Transcript

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Mia Christopher:

Good afternoon and welcome to the RECOVER Research Review or R3 seminar. My name is Mia Christopher, and I'm a research epidemiologist with the RECOVER Administrative Coordinating Center and the moderator of today's seminar. The goal of the 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 questions that arise during today's presentation using the Q&A feature in Zoom. At the end of the seminar, we will answer as many questions as possible. A Q&A document will be posted with the recording of the seminar on RECOVERCOVID.org. It will include answers for the submitted questions relevant to today's presentation. Questions about other scientific topics will be addressed in future seminars and answers to broader questions about the RECOVER will be available in the FAQs at RECOVERCOVID.org. As a reminder, we cannot answer individual questions about clinical care.

Our presenters today are Dr. Nora Singer and Dr. Dimpy Shah. Dr. Singer serves as the Medical Director of the MetroHealth System Clinical Trials Unit, and the Director of the Hub Research Capacity for Metro Health Site of the NIH-funded CWRU Clinical Trials and Science Collaborative, as well as the Director of Rheumatology for the MetroHealth System. She is a board-certified pediatric and internal medicine rheumatologist, and a CWRU Professor of Medicine in Pediatrics. Dr. Singer served on RECOVER's Commonalities with Other Post-Viral Syndrome task force, the adult cohort, and the ENT adult cohort ad hoc working group, and as a translational researcher whose portfolio now includes studies in Long COVID.

Dr. Shah leads the Cancer Epidemiology Research Program at the University of Texas Health Science Center at San Antonio, funded by the NIH, American Cancer Society, Oak Research Foundation, American Society of Clinical Oncology, HRSA, Biomedical Advanced Research and Development Authority, and other industry partners. The program develops epidemiological cohorts and conducts real-world evidence studies to improve population health.

In addition to her research activities, she serves as the Vice Chair for the Texas Cancer Registry Advisory Council, a member of the Shared Resources Oversight Committee, Founding Chair of the Respiratory Viral Infection Consortium, a member of the Hepatocellular Carcinoma Epidemiology Consortium, a steering committee member, and the Chair of the Epidemiology Corps for the COVID-19 Cancer Consortium. Dr. Shah also served on RECOVER's Adjudication Committee and Population Science task force, and as co-chair of the Risk Factor Writing Committee. She is additionally a founding board member of the patient advocacy nonprofit, Advocates for Collaborative Education.

The topic of today's seminar is Sex Differences in Long COVID. Today's speakers will discuss findings from the RECOVER Adult Observational Cohort study about how a person's sex may affect their

risk of developing Long COVID. They will also discuss possible reasons underlying their findings about sex differences. And with, that I will hand it over to Dr. Nora Singer.

Dr. Nora Singer:

We're here to talk about the role of sex at birth in Long COVID, and I'd like to give you some background information about COVID and Long COVID.

So, we know that SARS-CoV-2 has infected more than 700 million, with a high level of death, up to 7 million. And that a substantial portion of those persons who get COVID also develop Long COVID, or what was called previously post-acute sequelae of SARS-CoV-2, or PASC. And we know that Long COVID is not limited to any one particular population, and it can affect anybody who is infected with the SARS-CoV-2 virus. And the risk may differ among individuals. RECOVER is a study that was created to understand who gets Long COVID, how often it happens, and to understand what happens over time. Next slide.

So, in terms of the number of participants, the total number of participants in what's called the Adult Cohort is 15,161, and those are all patients who are 18 years or above. And a little more than 12,000 of those persons were infected at some point with SARS-CoV-2, and had symptoms of COVID-19. So, to come in as somebody who was infected, you can't have been symptomatic and had a negative test, though you could have been symptomatic and not tested at all, and that's an important distinction. And we enrolled almost 3,000 people who had never had COVID at the time that they came into the study. Next slide.

This gives you an idea about where the RECOVER sites are; there are 86 total. It spans from Maine to California to Hawaii and Puerto Rico. Next slide, please.

