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 ***Sex Differences in Long COVID*** held on March 11, 2025 (videos for this and previous seminars are available from https://recovercovid.org/r3-seminar-series):

- ***Title***
  Dimpy Shah, MD, PhD
  Nora Singer, MD
- **Discussant: Mia Christopher, BS, MPH**

* Responses may have been edited for clarity.

All Presenters: Questions and Responses

Q. How did the researchers determine COVID variant: with sequencing data or generalizing by timeline?

Response:

Dr. Shah: That's a great question. We didn't have the sequencing history or sequencing done for this manuscript. We did it based on the generalized time of XYZ variant, and December 2021 was taken as a cutoff. Anything prior to that was considered Delta, and after that was Omicron. However, RECOVER does collect biological specimens and there is a plan for sequencing in the future. I mean, again, we did not have that data. Thank you.

Q. The results presented by Dr. Shah for women aged 40–59 show the relative risk (RR) for Long COVID was 1.45 for non-menopausal women and 1.42 for menopausal women, while both their risk difference (RD) (0.08) and propensity matching (0.18) were the same. These results do not appear to support your conclusion that menopause status makes any difference in risk of getting Long COVID. Why did you conclude that it does?

Responses:

Dr. Shah: We couldn't measure the prevalence of Long COVID in RECOVER, but after propensity score matching, the estimated proportion of males, not females, with Long COVID was 18%. So actually, that 18% is the proportion in males. And as I mentioned, all the risk ratios look similar, the confidence interval included 1. So, those who were menopausal were not at a higher risk for Long COVID. In fact, those who were non-menopausal were at a higher risk for Long COVID. And again, this could be also due to lower sample size in that category, but definitely non-menopausal females had a higher risk because the sample size was very large for that specific subcategory.
Dr. Singer: So, when the confidence interval crosses 1 and you're talking about relative risk, then we conclude that we're unable to statistically determine a difference.

Q. How does hormone replacement therapy affect women with Long COVID, particularly those experiencing perimenopause?

Response:

Dr. Singer: I don't think we know the answer to that. In RECOVER, if you remember, we designed the study as the pandemic was ongoing. So the coordinating center primary investigator (PI) used to like to say, "We're building the plane as we're flying it." The result of this is the medications are in natural language and will need to be entered into discrete fields to do that kind of analysis. As to whether or not that's currently being done, I haven't gotten a recent update. But people were talking about whether or not AI could be used to do that or other things, because it could take somebody several months by going through each participant's medication. If we could somehow get these medicines into classes, then we could potentially answer that question, depending on how large the sample is of people who are on hormone replacement therapy.

Q. Have you found women with hormonal imbalances, such as polycystic ovary syndrome (PCOS), more likely to develop Long COVID or have complications from COVID?

Response:

Dr. Singer: I don't think we know the answer about PCOS. My contact PI here at Case Western is very interested in some of the metabolic changes that have accompanied COVID. But because PCOS is a clinical diagnosis, we may not have captured all the things that we need to capture.
I think I saw something in the chat about endometriosis too, and we were really unable to answer that question. I came across a review that referred to autopsy studies, finding that on autopsy up to 70% of women had some evidence of endometriosis. So, 30% of those who have it are symptomatic, but it's found in up to 70% of women. I'm not sure that we're going to be able to completely answer that question without really detailed studies and looking for it even in asymptomatic women. We may be able to answer a question about symptomatic endometriosis but not asymptomatic endometriosis.

Q. When describing the RECOVER cohort, a 6-month visit after index infection was mentioned. How is this different from the display of symptoms at 3 months post COVID-19 for enrollment?

Response:

Dr. Shah: That's a great question. This was a research study—it wasn't meant to identify those who develop Long COVID earlier, because it is very likely to have Long COVID before 6 months after infection. Also, it wasn't meant to identify or classify those who need access to some of the Long COVID programs or disability benefits. For research purposes, we were interested in those who had persistent Long COVID infection, and 6 months was chosen as a cutoff because having symptoms for 6 months or longer meant that we were capturing those who had persistent Long COVID symptoms. We completely understand and acknowledge that this limitation might lead us to have only a subset of participants who had Long COVID, as compared to those who could possibly have Long COVID much earlier. But for research purposes, we selected the 6 months cutoff.

And again, the research index is purely that, it's meant for research. I mean, we have to pick a time point to classify patients as Long COVID positive. And we were very specific in mentioning those who didn't have more than 11 symptoms or less than 11 symptoms were classified as Long COVID indeterminate—possible Long COVID, not negative. So, we don't say that these are negative, we just say that those who had 11 or more and we focused on that.

