.

I was asked to give a brief overview of our Northwestern Medicine Neuro COVID-19 Clinic, which opened in May 2020 in person or in televisit. Our philosophy at the time was to give full access or as big access as possible to anybody who wanted to see us, so we did not require physician referral or even a positive SARS-CoV-2 test to be evaluated in the clinic. Because we understood the limitations of testing at that time. We already know what to expect.

It started small, with just a fellow and me, and we put one web page on the website, like for any other new clinic. Before we knew it, the clinic was booked two-and-a-half years in advance. We had to increase staff, and everybody says hi. I just want to show that it was really a very big team effort from the start. All right.

This is the national reach of the Neuro COVID Clinic via telemedicine. During the COVID-19 emergency, we were able to evaluate 2,150 patients when we did not need to have a license to see patients in all those states. 70% came from Illinois and 30% from 44 other states. You see that we're covering most of the map, including places, to my surprise, like California and Florida, which is really not areas that we think of as lacking of neurology expertise. But apparently, those patients said that they were not able to find neurologists willing or able to care for them. As far as I'm concerned, they were underserved just the same, and we're happy to try to help them.

We saw all those patients, gave us the opportunity to report our finding. And I show this because, again, it was a very big teamwork with many other neurologists in our department and medical students who helped us with chart review of the first 600 patients that we saw in the clinic. The conclusion was that neurology manifestation of Long COVID really differ based on Acute COVID-19 severity. I'm just going to give you the punchline here, is that really, neuro-PASC patients have not all been treated equal.

On one hand, you have post-hospitalization neuro-PASC, or PMP, who all had COVID pneumonia. They are older, 54 years old on average. Some were intubated for pneumonia. They have a host of past medical history and comorbidities listed here, and we find that they have a broad neurologic and cognitive dysfunction, as well as in some time lack of insight in their cognitive problem.

On the other end of the spectrum, we have the non-hospitalized neuro-PASC patients, who had mild initial respiratory presentation, never hospitalized for pneumonia or hypoxemia. They are one decade younger than the other ones, 45 years old on average, tend to be more frequently female. And they have, as past medical history, a higher level of depression and anxiety. They have different symptoms and limited neurologic findings and deficits, predominantly in attention with a preserved insight in their cognitive problem.

As Dr. Spudich elaborated before, a possible mechanism in those two populations may be somewhat different. Those who are hospitalized with severe pneumonia may have incurred CNS damage during their acute illness. They may have had hypoxemia, multi-organ failure, cytokine storm, intravascular clotting, while the other ones who were never hospitalized had a mild initial disease, may be affected by autoimmunity, viral persistent, mitochondrial dysfunction, or potentially also have neuropsychiatric vulnerability.

The initial success of the Neuro COVID-19 Clinic led to the creation of our Comprehensive COVID Center, caring for all manifestations of Long COVID. You can see here that we have 12 different specialties, including pulmonology, cardiology, hematology, rheumatology and so on. And we had the opportunity to report our experience of the first 21 months of the Comprehensive COVID Center, where we evaluated a total of 1,802 patients seen across these 12 specialties, 2,361 new patients visit, with a mean number of specialties of 1.3 per patients, with a range of 1.6.

So, it's really a multi-system disease. The top three specialties visited were neurology, pulmonology, and cardiology, with neurology having 48.8% of all the visit, indicating that in the Long COVID spectrum, neurology really is predominant, at least in our center. I was told that in the last fiscal year, 80% of all the CCC visits came to neurology as fewer patients get hospitalized for pneumonia. As other centers have been closing their doors, we actually are ramping up and we've added a new specialty of OBGYN to care for those patients as well. All right. The parent grant for this study is an epidemiological study of Disparities in Sleep and Cognition in Older Adults. The acronym is DISCO. That is held by Dr. Knutson and Dr. Carnethon here at Northwestern. With the hypothesis, it's that inadequate sleep is one pathway linking race and ethnicity to cognitive problem and Alzheimer's disease. The aim of this parent study is to assess sleep and cognition function at baseline and 24 months later, in patients 55 years old and older, without severe cognitive impairment of diverse population. Our supplement for this NOSI is to do the same study in 55 year and older patients with neuro-PASC.

Our hypothesis is that sleep and circadian rhythm disruption and associated fatigue may affect the cognitive abilities in older neuro-PASC patients. And in addition, poor sleep may have a compounding effect on the alteration of the cellular immune response to SARS-CoV-2 and may cause a dysfunction of regulatory T cells and development of auto-immunity.