So, we recruited in 33 different sites and within the adult cohort is pregnant women, and there also is a pregnancy cohort. So some of the information we provide today will touch on pregnancy, but there are more detailed studies that have been performed by the pregnancy cohort, which we won't have a chance to discuss today. And the way RECOVER was organized is that everybody did some baseline surveys, labs, and a minimal physical examination when they came into RECOVER, and at specified intervals. Some patients would triggered what we refer to as tier 2 clinical tests, so those were things like echocardiogram or CT of the chest that weren't very invasive. Also, a smell test, for example, people who lost taste or smell might've triggered a smell test. And the tier 3 advanced testing, which is ongoing right now, is a smaller fraction of the total population, and people trigger it because they have persistence of symptoms, though there are also some asymptomatic patients for those symptoms who get the testing as a control. Next slide.

So, I wanted everybody to understand that we're studying infected people, and there are two ways initially you could come into the study, and then a third way to come into this particular study on sex at birth, even if you were in the healthy cohort initially. So, when we started the study, Alpha, Delta was at the tail end, that variant was at the tail end of our study startup. And most but not all of the people that were enrolled in the acute phase had the Omicron variant for virus. So, we enrolled some patients who had had Alpha, Delta, but had it prior to coming into the study and had it more than 30 days prior to coming into the study. And we had to arbitrarily set an index date so that we knew when the follow-up visits were. And that cohort is referred to as the post-acute cohort.

So, if you had COVID in March of 2022, but we started the study a year and a half later, you would still be able to come in. But for visit purposes, your index date was targeted not as March of 2020,

but as 30 days before, for purposes of the schedule. In the acute cohort are people who were infected within the past 30 days.

Now, we also enrolled "healthies," some healthy people who came in and had never had COVID at the time that they enrolled [but] got COVID subsequently. And those patients are referred to as crossover patients and they've crossed over into the acute cohort if we saw them within 30 days. Next slide.

So, we were asked to determine the role of biological sex, so that's sex assigned at birth, as one source of variability in the development, the trajectory and how Long COVID presents. As you know, Long COVID is very heterogeneous and can affect many, many organs, and not everybody has the same organ affected. Prior studies also lacked a full account of things like menstrual status, comorbidity, so illnesses that ran at the same time and may have predated COVID, vaccination status, which variant of the virus it was, how severe the acute illness was, and whether or not people had good health access. So, this is a crucial first step in beginning to understand the biological mechanisms and how it affects women and men differently, if it does affect them differently at all. And the study hoped to advance the development of effective interventions, guidelines for clinicians, as well as public health policies to alleviate the burden of Long COVID, which we know is substantial. Next slide. So, this is the paper that was recently published. Next slide.

And so, Dr. Shah is going to take over from here and discuss the questions and answers that we were able to analyze the data to present to you today.

Dr. Dimpy Shah:

Thank you. So, definitely our first question was, does the risk of Long COVID or post-COVID condition as it is also called, differ by sex? Next slide. And to do this, we aimed to evaluate the differences and the risk of Long COVID between males and female adult participants who had infection with SARS-CoV-2, while also adjusting for various baseline sociodemographic risk factors including age, race, and ethnicity. As well as clinical pathologic risk factors, including severity of the initial SARS-CoV-2 and the variant era. So, we wanted to ensure that we have included the factors that Dr. Singer mentioned that were missing in the prior studies, and we were able to do this because of the large cohort that we had access to. And we were able to understand once these factors were adjusted, how these differences stayed with regards to risk of developing Long COVID. Next slide.

And the design included all the adult participants who had previous SARS-CoV-2 infection and that were enrolled between October 2021 and July 2024. This included both the cohorts, the acute and the post-acute cohort, as we just saw. And the exposure is defined as self-reported sex assigned at birth. So this is the biological sex that is assigned at birth as either male or female. We did not have gender or intersex or any of these additional insights to be able to study how gender affects. So, our focus was just biological sex assigned at birth. And the current study was approved by the NYU IRB, as well as all the participants provided informed consent prior to their enrollment in this study. Next slide, please.

And I will not go into detail, but the subcohorts included in this study were those who belonged to the acute subcohort that were enrolled within 30 days after the index infection, and post-acute that were enrolled after 30 days of their index infection. And some of the participants who enrolled as uninfected but then later on developed infection while participating in the study were defined as crossover, and thus included in the acute subcohort because we had the exact date of infection for these participants. Now, the participants who were uninfected in enrollment but then eventually we had a positive antibody results were reclassified as infected and assigned an index date of 90 days prior to

that antibody test result. This was arbitrary because we did not have the exact date of infection, but there were a few participants who turned out to be positive based on the antibody test results. Next slide, please.

