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 presentations at the R3 Seminar ***Characterization of PASC and investigation of biomarkers: Insights from the RECOVER adult cohort*** held on February 11, 2025 (videos for this and previous seminars are available from https://recovercovid.org/r3-seminar-series):

- ***Standard Clinical Laboratory Measurements Do Not Differentiate Prior SARS-CoV-2 Infection and Post-Acute Sequelae (Long COVID) among Adults in the RECOVER Cohort***
  Kristine Erlandson, MD
- ***2024 Update of the RECOVER-Adult Long COVID Research Index***
  Linda Geng, MD, PhD
- **Discussant: Grace McComsey, MD, FIDSA**

* Responses may have been edited for clarity.

All Presenters: Questions and Responses

Q. Was the arterial stiffness study inclusive of pregnant women?

Response:

Dr. McComsey: Unfortunately, it was not. In fact, the technique is so non-invasive that you could easily do it in pregnant females, but we didn't have any pregnant women on study.

Q. Does the mechanistic working group cover biomarkers too?

Response:

Dr. McComsey: Absolutely, yes. One of the key goals is that the scientific studies we're providing samples for from the six different clinical trials will be used for biomarkers as well.

Q. Dr. Geng, was there an effort to exclude those overlapping syndromes (myalgic encephalomyelitis chronic fatigue syndrome [ME/CFS], postural orthostatic tachycardia syndrome [POTS], and mast cell activation syndrome [MCAS]) not associated with COVID or were they included in the model?

Response:

Dr. Geng: It's very difficult to exclude these syndromes because a lot of the symptoms overlap with each other. For example, the fatigue and the post-exertional malaise are also cardinal symptoms of ME-CFS. I think the goal was to add these symptoms in response to patient and community feedback to recognize that these are common symptoms in Long COVID. What are the commonalities from these overlapping syndromes? Do they come to the forefront when we look for the differences between infected and uninfected groups? And indeed, some of the symptoms—for example, post-exertional soreness—are also on the top of the list in the most common symptoms that we ended up seeing in those who identified as likely Long COVID.

This raises a lot of questions about why are there so many similarities. Are these actually similar or shared syndromes that just can be triggered by different viruses? And as you may recall from the prior seminar from R3 series about the original paper, we used the WHO definition and there are some who may not have had confirmed SARS-CoV-2. It was likely that they had SARS-CoV-2, but it's also possible they may have had other infections as well. So I think this is an important aspect where different syndromes have a lot of significant overlap, and we need to do deeper understanding and digging into shared potentially common pathways as well as manifestations. So that's a great question.

Q. Is there any evidence for the 3-month timeline for Long COVID? Why isn't it shorter, like the initial WHO definition of 4 weeks?

Response:

Dr. Geng: This is a great point, and one of great debate. The National Academies of Sciences, Engineering, and Medicine (NASEM) definition comes from stakeholder input—from researchers to clinicians to patients, policymakers, researchers, et cetera. And this is of great discussion—it was in one of the workshops. There is a rationale behind it, but I think it's really important to understand that it's a spectrum and it doesn't mean that if you had 2 months and 15 days, that you're not suffering from post-acute sequelae. In fact, some people even propose we should we call it medium to Long COVID. I think it all is just to emphasize the importance that after an infection, what we define as the acute period, there can be lingering issues that occur in the post-acute period, be it days to weeks and months to years, and that's very clear.

The reason why 3 months seems to be selected or favored as a consensus is that when you look at the rate of potential improvement in overall population, there is data to suggest that the rate of improvement is quite significant and then it tapers off around 3 months. So the likelihood of somebody improving past 3 months and the likelihood of somebody staying in the chronic illness state is higher within the 3-month period. So that's part of the rationale. But again, I want to emphasize it's a spectrum.

Q. How did RECOVER control for those without prior infection? Is it just self-reported no prior infection?

Response:

Dr. Erlandson: Most of this time of enrollment was from a few years ago, and so we had a larger portion of individuals who had not yet had infection. At the time we were enrolling, it was challenging to still find people who hadn't had a COVID-19 infection or didn't believe that they'd had infection. We started with just self-report of individuals who thought that they didn't have prior infection and then that was confirmed with antibody testing. And then we had people who came in with acute infection, but that was a different group of people. So self-report confirmed by antibody testing.

Q. Do the studies include post-vaccination long haulers?

Response:

Dr. McComsey: I've had people reach out to me nationally saying, "Can you see us? Long COVID clinics do not see people who have Long COVID symptoms after vaccination." And I have to say this post-vaccination syndrome does mirror Long COVID and is now being more accepted. I remember the first patient, an individual who reached out to me. I was skeptical because we had a lot of hesitancy for the vaccine and a lot of rumors and so on. But now it is getting more and more accepted, and we are seeing more and more people say that they have symptoms that are Long COVID symptoms after vaccination even though T-cell testing proves they never had COVID infection. So it does happen. I think now it's more accepted.