This is the schematic condition. We have a SARS-CoV-2 persistent infection in neuro-PASC, or it may be, that may be causing endotheliocytes involving the CNS and inflammation. This may also cause immune memory dysregulation. Both may affect alteration of sleep and wakefulness center. That may lead to insomnia, fatigue, and brain fog, and finally, cognitive dysfunction. I will present our interim result because the study is not completed. We have enrolled a total of 40 participants in this cross-sectional study; 17 non-hospitalized neuro-PASC, 55 and older from the Neuro COVID Clinic at Northwestern, 17 COVID convalescent, people who got COVID got over it with no lingering symptoms, 55 and older from the DISCO Study. We wanted also to enroll 20 uninfected, healthy control DISCO Study participants. It turns out that out of six that we could screen, five had actually serological evidence of SARS-CoV-2 infection. There's no such thing as the healthy control any more nowadays, and so we call them asymptomatic seropositives or AS, and they've been included together with the COVID convalescent in this pool control group for the purpose of this study.

Patients got a neuro exam, quality-of-life cognitive evaluation. They underwent sleep and circadian rhythm evaluation with one night of a sleep profiler, as you can see here. And they wore an actigraphy watch for one week that measured their movements and exposure to light. They also had an immunological evaluation, where we looked at the T cell response to SARS-CoV-2 and T-regs. All right. These are the demographics of our study participants. We had 39 that we would evaluate, including 17 neuro-PASC and 22 in the control group. The time from symptom onset was 13.6 months in average. The patient's mean age was 64.4. There was no difference in gender between the two groups, but there was a trend for more diverse population in the control group, and that reached significance for ethnicity, which is based on the design of the parent DISCO Study.

In terms of comorbidities, you have what you can expect at that age; hypertension, cancer. There were no significant difference in the neuro-PASC and the control group. Of note, 11.8% of the neuro-PASC patients had pre-existing depression or anxiety. These are the neurological symptoms that our neuro-PASC patient had when they came to see us in the clinic. We asked all patients a simple question. What is your subjective impression of recovery compared to pre-COVID baseline? If you say 100%, you're back to normal. And they say that on average, they were only 34.1% recovered.

They had a median of eight neurologic manifestations or symptoms that could be attributed to PASC. 94.1% had four or more symptoms, and the most prevalent symptom was brain fog. All of them had brain fog, followed by blurred vision, headache, numbness, tingling, myalgia, dizziness, anosmia, dysgeusia, pain other than chest, and tinnitus. In addition, they had other non-neurologic symptoms and of importance for NIMH, 82.4% said that they had depression or anxiety at the time of the visit, 76.5% had fatigue, and as well as 35% had insomnia. All right.

I want to spend a little bit more time on this slide that shows the quality of life and cognition in our patients. On the top left, you can see the Patient-Reported Outcome Measurement Information System, or PROMIS measure, measuring the quality of life for domain of cognitive function, fatigue, sleep disturbance, anxiety, and depression. And based on a normative population in the US, normal results would be a T-score of 50, with a standard deviation of 10. Lower results mean worse self-impression of cognition and higher results for all the other domains, worse quality of life in those domains. You can see that the neuro-PASC patients in red compared to the control group have worse quality of life in all those domains; cognitive function, fatigue, sleep disturbance, anxiety, and depression.

On the other hand, all participants were tested with the NIH Toolbox cognitive test on a tablet. That takes about 30 minutes to perform. Again, based on the large population in the US that's taking the test, a normal result would be a T-score of 50, with a standard deviation of 10. Lower results mean a worse score, and the Toolbox has been adjusted for age, education, sex, race, and ethnicity. You can see that there was a range of results. Some patients scored two standard deviation above the norm, some two standard deviation below. There were no statistical difference between the neuro-PASC patient and the control group for any of these single tests. Two tests reached borderline significance for attention and executive function. But when we looked at the global cognition T-score, then there was a difference, with the neuro-PASC patient performing worse than the control group. All right. These are the results, some of the few results of a sleep profiler that we obtained. There was a decreased total sleep time for the neuro-PASC patient compared to the control group. On the other hand, there was no significant difference for the apnea-hypopnea index between the two groups. But we saw that one-third of the neuro-PASC patients actually had a high AHI index, although none of them had a history of obstructive sleep apnea prior to COVID.

