Transcript

Beth Linas:

Welcome to the RECOVER Research Review or R3 seminar. My name is Beth Linas and I'm an infectious disease epidemiologist with the RECOVER Administrative Coordinating Center and the moderator of today's seminar. The goal of this seminar series is to catalyze a shared understanding of the research within the RECOVER Consortium. I want to start by thanking everyone who submitted questions in advance. Please submit any additional questions that arise today during the presentation using the Q&A feature in Zoom. After the presentation, we will answer as many questions as possible. A Q&A document will be posted with the recording of the seminar on revovercovid.org. It will include the answers for submitted questions relevant to today's presentation. Questions about other scientific topics will be addressed in future seminars and answers to broader questions about RECOVER will be available in the FAQs at recovercovid.org. As a reminder, we cannot answer individual questions about clinical care.

The topic of today's seminar is Characterization of PASC and Investigation of Biomarkers Insights from the RECOVER Adult Cohort. Our presenters today are Dr. Grace McComsey, Vice President of Research and Associate Chief Scientific Officer at University Hospitals Health System in Cleveland, Ohio, and a professor of Pediatrics and medicine at Case Western Reserve University. An internationally known researcher in the field of HIV, she is currently serving as a principal investigator on four NIH grants focused on understanding the heightened inflammation and immune activation and its effect on metabolic and cardiovascular comorbidities in children and adults living with HIV. Dr. McComsey is the author of over 250 peer-reviewed publications and metabolic and cardiovascular complications of HIV and treatment.

Dr. Kristine Erlandson is a professor in the Department of Medicine Division of Infectious Diseases at the University of Colorado Denver Anschutz Medical Campus. Her research is focused on the complications of aging, understanding the mechanisms of successful aging and implementing interventions to ensure successful aging among persons living with HIV. She leads trials at the University of Colorado and is the chair of the Comorbidities Transformative Science Group within the Advancing Clinical Therapeutics Globally or ACTG, identifying high priority studies for comorbidities and HIV. More recently, her research has expanded into understanding the consequences of other relevant infectious diseases, including COVID. She's the site PI for Recovery at the University of Colorado within the Mountain States Hub and is co-investigator for the RECOVER clinical trials at the University of Colorado.

And also Dr. Linda Geng, a clinical associate professor of medicine at Stanford University in the Department of Medicine division of Primary Care and Population health. Dr. Geng is co-founder and co-director of Stanford's Long COVID collaborative about multidisciplinary clinical and research program that aims to advance understanding care and treatments for Long COVID. Dr. Geng's. Research includes clinical trials, testing therapies for Long COVID, including as part of the NIH RECOVER initiative and also improving clinical care for models for diverse patients and communities with Long COVID as part of AHRQ Long COVID Care Network. Dr. Geng also serves on the National Academies of Sciences Engineering and Medicines Forum on Advancing Diagnostic Excellence, which aims to improve diagnosis within the US healthcare system. Today's speakers will share our current understanding gaps in our knowledge and how RECOVER will contribute to filling these knowledge gaps. And with that, I will hand it over to Dr. Grace McComsey.

Dr. Grace McComsey:

Hi everyone. It's my pleasure to be here talking about a very important topic. So my goal is to put in perspective the two talks that will follow and why they're important. Next slide. So imagine having to treat diabetes without hemoglobin A1C or treat HIV without viral load. There is an urgent need to find biomarkers for Long COVID. Whether biomarkers at the time of acute COVID infection. So we can do studies to prevent Long COVID or biomarkers during symptomatic post COVID period to diagnose and be able to manage and treat Long COVID, as well as importantly, we need biomarkers to rule out sequelae of COVID infection. So even if people who are not symptomatic, they don't have Long COVID symptoms, we don't know yet what's happening in their bodies and we need to understand if there are biomarkers because we hear more and more reports of COVID triggering autoimmune disease and diabetes and cardiovascular disease. So even these biomarkers in asymptomatic COVID survivors are very important.

Next. So in Long COVID clinics, most people who are seeing undergo a list of tests and outside of COVID there has been studies showing as you see some increases in some biomarkers, some decreases. These are what I call the clinical biomarkers. Every Long COVID clinic does a battery of biomarkers. Next. Let's see the slide. So you see here we have an opportunity because the studies available to date are either small and prospective or large relying on EHR data. So we had in RECOVER a great opportunity to look at more than 25 different clinical tests that we did on all the recovered participants to be able to study if any of them are good biomarkers for Long COVID. So Dr. Erlandson will tell us about that study. And if you read the paper, you know that no biomarker from that list was a big winner or a big hit, although there were some interesting findings that you'll hear about from Dr. Erlandson.