So, this is the study cohort. We had 15,161 adults who were enrolled in RECOVER as of September 2024 when the data log happened for this particular study. And those who were enrolled without a SARS-CoV-2 infection or did not have any infection during the study were excluded, that were equal to almost 1,400 participants. Now, we had 4,411 in the acute period, 8,291 in the post-acute period, and 981 who belonged in the crossover cohort. So we had a substantial proportion of patients or participants who belonged to this crossover cohort. Now, those who did not start the protocol or had all the visits at more than 6 months that were within the reinfection window were excluded, and we only included participants if they had an eligible visit at more than or equal to 6 months from the date of infection. We also did not include anyone who did not start a survey at a visit more than equal to 6 months, had missing sex that was assigned at birth, or were intersex. And we had very few, just five participants who had intersex defined at birth.

And finally, we had 3,814 included in the analysis that belonged to the acute cohort, 7,691 that belonged in the post-acute cohort, and 771 in the crossover. The post-acute period had almost half before and after Omicron, which is a December 2021 cutoff. And mostly in the acute and crossover were Omicron, understandably, because as Dr. Singer mentioned, the study started at the tail end of the Delta. Next slide, please.

So, the outcome was defined as Long COVID at the first visit 6 months or after the index infection, and it was ascertained using a previously reported symptom based scoring algorithm. This was published in JAMA and then refined in 2024 for NIH RECOVER cohort. And using this Long COVID research index, participants were given a score from 0 to 30, 30 being the highest. And anyone who had more than or equal to a score of 11 was classified as Long COVID positive, and less than 11 was classified as Long COVID indeterminate. And these are the symptoms that were included in defining the research index, including malaise, fatigue, brain fog, dizziness, palpitations, loss or change in smell or taste, thirst, chronic cough, chest pain, shortness of breath, and snoring or sleep apnea. Next slide, please.

So, the statistical methods were really elaborate for this study because we wanted to do an in-depth analysis of how sex differences account for the risk of Long COVID. So, we stratified based on demographic and clinical characteristics, looked at individual symptom frequencies between male and female participants, and we also identified the proportion of participants who met the 2024 Long COVID research index. We identified the mean and median scores based on this research indices for males, as well as females. And we also tried to examine the differences in the Long COVID subphenotypes of clusters, symptom clusters among participants. Now, these subphenotypes were also defined along with the Long COVID research index for RECOVER cohort, and then they were refined in another paper in 2024. Next slide, please.

We used propensity score matching, and propensity score matching is also used in causal as well as noncausal observational studies, which allows us to balance on various covariates. Understandably, a large adult cohort such as this one had multiple variables that we were interested in that could have an impact on the risk of Long COVID, but it may also be associated with the exposure that we are interested in, male versus female. However, not all the covariates need to be confounders when adjusting and propensity score model matching. So, we tried to balance on multiple covariates to identify, once balanced on these various covariates, how does sex have an impact or sex affect the risk of Long COVID? And this was our primary model, which included most of the covariates.

And we also examined a primary reduced model, which only included age, race, and ethnicity, but did not balance other covariates. And this choice was done to understand how the impact of the variables which are downstream from sex. And sex is assigned at birth, so almost all of the variables are downstream might have an impact on Long COVID. So when we remove that, we reduce the attenuation that could have been caused due to those downstream covariates. Next slide, please.

And this is the Love plot, which evaluates the covariate balance. As we can see, we adjusted on multiple covariates such as age, race, ethnicity, the variant era, pre-Omicron versus Omicron, the type of referral to the Long COVID clinic, various social determinants of health, marital status, disability, homelessness, unemployment, Medicaid, losing insurance during pandemic, and household income, as well as if they had difficulty covering expenses or skipped medical care or were food insecure. So, this is one of the strengths that we were able to adjust for so many covariates and still identify how sex is associated with the risk of Long COVID. Next slide, please.