These are the results of the actigraphy that we obtained, and although patients said at the lower total sleep time with the polysomnography testing, they tended to spend more time in bed. In addition, we saw a difference in the CAR, which measured the robustness of the circadian rhythm activity. Lower numbers are worse, showing a disruption of circadian rhythm between neuro-PASC patients and the control group. In addition, we saw that some of the neuro-PASC patients had a increase in sleep onset latency. All right. We also looked at the immune response of these patients. These are very preliminary results since we haven't tested all the sample which needs to be batched for testing. We saw an enhanced interferon-gamma and IL-17 production from T cell subset in the neuro-PASC patients. That shows that they have elevated cellular immune response to autoantigen that are linked with systemic lupus, rheumatoid arthritis, MS, and other autoimmune disease. On the other hand, the COVID coalescence had a higher production from CD4, FOXP3, T-regs, and from CD8 T cells compared to the neuro-PASC patient, which revealed a more tolerogenic response to autoantigen stimulation.

In conclusions, an average 14 months after symptom onset for older neuro-PASC patients, had lower quality of life in domains of cognition, fatigue, sleep, anxiety, and depression, compared to the COVID coalescence and asymptomatic seropositive in all control groups. The neuro-PASC patients have also a decreased global cognitive score compared to the control group, although the cognitive deficit is much lower than what would be expected compared to their subjective symptoms.



Neuro-PASC patients have decreased total sleep time, despite the trend to spend more time in bed in the control group. We also saw that they tend to have a disrupted circadian rhythm compared to the control group. We, to our surprise, a third of neuro-PASC patients have a high apnea-hypopnea index, despite having never been diagnosed with sleep apnea, and they could potentially benefit from CPAP.

On the primary immunological findings, we have an increased Th1, Th17 autoreactive T cells response in NP patients, which suggests some autoimmunity, while an increased IL-10 production from the T-regs in COVID coalescence reveals a more tolerogenic response to autoantigen stimulation. How alteration of the T-reg function may prevent NP patients to regulate inflammation and impact their sleep quality deserves further study. We have mechanistic studies that are ongoing to study the link between the T-reg function and sleep through transcriptomic analysis, including clock gene expression with single cell RNA sequencing. What are our future plans? Well, we-

**Claire Quiner:**

I'm sorry, Dr. Koralnik, you're just at time right now. Could you wrap it up in just about 30 seconds?

**Dr. Igor Koralnik:**

Sure.

**Claire Quiner:**

Thank you.

**Dr. Igor Koralnik:**

18 minutes. We'll continue enrollment until the end of the no-cost extension. We hope that strategies to improve sleep would become a therapeutic intervention for Long COVID, and that this could open new avenues of research in this field. I think that, especially for NIMH, mental health issues are an important aspect of neuro-PASC that point specific management. We have now evaluated about 2,400 patients in the Neuro COVID Clinic, and we're evaluating 60 to 80 new patients every month. And we are glad to collaborate on all aspects of neuro-PASC research.

These are our collaborators from sleep, actigraphy, immunology, clinical coordination. We're very grateful to all our patients and their families for participating to this research. And these are our study contact. Thank you very much.

**Claire Quiner:**

Thank you so much, Dr. Koralnik, for that really interesting and detailed talk. Next up, we have Dr. Shibani Mukerji.

**Dr. Shibani Mukerji:**

Thank you, Claire. Thank you to the organizers for inviting our team to share work today. I'm Shibani Mukerji. I'm a Neurologist and Researcher in neuro-infectious diseases. I'm speaking on behalf of the branch study team. I am very grateful for both Dr. Spudich and Koralnik, who has now beautifully set up the stage for discussing the breadth of symptoms and neuropathogenesis in Long COVID. So, I'm going to focus the next 20 minutes of our talk to discuss some of the experiences that we have using an imaging biomarker to investigate neuroinflammation, and that's specifically called TSPO PET.

I'll provide some of the insights that our group has used using TSPO PET in other multisymptomatic chronic disorders, and I'm going to share our preliminary findings that use TSPO PET in Long COVID. I'll end by some of our early impressions and some considerations. I want to clarify that in this talk, when I'm speaking about people with Long COVID, I'm discussing individuals who have had at least one persistent neurological symptom. I think many people have experienced that there is a mismatch between the burden of symptoms that people with Long COVID have and the relative lack of imaging findings that the majority of people who try to obtain a conventional clinical MRI have. There are recent studies that suggest that perhaps there are both neuronal as well

as glial abnormalities that you can observe in people with Long COVID using more advanced neuroimaging. And that might provide some increased sensitivity in trying to distinguish between people with Long COVID and those individuals who do not have Long COVID symptoms.