So next. It is very important to recognize upfront it is not going to be an easy task. This is from one study that looked at the different biomarkers, very in-depth cellular and soluble biomarkers of inflammation immune system. So it is going to be pretty complicated. And one thing I wanted to point out with this study is as seen on the slide we have to look at Long COVID, not only as one disease, no doubt some biomarkers will better fit specific symptoms or even clusters of symptoms, and this is how we should think. It's not going to be a yes-no biomarker for Long COVID. Next slide. Even more, it is important to have biomarkers that signal end-organ damage or predispose factors to new onset conditions like autoimmune and cardiovascular. So this study from my group showed that a biomarker of arterial stiffness, a predisposing condition to heart disease and hypertension was seen with Long COVID with large differences seen in people who had Long COVID versus survivors without symptoms or even people who are not infected.

On the right hand of the slide, you see that after adjusting for a comprehensive battery of immune factors, gut integrity markers, what we found is that female sex was independently associated with arterial stiffness. And even though sex modified the relationship between Long COVID and arterial stiffness so that women with Long COVID had much larger and much worse arterial stiffness than men with Long COVID. So not only we have to look at biomarkers with specific symptoms and clusters, we have to look at biomarkers by sex differences and even more by racial and ethnic differences.

Next slide. So we have a large treasure in important biorepository of samples that will be used to understand Long COVID and including already actually they're being used in dozens of ROAs and different scientific studies that are funded through RECOVER, and this work is happening as we speak. Next slide. We also have a lot of other opportunities to study biomarkers in RECOVER. This list is what we call tier two test. Tests that will be very important to shed light on end-organ damage that we see in Long COVID. Next slide. So even more there are tier three tests. These studies will help us a lot in deciphering mechanistic pathways for why Long COVID happened. As you see on the list, tests like

biopsies of the gut and muscle and skin and bronchoscopies or biopsies. Very, very important to understand the mechanisms as well as end organ damage that COVID or Long COVID may cause.

Next slide. Next. So I have to mention actually that RECOVER is not only observational. We have a lot of clinical trials as listed here that are either completed or ongoing within RECOVER. And these also will be very important to shed light on mechanisms and on biomarkers. We have a new task group or task force, which I'm a member of that's putting together a process that will make those samples available for scientific studies so stay tuned for that. I think this is a very important initiative as well. Next slide. So we're left, what? February 2025 without a biomarker to use for diagnosis. So the diagnosis so far relies on symptoms and as we know, Long COVID is elusive. Every organ system may be involved. Very variable presentations among people with Long COVID. Some symptoms are very common like fatigue, post-exertional malaise, brain fog, and others, but they're also nonspecific to Long COVID.

So in 2023 ... Next slide. Next. All right. So RECOVER published in JAMA, I would say the first attempt at defining Long COVID by identifying a research index. So by using models that selected symptoms that were different between COVID survivors and people who never had COVID, we were able to define threshold for that index. However, we were very careful to not call someone who doesn't have these specific symptoms as not having Long COVID. And even if people didn't quite reach the threshold that was 12 in that version, we still did not exclude Long COVID on them. We called them indeterminate. So it is important to remember that. And I'll tell you why in the next slide.

So next slide. And Dr. Geng is going to tell us about the differences between the '23 and the new version that was just out in JAMA in December '24. A refinement of that score with a little bit of difference in the clusters. So in '23 there were four clusters. The easiest way to remember them is the first one was only people who had smell and taste issue. Second one was post-exertial malaise and fatigue. Third one added to that brain fog. And then the fourth cluster was very symptomatic people. Pretty much symptoms in every potential organ system. So Dr. Geng will tell us about the refinement of that score and the refinement of the clusters that we found in 2024.

Next slide. So this is my last slide, but it's perhaps the most important thing. When you hear about the COVID, the RECOVER index. Again, and I know I said that we were very careful in our studies not to say that people who have some symptoms but didn't reach the threshold required that they don't have Long COVID. We rather use the terms indeterminate or PASC possible. The reason for that is we believe the recovery index is important to use in research, not necessarily in the clinic. The NASUM definition of Long COVID, which is much more inclusive, should be used clinically. And this is what the NASUM definition said in front of you. And as you see, it is pretty inclusive of the course of the symptoms, whether they're intermittent, whether they're progressive, as long as they happen for at least three months after a COVID infection and can affect one or more organs. So it's a very wide definition, but I think that's the best definition to use now, clinically leaving the RECOVER index for research. And as you see in the figure in the NASUM, it really depicts how complex the diagnosis is. Pretty much any organ system can be affected and the diagnosis for now, that's why it's clinical. With that, I'm going to leave you to present to us the first paper, the biomarker paper within Recover. Kristine.