In RECOVER, we have not added post-vaccination induced Long COVID. Honestly, by now it's like everybody had the infection so it's really hard to be able to tease out the difference between the two. I know that there are some cohorts like the Yale cohort which is focusing on trying to understand the post-vaccination Long COVID. And I think that is important because there are a lot of people who are suffering from that. So that said, again, because it's vaccination, I want to clarify that contracting Long COVID after vaccination is much rarer. The prevalence is way less than Long COVID after COVID infection. So this is not an anti-vaccine statement. It's clear that some people have it, but it's much worse to have an infection than to have the vaccination.

Q. What are your thoughts about other inflammation markers that seem to correlate with Long COVID?

Responses:

Dr. Erlandson: Interleukin-6 (IL-6) has always been of interest. Some of the early studies targeted IL-6 in acute COVID, as we know that it has an important role. We've learned a lot about inflammation in the HIV world and chronic consequences of HIV infection. And when we focus in on one biomarker, we tend to have negative studies—and it may be the interaction of how all of these different inflammatory markers are working together, perhaps one suppressed, but then there's another pathway or two that gets upregulated. Looking at how these biomarkers all interact with each other is probably more relevant than just focusing in on one biomarker.

Dr. McComsey: That's a great answer, Dr. Erlandson. You can tell that we do a lot of inflammation studies in HIV. One question I had on my list to mention relates to kynurenine, which is a metabolite of tryptophan. And Dr. Erlandson, I know that that actually is the same story in HIV. It seemed like that may be a biomarker and it correlates with inflammation like IL-6, so it could be one of the potential metabolic biomarkers that are important in Long COVID as well. It is definitely something that requires further testing. I don't know that we have definite plans yet within RECOVER, but that will be something to look into if we don't already, so that's one good potential for biomarkers.

Another one I want to mention is oxidative stress. I know my group has done a lot of studies with oxidized LDL. And starting with HIV we found that oxidized LDL not only is high, but really predicted all the monocyte activation and inflammation. We're seeing the same thing with Long COVID. Oxidized LDL is very different. This is only one marker. There are a lot of other markers of oxidative stress. I think that is another pathway that's definitely worth investigating in Long COVID. And we do have plans within RECOVER to look at more oxidative stress.

Q. How can we look at micro-clotting even more?

Response:

Dr. Erlandson: I think there is a similar question on looking more at platelet hyperactivity and changes in platelets over time, which probably fits together. Some of our findings may have been related to timing post-infection, and we had a variety of individuals, some that had had infection within the last 6 months and some closer to 6 months, and some that were really a couple of years out by the time they enrolled. And so some of those findings might've been a bit diluted just by the range of different time of infection or the range of severity of symptoms. But I absolutely think it's worth further investigation looking at the activity of platelets well beyond just the total platelet count.

Dr. McComsey: Correct. So it's not just number, it could be the platelet hyperactivity. One study implies that the clots have inside of them different platelet hyperactivity, different amyloid. There is a way to stain them, and the

investigator was able to show that in less than 100 people with Long COVID (but still a significant sample size), all their blood had micro clots that she was able to see under the fluorescent microscope. I think it's interesting, with the platelets being hyperactivated and those micro clots that you can see, it's going to be those tests probably that will show us something much more than D-dimer or fibrinogen. I think it is getting more and more sophisticated tests rather than just a regular blood test that's going to give us the answer. Another [study] that came out is on autoantibodies. So we all hear about different autoantibodies that are seen after COVID.

Q. Are autoantibodies after acute COVID linked to Long COVID?

Response:

Dr. McComsey: It's a great question. Yes. There are a bunch of autoantibodies, including autoantibodies to specific organs and systems. It's unclear actually which one is meaningful. However, we did a study in Cleveland where we looked at new onset autoimmune disease in people who had COVID, regardless of symptoms, if they were at risk of new onset autoimmune disease. And we looked at the value of autoantibodies and predicted that. And what we found actually is yes, even with a good control, people around the same time period, demographically controlled, who had COVID seemed to have a trigger of more new onset autoimmune disease. And the anti-nuclear antibodies stuff you see with lupus and other autoimmune disease predicted who's going to develop autoimmune disease. It was very interesting. Obviously it's one study, it needs more data on it, but these autoantibodies, at least some of them seem to be predictive of outcomes. We have to do more of these autoantibodies for sure.