I'm going to highlight two papers that are led by other groups, to use as examples. The study on the left is by Linda Chang's group, where they use MRI with spectroscopy to try to understand or identify differences at the level of neurometabolites among people with Long COVID. And they've identified lower levels of a neuronal metabolite in areas and regions of cortex. The paper here on the right is by Jeffrey Meyer's group that has used TSPO PET imaging to investigate areas of neuroinflammation in people with Long COVID. This study had shown that there was increased TSPO signal in both cortical, as well as subcortical regions, and that included prefrontal cortex that includes striatum and hippocampus. And they make the argument that there's increased neuroinflammation in people with Long COVID.

Our team specializes in trying to combine these two imaging techniques of both of these studies. So, combining TSPO PET with MRI and spectroscopy to investigate neuroinflammation and glial dysfunction in chronic disorders. Our group's interest is keenly in disorders that really are heterogeneous in nature, and that may have neurological symptoms that are challenging for investigators to localize in the brain. That includes fatigue, that will include pain, and that can include also diminished cognitive focus.

I want to spend a little bit of time to briefly walk through the methodology of the imaging. For that PET imaging, what we use is a radiotracer called PBR28 that binds to a protein called TSPO. TSPO is naturally expressed on the outer membrane of mitochondria, and for reasons that we don't fully understand, TSPO upregulates in cells during inflammation. And in the brain, it's primarily upregulated in microglia and astrocytes, both of which are glial cells. The upregulation of TSPO has been well-documented in preclinical models. And in humans, it's actually been hypothesized that TSPO PET is a marker of glial density, and that this increased glial density may also be related to inflammation.

Typically, healthy brains will exhibit very minimal TSPO signal, as you can see here on the left. In certain disease states, like ALS, which is a neurodegenerative disorder that affects motor function, we can observe TSPO uptake in regions that include premotor and motor cortex. We can also observe focal TSPO signal in other chronic disorders that may, in fact, have a central neuroinflammatory component that includes chronic low back pain. Here, what you're observing is an increased TSPO signal in the thalamus, a region that we know modulates at least pain information.

I'd like the audience to contrast this concept of fertility with our findings that we see in people living with HIV. Our group has previously documented a more widespread pattern of TSPO signal in a study where we had investigated people living with HIV, who are virally suppressed and on antiretroviral therapy. Here, instead of us seeing more focal areas, what we're seeing are large swaths of TSPO uptake, both in cortical tissue, as well as several cortical regions. And here, the cohort has multiple neurological symptoms, many of which can be a challenge for us to pinpoint anatomically in the brain. That included fatigue as well as neuropathic pain. We can start to think about taking approaches on whether or not we see increased TSPO uptake can actually correlate with some of these symptoms.

The strategy has been effective in our hands for another disorder, fibromyalgia, and it's a disorder that we observe increased TSPO signal in multiple cortical regions. If we focus specifically on specific regions that include, for example, the middle cingulate cortex, we can start to see the TSPO levels are, in fact, proportional to fatigue severity, with the highest TSPO signal associated with more severe fatigue scores; lending us to the possibility or at least the idea that we could potentially identify a neural signature of something as general symptom like fatigue using TSPO PET.

To add another layer and going beyond the scope of PET, we can use other imaging modalities to try to assess the health of an underlying brain tissue without the need of, for example, a biopsy. Our research group has expertise and really understanding at least probing tissue metabolism using spectroscopy. And within regions of interest, one of which is shown here, the posterior insula, we can evaluate a spectrum of markers. That includes inflammation indicators, neuronal markers, as well as a concentration of both excitatory as well as inhibitory neurotransmitters. I think it's by trying to integrate some of this data from multimodal imaging techniques, here shown TSPO uptake, neurometabolites, as well as including participant-reported symptoms, we can start to try to construct a more cohesive narrative of potentially disease mechanism.