So, in addition to our primary full model and primary reduced model, we also conducted two secondary analyses. One where we stratified by age group of 18 to 39 years, 40 to 54 years, and 55 years or older. And this was again our aim to understand how the various subgroups or age strata, as well as age and menopausal strata, affect the risk of Long COVID. So, the secondary analysis focused on age and menopause, and this was again 18- to 39-year-old nonmenopausal females. 40 to 54 was stratified into nonmenopausal as well as menopausal, and most of the females who were 55 years or older were menopausal so we only examined that subgroup.

We also conducted five sensitivity analyses, which included the acute subcohort. That means only the participants who either belonged to the acute cohort, including the crossover participants, were examined. Any participants who were pregnant from the time of their index infection all the way to the follow-up visit, if they were pregnant during this time, they were excluded. So we only examined how nonpregnant females had a risk of Long COVID, compared to males.

Finally, we also added comorbidities in one of the secondary analysis. Now, comorbidities were not included in the primary analysis because it was considered to be a mediator in the causal pathway between biological sex and Long COVID. So, many of these comorbidities are linked with female sex, and then they also linked with perhaps a higher possibility of developing Long COVID. Thus, it was considered to be in the causal pathway and if we were to add it in our primary model, it would lead to overcorrection or overadjustment and does attenuate and not give us the actual risk that the biological sex may have on Long COVID.

We also stratified by pandemic wave era, which is pre-Omicron versus Omicron. And we also stratified by the severity of illness, acute illness, by examining the hospitalization status at the time of acute illness, so those who were hospitalized versus those who were not hospitalized. And all of these sensitivity analyses examined how it affected the association between biological sex and risk of Long COVID. And sensitivity analyses are done to identify how by focusing on one variable, we can identify how it impacts the outcome, so to understand how it influences the outcome. Next slide.

So, we ended up with 8,969 female participants, 3,307 male participants, which is overall of 12,276 participants from the RECOVER adult cohort that were included in the final analysis. Males were slightly older compared to females, and we had almost equal distribution of race and ethnicity between males and females. And we did not identify major differences between the infection cohort or enrollment subcohort and era. Next slide.

Similarly, we did not identify major differences between the hospitalization status and the vaccination status at first infection. And these were examined exactly as the previous manuscript, looking at the unvaccinated, partially vaccinated, fully vaccinated, or having missing data. And also by the visit month, they were pretty similar between male and female participants. And all of these were adjusted and the propensity score matching, so we were able to account for those balances in our primary, as well as secondary analysis and sensitivity analysis. Also, the missing data for all the covariates were very similar between male and female and they were considered to be random. Next slide.

These were the comorbidity differences. We do identify some of the differences in comorbidities between male and female, with regards to asthma, mental health disorder, and definitely obesity. So there were some differences between male and female, and as we mentioned that some of these have preponderance based on the sex assigned at birth. So, these were considered as potential mediators rather than confounders for this association. Next slide.

So overall, females had higher proportion of Long COVID positivity based on the research index, 21% as compared to 16% of males who had Long COVID positivity. And again, females had slightly higher mean and median Long COVID scoring, and we did not identify major differences in the subphenotype cluster among Long COVID positive participants, with cluster 1 being quite similar between male and females. We do see some differences with regards to higher cluster 2 and 3 in males versus females, and higher cluster 4 and 5 in females versus males. And there might be some differences based on symptoms, but we did not have adequate power to examine each subphenotype, so we only examined the entire positive versus negative Long COVID. Positive versus indeterminate Long COVID, as compared to various subphenotypes or symptom clusters. Next slide, please.

So, these are the symptom frequencies stratified by sex and they are listed based on those that were included in the research index and those that were not. And cluster 1, so there's the heat map and on the left side we have clusters 1 to 5 within females and each symptom, and cluster 1 to 5 in males and each symptom. So, we do see that most of these frequencies are similar with some differences in some of the symptoms between male and female. However as I mentioned, we did not have adequate power to examine each specific symptom and how that differs between sex. We just did not have the power to look at each individual symptom. Next slide, please.

So, this is the primary and the secondary models. And the primary model shows that the risk ratio was 1.31 and it was significantly higher in females compared to males, with females having 31% higher risk of developing Long COVID. The right column shows absolute risk difference and in the reduced model when we actually removed some of these downstream covariates that were adjusted and we only adjusted for age, race, and ethnicity, we saw an increase in the risk. So, some of these downstream factors were actually attenuating the risk of Long COVID. So, the reduced model actually showed that females have 44% increased risk of Long COVID compared to males.