Q. A slide showed the difference or higher rate in albumin/creatine. Did you see a rise or decline from the start of Long COVID over time? Did you monitor this and what was any change attributed to any treatment?

Response:

Dr. Erlandson: This was really just the first laboratory assessment or that initial time point, just a cross-sectional look at the laboratory measures with either no COVID or with prior COVID infection or with the Long COVID index. In the way that laboratory measures are done, there are a certain number of people that will "trigger" repeat assessments only if they have an abnormal initial testing—then they'll have a repeat of just the abnormal tests over time. And so, we will have longitudinal trajectories for participants who had initially abnormal tests over time. We did not look at it in this current manuscript, but we certainly plan to look at how these measures change over time. I think there was a similar question related to platelets or another lab measure. Similarly, this is certainly of interest to look at how these measures are changing over time.

Q. I have read and heard about people having long influenza and other prolonged symptoms post-viral infections. Is this similar to Long COVID?

Response:

Dr. Geng: That's a great question. And it goes back to that umbrella term of infection-associated chronic conditions under which Long COVID falls. And there are many viruses—many infections, bacterial or viral—that can induce long-term sequelae or long-term consequences and symptoms and syndromes. And so influenza is also one of those. There have actually been great EHR studies looking at and comparing those who had influenza and those who had COVID, and then they looked at symptoms thereafter and they found a lot of similarities. The rates of the symptomatology seemed to be higher in those who had COVID, but still very similar types of fatigue and other cognitive issues and other symptoms that we associate with Long COVID but also we're seeing in those with influenza just at a lower rate. So, again, I think this goes back to these overlapping syndromes, and that viruses and other infections can trigger whatever the downstream pathways are that often manifest in very similar ways, be it fatigue or cardiovascular or pulmonary or multi-system. This is an area where we really need to do deeper biological investigations about all the different post-viral syndromes of post-infectious illnesses.

Q. Given that many studies found endogenous carbon monoxide was increasing during acute COVID infections, and given that all the symptoms of Long COVID are also reported in studies of carbon monoxide poisoning survivors, I'm wondering why these studies did not measure CO. Were any tests of CO in blood, breath, or skin considered?

Response:

Dr. McComsey: That's a great question. I'm aware of the studies during acute COVID, mostly in very sick ICU patients, but I'm not aware anybody's looking at it in Long COVID. We should look at it.

Q. Should research examine specific cellular processes, such as stress as indicated by H2S levels or kynurenine pathway biomarkers?

Responses:

Dr. Erlandson: Absolutely agree. This was really the first attempt to use the existing clinical laboratory measures that are already obtained in clinic to see if there's anything here that we might incorporate into the PASC definition that RECOVER is using for research purposes. We didn't see anything. I don't know that we were expecting to see marked differences in many of these routine clinical biomarkers, and we most certainly do need more of these mechanistic biomarkers, which are being currently investigated in many of the Research

Opportunity Announcements (ROAs) that Dr. McComsey mentioned as well as in some of the mechanistic sub-studies or mechanistic working groups within the RECOVER clinical trials.

Dr. Geng: This is from my clinician perspective. A lot of times, if you find a negative finding, it seems boring or like, "oh, well of course we need to look deeper." But it actually is important because a lot of patients will, in their clinical journey, get these tests, and the doctors and patients see that results are normal. Well, why do I feel this way if this is all normal? And I think this is evidence from people we know from our cohort—that these aren't necessarily the signals that we need to look for. We just haven't yet developed or haven't validated a clinically available test as a biomarker for Long COVID. But it's important to educate a lot of our clinicians out there in practice who are running these tests, not to dismiss that there may be other underlying biology pathways. We just haven't established those yet for Long COVID. But just because somebody has negative findings in the list of common routine laboratory tests doesn't mean they don't have Long COVID and it doesn't mean that there isn't something biological going on. We just haven't established those yet. So it's an important framework for clinical practice as well.

Dr. McComsey: I couldn't agree more, Dr. Geng. That's a great point, because clinicians who are seeing people with symptoms a lot of times dismiss them saying, "oh, your labs are normal." This is a very important negative study and clinicians should not dismiss people just because their labs are normal. One thing we didn't mention, and I have to mention because I started my career looking at mitochondrial dysfunction—there are studies of mitochondrial dysfunction, whether in the peripheral blood mononuclear cells (PBMCs) or in the blood of people or in the muscles. I think definitely mitochondrial dysfunction is one of the potential mechanisms we're looking at in RECOVER. We even have muscle biopsies that will be done within RECOVER, and obviously a lot of blood that we could look at. So that's another one that we didn't mention, but I want to assure people that we have this in the back of our mind. We didn't forget about it.

Q. Are there other tests that the team is brainstorming or considering after seeing that the basic tests are not showing clear differences between Long COVID and non-Long COVID patients?