A research question for this supplement in Long COVID were largely the following. We anticipated that people with Long COVID, who have neurological symptoms, would have higher glial activation or at least TSPO uptake, and we aim to try to identify that pattern of uptake using PET. We also anticipated that neurological symptoms, as well as the level of certain neurometabolites, using spectroscopy would, in fact, correlate with our TSPO uptake. And then, given our prior work in people living with HIV, we wanted to understand whether or not infection with COVID-19 would alter that TSPO pattern such that individuals living with HIV would have a synergistic increase in neural inflammation, would have an ultimately different pattern of TSPO uptake. Individuals that are included in our study are people with Long COVID, who have at least one neurological symptom, and are at least two months after infection. During their visit one, we obtain a medical history review, we obtain history of acute symptoms, as well as understanding of Long COVID symptoms. We obtain understanding of HIV status, T cell subsets, COVID antibody, as well obtaining a TSPO genotype testing for polymorphism of the TSPO gene, which is known to affect binding for several of our TSPO radioligands that includes PBR28, the radioligand that we use.

Just to note that about one in 10 individuals will have a TSPO genotype that's predicted to be a low-affinity binder for PBR28, and unfortunately, we are not able to include those individuals for PET scanning. But we can also use this polymorphism in the genotype to match our participants with Long COVID to controls of similar genotypes.

During visit two, we also have a structured neurocognitive evaluation that tests for processing speed, executive function, and motor control, and we use several instruments for fatigue, including the Multidimensional Fatigue Inventory, and we use several pain inventories that we regularly use in other studies, as well as depression scales. In addition, we also use a Von Frey sensory testing method, which is a standardized test for pain sensation. It uses a small plastic filament to understand an individual's pain threshold. And finally, we do cryopreserve whole blood, both cells as well as plasma, for future research studies.

I'm going to go through our screening thus far and our individuals that have gone through our study. We've pre-screened 86 individuals, consented 44, and 35 individuals have completed their visit one. The median time between those individuals who have come for visit one from between their acute COVID-19 infection and their visit is about a year-and-a-half. There's about 18 people who have completed our PET imaging study. Thus far, we've reconstructed the first 10 scans of the cohort and genotyped and matched to age, sex at birth, and TSPO genotyped to a control group that did not have COVID-19. I'm going to be showing data for individuals who are on average about in their 40s. They have a wide deviation of age, and the majority of whom are female sex at birth. I wish to acknowledge an incredible member of our lab team, Ellie Kim, who's really worked hard to analyze and present these statistical maps. What you are seeing is a statistical comparative map that's showing the brain regions where there's increased TSPO signal in people with Long COVID compared to the control group.

What I'm going to show you are two main points. First, I think the audience can appreciate that the TSPO signal that we're observing is more widespread, not focal. And thus, in our hands, what we're actually seeing is this wide swaths of increased TSPO across several brain regions. Second, and I think is a more subtle point, is that we are observing TSPO signal in cortical regions that includes both posterior mid-cingulate cortex, for example, pre-general anterior cingulate cortex, as well as subcortical regions like the striatum, as well as the thalamus. In addition to gray matter, we're also observing increased TSPO uptake in frontal white matter, as well as in the corpus callosum, which is a white matter tract that connects both hemispheres. At this point, we can only offer speculation as to why we're seeing such an unrestricted pattern of TSPO uptake in participants who are nearly a year-and-a-half out after their acute infection. Our understanding of glial biology really is that these glial cells are actually very highly sensitive to their surrounding microenvironment.

We strongly suspect that these large swaths of TSPO signal uptake could be actually due to more underlying abnormal or aberrant neural networks. It's a concept actually of neurogenic neuroinflammation, and so whether or not there's abnormal underlying neural network activity really still remains to be dissected, and trying to figure out why an individual who is nearly a year out will have such abnormal, aberrant neural network activity. Our speculation had initially been that there was maybe underlying inflammation, and that maybe those are one of the reasons why we are thinking and we're seeing this increased TSPO uptake. Increased inflammation, meaning either signal from cytokines or cellular-immune infiltrates, were one of the reasons why we had speculated and things that we had thought about. We've also more recently started to speculate that there may be vascular

abnormalities that are underneath it, and maybe that's the reason why we're starting to see or at least see abnormal TSPO uptake.