Now in the secondary analysis we identified that participants who were 18 to 39 years old, they did not have a significantly higher risk of Long COVID compared to males of the same age group, but the highest risk was observed in females who were 40 to 54 years, with 48% higher risk of Long COVID compared to males. And the next group was more than/equal to 55-year-olds, who had a 34% risk of Long COVID.

When we stratified by age and menopausal status, again the nonmenopausal 18- to 39-year-olds, who were most of that subgroup, did not have a significantly higher risk, but within 40- to 54-year-olds, nonmenopausal females had a significantly higher risk. Again, 45% higher risk of Long COVID

compared to males of that age group. And menopausal women, they did not have a significantly higher risk. So, although the risk looks similar, it includes a confidence interval of 1.0, which shows that it was not significantly higher. And this could be probably due to the impact of menopause or it could also be due to a lower sample size in that subgroup. Next slide.

Now within sensitivity analysis, when we only restricted the analysis to acute and crossover subcohort, we identified a much higher risk of females developing Long COVID. And the risk ratio was 1.58, which translates to 58% higher risk of Long COVID compared to males. So, acute or crossover cohort is considered to be a more pure or lack of selection bias cohort because we knew exactly the date of infection in these participants, and thus, when we remove that selection bias, the risk actually increases. So, this is very reassuring that the results that we are seeing are not by chance but they actually stand true even in the sensitivity analysis.

Again, when we excluded pregnant females, if they were pregnant between index and study visit, the risk increased. So nonpregnant females had much higher risk of 50% of developing Long COVID as compared to males. And when we added comorbidities, as expected, the risk ratio became nonsignificant. Thus, when we adjust for comorbidities, the difference between male and female with regards to risk of developing Long COVID becomes nonsignificant. There's also kind of pointing that comorbidities might be acting as mediators in the causal pathway.

Now, when we stratified by other infection era or stratified by hospitalization status, we did not see differences between pre-Omicron or Omicron era when it comes to the risk of females developing Long COVID. And we also did not see the difference with regards to severity of acute illness when it comes to females having a higher risk of Long COVID. Thus, all of these sensitivity analysis point to the fact that females do have a higher risk of Long COVID. Next slide, please.

So as any cohort, there were also limitations that were present in our study with regards to reporting bias, which might be that females might be more likely to report symptoms, or selection bias if females were more likely to enroll in the cohort. And this could be because they had Long COVID and this could be possible in the post-acute subcohort, but not so much in acute and crossover. That's why doing the sensory analysis just by acute and crossover was important, because it showed us that when we took away the selection bias, we still had, we actually had much higher risk of developing Long COVID in females compared to males.

We also did not have data on sex hormone levels, the timing of the menstrual cycle with regards to infection or Long COVID, various hormones, medications, number of pregnancies and its complications. So, there is another cohort that only examines pregnant females within RECOVER, and they examine in much depth of how pregnancy affects, comparing nonpregnant females to pregnant females. In our study we compared females to males because we were interested in sex differences. There might also be differential dropout by sex due to symptomology as well that might have occurred, but again, these were not very different between males and females and we do not think that that kind of affected based on all of these analysis. We also had inadequate representation of participants assigned intersex at birth or who had undergone gender-affirming medical care. So again, we were only limited to examine biological sex that was assigned at birth. Next slide, please.

Despite all of these limitations, there were definite strengths to this study. One of them being that we were able to identify a variety of time points before and after infection, and that showed that how the sex-based differences occurred at various time points with regards to the COVID infection. The RECOVER cohort is also very large and very socioeconomically diverse compared to many of the other previous cohorts that examined sexual differences between, with regards to developing Long COVID. We

were, again, this was one of the most important strengths, is that we were able to balance for multiple covariates, such as demographics, the variant era, the vaccination status, severity of acute illness, and so many social determinants of health. And many of the previous papers did not, were not able to account for these differences. And once we, based on the propensity score matching, we were able to account for a balance between the two groups, males and females, and still able to identify how that risk differs with regards to Long COVID development.