Response:

Dr. McComsey: I would say, everything we mentioned—from the oxidative markers to the tryptophan metabolism to the inflammation. We have an exhaustive list of potential mechanisms that we're looking at. And I have to mention one—I know it didn't come up yet, but the whole antigen assay. RECOVER was part of a paper that came out looking at different spike and nucleocapsid antigens in the blood of people with Long COVID. And although in asymptomatic people who had COVID at some point but have no symptoms, about 20% did have those antigens—for the symptomatic people, it was like 80%. There was a relationship between having the circulating antigen and

having symptoms. So that's another line of investigations looking both at mRNA, looking at antigen to see how much of a potential reservoir SARS-CoV-2 is building in the bodies and where the reservoirs would be. It's like thinking about HIV again. Brain lymphocyte and Gut are very important in Long COVID. So there are a lot of different issues that we will be looking at in RECOVER to try to decipher if there is really a reservoir of the virus hiding somewhere.

Q. Has RECOVER looked at the damage done to NMDA receptors from Long COVID?

Response:

Dr. McComsey: I'm not aware that that's actually part of any study, but I want to encourage people. If you are a scientist with a lab and you have ideas, as I said, we have tons of blood. There is a mechanism to be able to do studies using RECOVER samples. We would love it if people submitted thoughts and collaborated with one of the investigators in RECOVER to be able to learn more. We want to learn as much as possible from RECOVER.

Q. What have you discovered about the differences between Long COVID patients infected in 2020 prior to vaccinations and those who developed Long COVID after at least one vaccine was administered?

Response:

Dr. Geng: I think the whole vaccination discussion is really important—both in terms of what Dr. McComsey already talked about regarding post-COVID vaccination syndrome, and also the impacts in a lot of literature that suggests that it's protective against developing Long COVID. What we found in the index research, as I mentioned, is that hybrid and subtype of subtype five where individuals in that group really have a significant number of symptoms, and that seems to correlate with those who did not get vaccinated as well. So it may be that there are more severe phenotypes that occur not just the increased risk of Long COVID, but increased risks of more severe Long COVID as well. I think the relationship is complex, as we've discussed and alluded to, so we really need to understand. And then the vaccination can occur in so many different phases. Our group was also trying to study what happens if you have Long COVID and then you get vaccination after Long COVID as well. So there's the pre-COVID and then, independent of COVID, getting the vaccine and maybe having symptoms—and then there's the post-COVID where it could have potentially beneficial effects or potentially worsening effects as well. That all requires teasing apart from a biological level of understanding the immune pathways likely that are being triggered or dysregulated.

Q. Is it thought or suspected that individuals with Long COVID become more susceptible to inflammatory processes than, say, someone with a different viral infection initially?

Response:

Dr. McComsey: I think obviously it will be just postulating. But I think that is the concern. As we learned in HIV, people with HIV are not dying from HIV. They're having all kinds of comorbidities because of this sustained chronic inflammation that they have. So going back to Long COVID, that's the concern that most researchers have. How much of this inflammation is going to end up with a lot of comorbidities? Not only cardiovascular and diabetes, but even cancer, even premature aging. So there's a lot of concern about this chronic inflammation and what the long-term effects are. And this is why it's important for the cohorts not to just look at short-term. Long COVID is not going anywhere. Most people are not getting better. So we need to understand not only Long COVID, but also, as I mentioned, what is the effect of the virus even in asymptomatic people. Are we going to start seeing a lot of cancer and heart disease and clotting? To me, that's a huge concern as well.

Q. If you do not propensity match on thrombocytosis, which is driven by IL-6, would you see thrombocytosis in any of the PASC subtypes?

Response:

Dr. Erlandson: Propensity scores did not match on thrombocytosis, but were calculated based on age, sex, race and ethnicity, SARS-CoV-2 variant era, referral source, vaccination status at the index date, comorbidities before the index date (not included for the PASC subtypes), homelessness, employment status, insurance status, income, level of difficulty in covering expenses, last visit to a physician, and food insecurity.

Q. This discussion obviously has focused on biomarkers, but is there discussion about other types of diagnostic testing (physiologic testing, nuclear medicine tests, etc.) as a means to distinguish Long COVID patients from non-Long COVID patients?

Response:

Dr. Erlandson: Yes, several other physiological tests have been obtained as part of RECOVER, and analyses are currently underway. We expect these data to be presented and hopefully published soon.

Webinar Slides

To request a copy of the R3 Seminar slides, please email RECOVER_ACC@rti.org.

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/covid/long-term-effects/
- For medical/scientific terminology: https://medlineplus.gov/healthtopics.html