Q. Have you observed an overlap between environmental illness, postural orthostatic tachycardia syndrome (POTS), and Long COVID, particularly with an increase in female patients? And have you found testosterone to be a helpful treatment?

Response:

Dr. Singer: I'm particularly interested in POTS because I've seen it for many years in children and adolescents that I sometimes care for, especially after mononucleosis. The problem with POTS is that you may come to POTS from many directions. The studies of commercial antibodies against something called G-proteins have not proven very successful. The G-protein-coupled receptor, which antibodies are thought to be directed against, is a very difficult receptor to study because it transverses the cell membrane seven times. To recreate it in a lab is difficult, but it's being worked on. We're actually funded to look at some patients and some of the transcriptomics from them. But I think that you can get POTS from genetics. We know that there are people who have heritable disorders that resemble POTS. We know that you can get there after a viral infection, and probably you can get there after certain environmental threats yet to be determined.

I don't think there have been rigorous studies of testosterone on POTS. Much of the time, clinically, patients who have POTS have a rise in heart rate and no drop in blood pressure with a head-up tilt table. But many patients are diagnosed with POTS-like illness without actually undergoing the tilt table test, which is part of the definition. And so we don't know whether, in patients who have symptoms that are similar to POTS but are tilt table negative, it's just a spectrum and a curve. And POTS is only one of the dysautonomias that we can see post virally. We've seen arrhythmias and tachycardia or fast heart rate, we've seen gut mobility issues. There are a number of these dysautonomias we've seen, sensory things, and we're only beginning to do some sensitive testing to look for that in the RECOVER cohort.

But until we really define what we're focusing on for Long COVID and which of the dysautonomias, we're not going to be able to do rigorous trials of testosterone. I don't know of any placebo controlled randomized studies of testosterone in any of the dysautonomias. If somebody else does, I'd be glad to read about, but I think that's an unknown.

Q. Does your cohort include post-viral infection status, such as having prior exposure to Epstein-Barr virus (EBV)?

Response:

Dr. Singer: I'll answer that in two ways. One, patients with some of the symptoms that you get with Long COVID were pretty rigorously asked when they enrolled about whether or not they also had those symptoms before. Sometimes people have preexisting symptoms that have worsened. So we have a pretty good understanding of who might've had some dysautonomia symptoms when they came into the study.

There have been a series of two rounds of studies that have been funded that accompany RECOVER that are what are called pathobiology studies. They are meant to try and understand how the symptoms of Long COVID arise. And there are at least two awards at Stanford that I know of. One is looking directly at EBV, and one is looking at some of the other targets of antibodies, some of which might be implicated in Long COVID. But we don't have those answers yet.

Q. Can you define partial versus full vaccination status?

Response:

Dr. Shah: Vaccination status was defined in our previous manuscript from RECOVER that actually also defined the Long COVID research index. For this cohort, individuals were considered fully vaccinated if they had a final dose of their primary series, second dose for all vaccines except J&J, administered 14 or more days before indexing date of infection. Individuals are considered partially vaccinated if they only had one non-J&J dose before 14 days of their index infection, or had their first vaccine dose, any type, in the 14 days leading up to their index date. And if they

had zero doses before index date of their infection before December 1, 2020 (when vaccines started to become generally available), they are considered unvaccinated.

We understand that this does not account for the boosters. But again, the surveys were done at the time of enrollment, which was way back when we didn't have boosters—or even in the post-acute cohort, when the vaccination happened well before. So we were trying to understand the vaccination status at the time of their index infection or in relation to that acute COVID.

Q. Has there been an analysis of pre-menopausal females that have hormonal or menstrual diagnoses like PCOS, endometriosis, and so on? Is there anything further you'd like to say on this?

Response:

Dr. Shah: No, as Dr. Singer mentioned, this was such a Herculean task just to examine so many subgroups and so many sensitivity analyses, for us to first decipher whether even there is an increased risk with regards to biological sex. Now that we have a pretty good understanding, or at least our data show that yes—females overall and specifically some of the subgroups have a higher risk of Long COVID, I think it would be worthwhile. And that is what we conclude in our paper, that our future directions would be to definitely look at even more granular detail with regard to hormone replacement, contraception, sexual hormonal variations, and also some of these PCOS or endometriosis conditions that are very specific to females. But I believe this is the very first step in that direction, and we hope that this is an impetus for doing more studies, research wise—but also a call to action with regards to public health policy and increasing awareness that this is a high-risk group for Long COVID.

Q. With potential effect of whether in African American women particularly, is there an earlier age range for the sex selection seen for Long COVID in that group compared to their white and or Asian counterparts?