Members from our group separate to the supplement had also imaged an additional 12 individuals with Long COVID. And in these statistical maps with a larger comparative control group, we also see this widespread TSPO signal, both in gray matter as well as white matter. The whole brain TSPO signal actually correlates here in this study with markers of vascular disease. I was interested to look at Dr. Spudich's study, thinking about vascular disease, or markers or plasma markers of vascular dysfunction, including fibrinogen or haptoglobin. In this study, increased levels of those markers correlated with at least increased TSPO signal.

What at this time did not relate statistical significance were levels of cytokines or other markers of inflammation and their relationship to at least TSPO uptake. I don't wish to oversimplify or overstate. What we're seeing here are cross-sectional associations in a small cohort, and we don't yet understand causality. But I do think we can start to investigate some of these regions in the brain where we're observing at least this increased glial activation.

As I mentioned earlier, one of our primary goals is to investigate how TSPO signal is related to symptom burden for people with Long COVID. So, we gather information on fatigue, and one of the measures we use is the Multidimensional Fatigue Inventory. The MFI consists of five subscales, including mental fatigue. The mental fatigue subscale yields a total score of 20, with higher scores indicating higher levels of fatigue. In this scatter part, what you're seeing is TSPO signal in the hippocampus, correlating with the severity of fatigue in people with Long COVID.

The hippocampus is actually a critical region in the limbic system. It's a region that encodes for episodic memory. It's also a critical region in the brain that helps us navigate spatial memory of an event, and it's particularly interesting that we are seeing some associations, at least with mental fatigue. One of the key reasons I think we're fascinated by this correlation between a TSPO signal in the hippocampus and mental fatigue is an observation that we had in a control population.

I do like Dr. Korallnik's point to be made that we don't really have controls in the post-pandemic era, but in this pilot study, which is a cross-sectional study, people who are considered control groups in the pre-pandemic area had some degree of TSPO uptake. Post-pandemic, those control individuals where they were not known to have COVID-19 after testing, they still had an increase in mental fatigue. And that increase in burden or symptom burden in mental fatigue was associated with increased level of TSPO uptake, particularly in the hippocampus. What we're speculating is that glial activations in areas that include the hippocampus may relate to mental fatigue, and it's possibly involved in at least a fatigue network in the post-pandemic era.

As I had mentioned, we're interested in understanding special populations with Long COVID, and that includes people who live with HIV. Thus far, we've reconstructed images for our first four individuals who live with HIV. Those individuals are about 10 years older, and there's equal between men and women sex at birth.

We don't have the numbers quite yet to have statistical comparisons, so what I'm showing you are the qualitative images looking at regions of TSPO signal. Again, these are highly preliminary observations, but as we continue to scan people with HIV who have Long COVID, we notice a few key findings. One is that we see at least observed increased signal in the right prefrontal cortex and potentially, we have higher signals in thalamus comparatively from what we have observed with individuals with Long COVID who don't have HIV, as well as maybe some prior work that we had in people with HIV pre-pandemic.

For now, what we're anticipating is that there will be regional differences in TSPO uptake in people with Long COVID who have pre-existing disease. We do anticipate that there's going to be a heterogeneity as we start to try to develop these neuroimaging patterns of Long COVID, and this will be a major point to consider as we start to think about interventional studies.

We are part of the COVID BRAIN Consortia, which is a longitudinal imaging study that spans five locations across the United States. This study employs advanced MRI to try to look at microstructural neurometabolite, as well as vascular dysfunction in the individuals with Long COVID who have neurological symptoms, and they will perform brain MRIs at two years apart. Thus far, they have recruited over 100 individuals, majority of whom are female sex at birth and in their 40s and early 50s.

**Claire Quiner:**

I'm sorry, Doctor, you've got about one minute left for presenting.

**Dr. Shibani Mukerji:**

Thank you.

**Claire Quiner:**

Thank you.

**Dr. Shibani Mukerji:**

Cerebral blood flow is actually a measure of brain tissue perfusion that's been extensively characterized as a marker of hemodynamic function in cerebral vascular disease. Arterial transit time represents the time it takes for arterial blood to arrive at the microvasculature. I'm just briefly going to go over that the data from the COVID BRAIN Consortia does show that at least in non-hospitalized participants, there's at least a trend of lower cerebral blood flow and longer arterial transit time in the brain stem, cerebellar white matter, as well as cerebellar cortex, suggesting that in some people, there's potential involvement of cerebral vascular dysfunction.