Again, one of the important strengths is a prospective data collection through a standardized questionnaire, which is definitely a major strength and it does not have some of the biases, reporting biases that are present in the cohorts that depend on clinical care or electronic health records, because that might be based on differential access to care or the reporting bias on what is recorded in the electronic health records. So, definitely prospective data collection through a standardized questionnaire and standardized visits for all the participants was a major strength, allowing us to have a better picture of the natural history of Long COVID development.

And both the protocol, as well as the current analyses, were developed in collaboration with patient representatives, understanding what is the most important and most relevant questions, burning questions in the patient community, and how we are answering the questions that are relevant to those that are affected with COVID or Long COVID. Next slide, please.

And now, Dr. Singer will discuss— next slide, please. Now, Dr. Singer will discuss, how do we infer these results and what are the next steps? Thank you.

Dr. Nora Singer:

So, I think Dr. Shah touched on the first two points. Menopausal females did have similar risk as males, and so we hypothesized it but were unable to measure sex hormones, that high estrogen and low testosterone might predispose to a high risk of Long COVID. Next slide.

So, we did demonstrate that comorbidities lowered or attenuated the high risk when you took them into account. So that if you had autoimmune disease, we know female sex is a risk for autoimmune disease and female sex is a risk for Long COVID. So you don't want to account for both because you may over-correct, and that's the risk of correcting for all the comorbidities when it may be in the causal pathway. Females also we know have a higher risk of other postviral complications compared to males. So, as a rheumatologist for a number of years, probably my entire career, I've seen adolescents and young adults and some older adults with complications after mononucleosis, for example, that can be associated with chronic fatigue. And we're now learning is associated with some autoimmune disease as well.

So, whether or not if we studied sex steroid therapies, we could manipulate any of them for acute COVID or Long COVID is really unknown. Sex steroids are hard to study because they have some variation dependent on time of day or diurnal variation. We know this for other steroids, such as cortisol as well. And so most studies that look at sex steroids or glucocorticoid endogenous production of steroids such as cortisol, are very prescriptive about when and how the blood was drawn. And since this was not the main aim of RECOVER, whether or not the samples are completely suitable for study of sex steroids remains to be determined. Next slide.

So, we know that females had a higher risk of developing Long COVID, and that the risk is age, pregnancy, and menopausal status dependent. The clinical and public health implications are substantial, and it's really going to be important in the future to try and disentangle the risk of aging, the risk of having one sex hormone or another, endogenous and exogenous or therapeutically administered,

as well as inflammatory response and comorbidity. So for example, we don't really know the effects of replacement hormones during menopause or oral contraceptives during the premenopausal years. But understanding the biological mechanisms contributing to sex specificity can help us risk stratify, and may be able to help us with targeted drug development and improved management of not only Long COVID but other postviral illnesses as well. Next slide.

So, I think future directions do include study of some of the medications that people have brought up in the chat. We have two different arms of the immune system that we commonly think about. We commonly think about the innate immune response, and that's your flight-or-fight response, something called type 1 interferons are often involved in the immediate response to a virus and are involved in the immediate response to COVID, but the sex hormone influence on innate immune responses, we don't know. We also have an arm of the immune system called the adaptive, which is the slower arm, but produces the antibodies against threats, so they are responsive. The adaptive immune system produces the antibodies when you get vaccinated or when you get infected.

So, we would also like people to decipher the underlying metabolic profiles and comorbidities as potential mediators of the observation of sex-based differences that we had today. We'd love to understand if there are preventative interventions before the onset of Long COVID, and are their effects sex dependent, so that we're treating the right population with the right medications. Identifying groups at risk for targeted intervention is important and really personalizing Long COVID therapy, based on age and sex differences is really the goal. Next slide.

I think we'll stop there, thank the audience, and take questions.

Mia Christopher:

Thank you, Dr. Shah and Dr. Singer. Now we have some time for Q&A. Some questions may be directed to either Dr. Singer or Dr. Shah, while other questions are more general. Panelists, if you have insights to share, feel free to jump in, even if the question was not directly asked to you.

The first one is for Dr. Shah. Dr. Shah mentioned variant era [was] included. Does that mean that there was testing to see participants' variants sequencing history, or was it generalized based on the time XYZ variant was most circulating lining up within section?