Response:

Dr. Shah: We didn't do that, and we didn't have enough data or substantial evidence to select a different age category, if I'm understanding. The question is if we selected an earlier age category for black patients compared to their counterparts non-Hispanic, white, and Hispanic. We didn't have substantial evidence to do that, to justify that. However, as we showed in those propensity score matching, we kind of balanced it, with regards to race and ethnicity. So when we balanced it with all of these factors, we are trying to see if there is an adjustment for all of these covariates, and then how it matters.

Again, the scope of this manuscript was not to compare various races and ethnicities, but to compare females with males. I'm sure there is work done in RECOVER with other studies. It's such a large cohort with so many covariates that there are multiple studies coming out looking at multiple infection, the role of age on Long COVID, the role of race and ethnicity, where they can do these kinds of deeper dives. But we didn't do that in our study.

Q. Can you please clarify the effect of comorbidities.

Responses:

Dr. Shah:. I'll try to explain it in a causal mechanism pathway. There are some factors that are assigned at birth like age. I mean, age just moves along as time progresses. Or sex, biological sex at birth, whatever they're assigned at birth—or even race and ethnicity, that doesn't change with time.

Now comorbidities, they change with time. And one of the factors for these comorbidities, what we are trying to say is that some of these comorbidities have a predilection to be present in females and some of the comorbidities have a predilection to be more present in males. So when we're doing statistical analysis, when we're trying to look at whether there are differences with regards to sex on developing an outcome, if we were to adjust for things that are in the pathway, then we are over-correcting. So what we're trying to find out is, given that they have equal comorbidities, is if males or females have higher risk of Long COVID. And we found that they don't [i.e., there is no difference], because Long COVID is associated with--or the risk is increased by--some of these comorbidities.

Now, we have not examined which comorbidities, and our hope is that the future papers will do a deeper dive like we did with regards to sex they would do a deeper dive on comorbidities.

But what we are saying is that the beginning is the sex, the biological sex that leads to an increase in comorbidities, which eventually may increase Long COVID—or even without comorbidities, the female sex could lead to higher Long COVID, and that is probably because of the hormonal levels. So, these are the two factors we feel are the mediators or the mechanism, the underlying biological mechanism of why females might have higher risk of Long COVID. It could also be related to immune system and the various stages in the sex or in the life of a female with regards to pregnancy, menopause, and so forth, that changes these hormonal levels that might have an impact on the risk of Long COVID.

Dr. Singer I think about it as a clinician, that if you're thinking about atherosclerotic disease, if you try and match on atherosclerotic disease, you reduce your proportion of women since we have a lot of middle-aged women. Whereas cardiovascular disease is much more common in men, or at least until recent years has been much more common in men. So that goes in this direction, but if you're looking at something like BMI, it might go in this direction with women having slightly higher BMIs. So you're putting together things that go in opposite directions and they might balance each other out and you might also miss something that is significant if they're not the same comorbidity going in the same direction for everybody.

Different things go in different directions as risk factors for men and women, it seems. And so you might actually miss something important if you try to take into account all these things. You might end up with a mishmash of

things that you fail to recognize as true and you said were false or were not statistically significant because you had something else compensating in the opposite direction that was more common in men or women. I think that was our concern.

Also, when you propensity match on cardiovascular disease, you have a much lower sample size. You reduce your sample size because there are many fewer women in the RECOVER cohort who started out with some elements of cardiovascular disease than men, or with other autoimmune diseases which are more common in women. So, I think that the statistical analysis plan was not designed to look at all these different factors separately, and we worried about losing power by doing so, and not really coming up with anything at all. The more you slice and dice, the more problems you have showing statistical significance, if that makes sense.

Dr. Shah: And I think also with regards to if A leads to B, and B leads to C, then B does not really lead to C, it's A that is the driving force. So I would just say, we wanted to begin at the starting point, like, what is the driving force of increasing the risk of Long COVID? And the driving force is sex, not really comorbidities. It's the sex that is driving higher comorbidities, which might have an increased risk of Long COVID. So, we want to start at the most proximal level of, what is the driving force or the causal factor for developing a particular outcome?

Q. Which specific sex steroid-based therapies for males were you referencing?

Response:

Dr. Singer: I don't think I was particularly thinking of any specific ones. There's a lot of debate about testosterone therapy even in males with low testosterone, and it may be only symptomatic males that sometimes get treated. So, I wasn't thinking of anything specific—but more about whether things like hypogonadism might occur at a higher rate, or since we've seen senescence in immune cells, whether the symptoms associated are seen more prominently. But I didn't have anything specific other than that in males.

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