In the same study, amongst the hospitalized group, there is a decrease in total creatinine, which is a marker of energy metabolism, glutathione, which is an antioxidant marker, as well as total NAA, which is a neuronal marker, despite months being out after hospitalization. The hospital groups are individuals who are actually not intubated or had organ failure, but these are individuals who required supplemental oxygen. While the numbers are still low in this category, we do see if whether or not trends persist with larger sample size and whether they persist two years later on a subsequent MRI.

In our cohort, we've observed widespread both cortical and subcortical increased TSPO signal in people with Long COVID compared to people without COVID-19, we see increased TSPO signal, at least it correlates with mental fatigue in the hippocampus, and we're qualitatively observing regional differences in TSPO signal in people with Long COVID and HIV that requires further investigation.

I made a comment in some of the considerations. We do think that because glial cells are so highly sensitive to alterations in the surrounding microenvironment, that it could be that these widespread TSPO signal may be due to underlying abnormal or aberrant neural activity, a concept that we can speculate is neurogenic neuroinflammation. We do recognize that Long COVID is a heterogeneous disorder and speculate that using advanced neuroimaging will actually be helpful in identifying endophenotypes in Long COVID. That would then be used to inform clinical trials. One could consider TSPO PET as a translational tool for preclinical studies. It may also be actually a potential imaging endpoint for clinical trials in Long COVID.

So, with that, I want to thank our study participants who have dedicated themselves to understanding Long COVID. We want to thank the Long COVID Justice for getting the word out, our branch study team members, highlighting Ellie Kim, the support of the NIH and the RECOVER Supplement. The studies we talked about are from the parent R01, the HOPE Study. I talked about branch, as well as the COVID BRAIN Consortium. So, thank you for your time.

**Claire Quiner:**

Thank you. With that, we'll pass it over to Dr. Joseph for discussion, questions.

**Dr. Jeymohan Joseph:**

Yeah. I want to thank all the speakers for the excellent presentation. Before we get into a discussion, I want to give a really short summary of the key points of each of the presentation.

Dr. Serena Spudich provided an excellent summary of the cerebrospinal fluids in past from a cohort at Yale. Some of the baseline results indicate no elevation of clinical markers of CSF, of inflammation, or blood-brain barrier breakdown, or CSF cytokine chemokine levels. However, there was evidence of perturbed plasma markers of vascular and endothelial dysfunction, no evidence of viral antigen persistence in plasma or CSF.

Dr. Koralnik discussed his studies of neuro-PASC in older adults. His work was done at Northwestern, where he evaluated 21,050 patients during COVID. A key hypothesis of his studies was that sleep and circadian

rhythm disruption and associated fatigue results in cognitive problems in older neuro-PASC patients. And older neuro-PASC patients have lower quality of life in domains of cognition, fatigue, sleep, anxiety, and depression. And decreased sleep time may be linked with dysregulation of T regulatory function and inflammation.

Finally, Dr. Shibani Mukerji presented her work examining brain neuroglial dysfunction, using multimodal imaging in patients experiencing PASC. She also used TSPO PET, which captures activated microglia. Cortical and subcortical brain regions showed increased TSPO uptake in Long COVID patients experiencing neurological symptoms, and increased TSPO uptake correlated with mental fatigue.

Okay. Now, we'll go to some questions. First, to Dr. Spudich, so your study showed no evidence of viral persistence in PASC patients in CSF. But there have been some studies from other investigators, showed that viral RNA persists, for example, in colorectal tissue for up to two years. Viral protein has been identified in plasma for up to 16 months. Do you think these persistent RNA or protein in peripheral tissue could have some impact on CNS pathophysiology? What are your thoughts on that?

### **Dr. Serena Spudich:**

Yeah, I know. That's a great question. And I'd like to just make a statement that so far, we have a small study. We do have a small study, and looking, for example, at CSF antigen is just one marker of even persistence in the nervous system. I think that the clinical data from Acute COVID, though, even in Acute COVID, 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 that we thought would be something we might find as positive in post-neuro-PASC. But I don't think we've proved 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 is a real potential connection between what's happening in the gut and the brain. On the other hand, we haven't detected from just a small panel of markers 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, that was not actually associated with Long COVID. That was simply found in people some months after COVID, but there was no necessary connection. So, even the persistence of that antigen in the blood that was 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, ongoing neuronal injury, and so far we're not seeing that. But definitely we have not laid the question to rest and we have other markers we're looking at.

### **Dr. Jeymohan Joseph:**

Okay. Okay. Thank you. Yeah. Dr. Koralnik, so 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?