Dr. Dimpy Shah:

That's a great question. So we did not have the sequencing history or sequencing done for this manuscript. We did it based on the generalized time of XYZ variant, and which was December 2021, that 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.

Mia Christopher:

Another question for Dr. Shah. Why did Shah et al. conclude that menopause status was a risk factor for path when Table 4 gives exactly the same 18% prevalence estimate for women age 40 to 54 who were menopausal, compared to nonmenopausal, and almost the same relative risk, 1.42 versus 1.45?

Dr. Dimpy Shah:

Sorry, I muted.

Dr. Nora Singer:

That's the confidence interval thing you referred to.

Dr. Dimpy Shah:

Yeah, we could not 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 1.0. So, those who were menopausal were not at a higher risk for Long COVID. In fact, those who were nonmenopausal were at a higher risk for Long COVID. And again, this could be also due to lower sample size in that category, but definitely nonmenopausal females had a higher risk because the sample size was very large for that specific subcategory.

Dr. Nora Singer:

So, when the confidence interval crosses 1.0 and you're talking about for relative risk, then we conclude that we're unable to statistically determine a difference.

Mia Christopher:

Thank you, both. This next question I'll pose to Dr. Singer. How does hormone replacement therapy affect women with Long COVID, particularly those experiencing perimenopause?

Dr. Nora Singer:

So, 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 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 so we are going to need to be entered into discrete fields to do that kind of analysis. And 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 if they were doing it by going through each participant's medication. But 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.

Mia Christopher:

And the next question is, have you found women with hormonal imbalances, such as PCOS, more likely to develop Long COVID or have complications from COVID?

Dr. Nora Singer:

So, 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, but just in general, when I came across a review that referred to autopsy studies, is finding that on autopsy up to 70% of women had some evidence of endometriosis. So, we have 30% of those who have it are symptomatic, but it's found in up to 70% of women. I'm not sure without really detailed studies and looking for it even in asymptomatic people or asymptomatic women, that we're

going to be able to completely answer that question. We may be able to answer a question about symptomatic endometriosis but not asymptomatic endometriosis.

Mia Christopher:

Thank you. Another question. Seeking clarification, 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?

Dr. Dimpy Shah:

That's a great question. So, this was a research study, it was not meant to identify those who develop Long COVID earlier or because it is very likely to have Long COVID before 6 months after infection. And also, it was not meant to identify or classify those who need access to some of the Long COVID programs or disability benefits. But 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 kind of meant that we were capturing those who had persistent Long COVID symptoms. Completely understanding and acknowledging that this limitation might lead us to have only a subset of participants who had Long COVID, as compared to those who had, those who could possibly have Long COVID much earlier. But for research purposes, we selected the 6-month 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 either Long COVID positive. And we were very specific in mentioning those who did not have more than 11 symptoms or less than 11 symptoms, they were classified as Long COVID indeterminate, not necessarily—

Dr. Nora Singer:

Possible, possible.

Dr. Dimpy Shah:

Or possible, 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. Thank you.

Mia Christopher:

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

Dr. Dimpy Shah:

Dr. Singer?

Dr. Nora Singer:

So, I'm particularly interested in POTS because I've seen it for many years in the 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 crosses, transverses the cell membrane seven times. And so to recreate it in a lab is difficult, but 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 to 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 think there have not been rigorous studies of testosterone on POTS. Much of the time clinically, patients who have POTS, so they 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, which is part of the definition. And so, we don't know if patients who have symptoms that are similar to POTS but are tilt-table negative, if it's just a spectrum and a curve. And POTS is only one of the dysautonomias that we can see postvirally. So we can see, 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. So I don't know of any placebo-controlled randomized studies of testosterone in any of the dysautonomias. So if somebody else does, I'd be glad to read about, but I think that's an unknown.

Mia Christopher:

We have another question about postviral infection. Does your cohort include postviral infection status, such as having prior exposure to EBV?

Dr. Nora Singer:

So, 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. So, sometimes people have symptoms before that have been worsened. And so we do 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. So they're meant to try and understand how the symptoms of Long COVID arise as a result of COVID. And there are at least two awards at Stanford that I know of that are, 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.