### **Dr. Igor Koralnik:**

Certainly. Dr. Joseph, you saw that today, all the presenters pay 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.

And so, I think it's very important to distinguish them. We have to study both of them, but we have to analyze the result 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 previously healthy, who may have viral persistence, may benefit actually from Paxlovid, so these should be analyzed separately.

As we go along in the pandemic, there are fewer and fewer patients being hospitalized, fortunately. 90% 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.

### **Dr. Jeymohan Joseph:**

Okay. Thank you. Shibani, so what specific advantage does TSP or PET imaging offer in assessing treatment efficacy and predicting patient outcomes in neurology-related clinical trials? Are there concerns regarding reproducibility and standardization of TSP or PET imaging across different sites or populations?

### **Dr. Shibani Mukerji:**

Yeah. I think that's an excellent, sophisticated question. One of the things that sites do is actually standardize protocols. This is not unique to TSPO. This would be something that would have to be done for MR imaging as well. So, protocols standardized across the sites. And so, ensuring that you're using the same radioligand, same protocols for cyclotron. I think those items, I think those are operational logistical items. So, yes, 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. And so, that in and of itself is true.

Then, the concept of variability, I think, holds true for almost any of our imaging modalities as well. There's going to be day-to-day just variability for an individual, as well as participant-to-participant variability. The question, I think, is how you're going to design that study trial, I think, will be incredibly important. Statistical design of these study trials, whether you're going to use a baseline versus... Two time points, pre and post treatment, is one study design. It could be another type of study design.

The group has used TSPO PET specifically, or are using it right now for a dietary interventional trial in a syndrome that also has fatigue, as well as diminished cognitive focus. Those design trials, again, had preliminary data to inform how they're going to look at regions of interest and things like that. So, the answer is yes, you would be appropriate to be concerned about variability. I think study designs will have to be thoughtful when you're using an imaging endpoint.

And then, to be very clear, that 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.

### **Dr. Jeymohan Joseph:**

Thank you. I think we're out of time for discussion, but I hope all these excellent studies will ultimately lead to clinical trials and treatment for Long COVID, particularly neurological aspects. Over to you, Claire. Yeah.

### **Claire Quiner:**

Wonderful. Thank you, Dr. Joseph. I think we have time for just one or two questions from the Q&A. We'll start with one from Dr. Spudich. 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?

### **Dr. Serena Spudich:**

The first question, so what we did is 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.

And some studies have begun to look at abnormal vascular dysfunction, which affects all organs, kidneys, heart, lungs, brain, 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, 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 during people with PASC versus people who have had COVID and don't have PASC. Our study is actually going to do longitudinal assessments at one year and two years, to try to answer that question of whether it resolves, but we haven't gotten there yet. That was a great question.

### Claire Quiner:

Wonderful. Thank you. Perhaps time for one other question. This is just open to the whole group. Someone's interested in hearing about how COVID-19 or SARS-CoV-2 affects small muscle ischemic disease and gliosis. Anyone have any comments on that?

### Dr. Shibani Mukerji:

I'm not sure, Claire, if I could maybe reframe that question. Is it, how do you affect cerebral small vessel disease? Is that the question specifically?

### Claire Quiner:

I think so, yeah.

### Dr. Shibani Mukerji:

Okay. Maybe I can tackle that. One of our interests, I think many people have discussed interest about 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 would be one of the great ways to really detect that. It sounds like other individuals, including Dr. Spudich, is 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, cerebral vascular disease in general. Hopefully, Claire, that answers the individual's question.

### Claire Quiner:

Wonderful. Thank you. With that, I think that we will move on here to our closing remarks. I want to say thank you so much to our wonderful presenters today, to our audience for attending the seminar and engaging with the Q&A, especially those who submitted questions in advance.

As a reminder, a recording of today's seminar will be available on [recoverCOVID.org](https://recoverCOVID.org) within a few weeks. We'll also be posting a Q&A document that has responses to the questions received today, including some of those that we did not have time to answer in our Q&A session today.

We do have some exciting topics coming up, and we hope to see you at future sessions. Please, check back on our website for upcoming dates and topics. Additionally, you'll see a short survey come up on your screen, which asks for your feedback on this seminar. We would appreciate if you could take a minute to fill out this brief survey.

With that, I want to say thank you to everyone, and have a great day.