Mia Christopher:

Thank you. The next question is, please kindly define partial versus full vaccination status.

Dr. Dimpy Shah:

Sure, that's a great question, I'll take that. So vaccination status was defined in our previous manuscript from RECOVER that actually also defined the Long COVID research index. At that time, the fully vaccinated, or for this cohort, fully vaccinated were considered 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. Now, [the] partially vaccinated were 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 unvaccinated were considered if they had zero doses before index date of their infection before 12/1/2020, when vaccines started to become generally available.

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 did not have boosters or even in the post-acute cohort when the vaccination happened much before. So we were trying to understand the vaccination status at the time of their index infection or in relation to that acute COVID.

Mia Christopher:

Thank you, Dr. Shah. The next question has been touched on previously, but has there been an analysis of premenopausal females that have hormonal or menstrual diagnoses like PCOS, endometriosis, and so on? Is there anything further you'd like to say on this?

Dr. Dimpy Shah:

No, as Dr. Singer mentioned, this was such a Herculean task just to examine so many subgroups and so many sensitivity analysis, 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 definitely look at even more granular detail with regards to hormone replacement, contraception, sexual hormonal variations, and also some of these PCOS or endometriosis conditions that are very specific to females and how that effects. But I believe this is the first, 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.

Mia Christopher:

Thank you. The next question is, with potential effects of weathering 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?

Dr. Dimpy Shah:

We did not do that, and we did not 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. So we did not 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 does that matter?

Again, the scope of this manuscript was not to compare various races and ethnicities, but the scope was 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 did not do that in our study. Dr. Singer, if you would like to add anything?

Dr. Nora Singer:

No, I think that answers the question, thanks.

Mia Christopher:

Thank you. I have another clarifying question. I remain confused, not being a statistician, on the effect of comorbidities. Might one of you clarify this for me?

Dr. Dimpy Shah:

That's a great question. I think most of us remain confused, even despite being statisticians. So, I'll try to explain it in a causal mechanism pathway. So what happens is there are some factors that are assigned at birth like age, right? I mean, age just moves along as the 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 sex. 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 are doing statistical analysis, when we are looking at causal pathways, if we were to adjust for— when we are trying to look at whether male and female, if 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 overcorrecting. So what we are trying to say is, given that they have equal comorbidities, do males and females have higher risk of Long COVID? And we found that they don't, because Long COVID is associated or the risk is increased by some of these comorbidities. Now, which ones, we have not examined that, 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, etc., that changes these hormonal levels that might have an impact on the risk of Long COVID. And I'm sorry if that's not very clear, but I don't know if Dr. Singer would like to try from a biological perspective of how do we—

Dr. Nora Singer:

Yeah, so I think about it as a clinician, that if you're thinking about atherosclerotic disease, if you try and match an 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 direction 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.

So, 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 and women. I think that was our concern.

You 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 the men or with other autoimmune diseases which are more common in women. So, I think that the way the statistical analysis plan was designed 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. Dimpy 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?

Mia Christopher:

Thank you, and I think we'll do one more question. Which specific sex steroid-based therapies for males were you referencing?

Dr. Nora 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 or not things like hypogonadism might occur at a higher rate, or since we've seen senescence in immune cells, are the symptoms associated seen more prominently? But I didn't have anything specific other than that in males.

Mia Christopher:

Thank you. Thank you so much to our presenters and thank you to our audience for attending this seminar and engaging with the Q&A. As a reminder, a recording of today's seminar will be available on https://recoverCOVID.org within a few weeks. We will also be posting a Q&A document that has responses to questions we received today, including some that we did not have time to address. Next slide, please.

Before we conclude, a reminder that the researchers both within and beyond the RECOVER Initiative can now apply to use RECOVER data for ancillary studies. This includes data from three RECOVER cohort studies, adults including pregnant adults, pediatrics, and autopsy and biospecimens collected from cohort study participants. Interested researchers must submit an ancillary study proposal and receive approval. Researchers must also have independent funding support to conduct the proposed study. To learn more and apply, visit https://recoverCOVID.org/ancillary.

This slide lists three topics for future sessions. We have some exciting topics coming up and hope to see you at future sessions. Additionally, you will 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. Thank you, and have a great day.