Dr. Kristine Erlandson:

Thanks, Grace. I think we have to unshare so I can share my slides. Thank you. Okay. Slides look okay. You can see them. Okay. Excellent. So as Grace said, I'm going to present the biomarker paper. This was standard clinical laboratory measurements do not differentiate prior SARS-CoV-2 Infection in post-acute sequelae or Long COVID among adults in the RECOVER paper. And this was published last

summer in the Annals of Internal Medicine. And as Grace mentioned, it was specifically focused on those clinical laboratory measurements that we're obtaining within the RECOVER cohort in the entire cohort. Not very specific mechanistic biomarkers of particular interest. So following up on the background that Dr. McComsey presented, there's currently no validated clinical biomarkers of Long COVID. But if we had these clinical biomarkers that could help distinguish people with or without Long COVID, these could be particularly useful both in the diagnosis, the prognosis, the prevention, and the treatment of Long COVID or specific Long COVID subtypes.

We also can use these laboratory tests to identify potential organ damage among people who may have minimal to no symptoms. For example, underlying renal disease or liver disease that may not be associated with any symptoms. And most of the studies that had looked at biomarkers prior to this had been quite small, had very limited follow-up after the initial infection, or didn't have a control population. In fact, many had 20 to 30 participants in different subgroups, and so limited conclusions that could be made from several of these prior studies. So our goals for this paper were twofold. Number one, we wanted to determine whether SARS-CoV-2 Infection results in persistent changes in common clinical laboratory tests. Those tests that are obtained in every participant, at least upon initial enrollment, regardless of their symptoms and individuals with prior infection compared to those without prior infection. And then the second part was to determine whether those with Long COVID symptoms defined by the PASC index or the PASC research Index had persistent laboratory changes compared to those that were unlikely to have Long COVID with the goal to be able to identify abnormalities driving symptomatic long.

And then this first paper was really looking at whether any laboratory markers or laboratory studies could be used to augment the RECOVER PASC index before we revised it, which Dr. Geng will present next. So RECOVER adult participants that had available laboratory samples and symptom data were included. We did exclude individuals who were pregnant during this part of the study since pregnancy itself can alter many of these laboratory values. Participants who enrolled as uninfected were included as long as they didn't have an infection for the first six months. And then we based the analysis on the laboratory values at the six-month visit or the enrollment visit if people were enrolled at least six months from their index visit. Subsequent visits were not included because for those of you who are involved closely with the study or participants in this study, repeat labs are often triggered based on abnormal preceding labs. And so we didn't want to bias by just looking at people who had abnormal labs at the onset.

So our first goal was to compare laboratory values between participants with and without a history of SARS-CoV-2 Infection. And then we for goal number two, we restricted to those who had had a prior infection and compared participants who had a PASC index score of 12 or more to those who had a PASC index of zero. We used something called propensity score weighted linear regression models, which is essentially a statistical method to try to make the two groups look as similar as possible. We adjusted for all of the variables that are at the bottom of this slide here as we know that many of these things can alter these laboratory values in of themselves. For example, we might see higher inflammation or lower renal function just associated with age. So we're trying to make these two groups look as similar as possible so we could really tease out the effect of Long COVID symptoms based on the PASC index or based on the prior COVID infection.

We use the PASC index as Dr. McComsey mentioned with a score of 12 or more for the comparison group versus those that had a score of zero. So the study population that we included in this paper, the first goal as I mentioned, was comparing by prior SARS-CoV-2 Infection, yes or no. And of the 10,000 participants, we had about 8,700 that had had prior SARS-CoV-2 Infection, 1300 that had not. And then we divided those that had prior infection into those that had a PASC index of zero, which was

almost 40%, and those that had a PASC index of 12 or more, which was just over 20%. So here we're comparing by Long COVID as defined by the PASC index. This is a much busier slide that's in the paper if you're interested in who was excluded from different components. I won't go through this in detail, but I'll leave the slide here for reference if anyone has interest in this.

So to jump right into the results, this is the long list of laboratory measures that we included in this analysis. You can see we looked at kidney function, liver function, some urine measures that Dr. McComsey mentioned. Some lipid measures. One inflammatory marker, which is the high sensitivity CRP, D-dimer, which we see in some prior studies, hemoglobin A1C looking at diabetes, as well as some other measures that are listed here. I've highlighted the only values that we actually saw differences between those that had prior infection as shown in the left column here and those that had no prior infection as shown in the right. And these are the adjusted values. So this accounts for that propensity score matching that I commented on. So we're trying to make these two groups or the propensity score weighting, trying to make these two groups look as similar as possible.

And what we show here is the estimated average in this group and then the confidence around that average. So this is showing 95% of the values lie within this range. And so for example, we saw a difference in platelets. Those that had prior infection had a platelet count of 265 and those that had no infection had a platelet count of 275. 95% of those values fell within this range for those that had prior infection and 95% fell within this range for those that had no prior infection. Notably, these values are both within the normal range of what we'd expect with platelets and are pretty minimally different when we think of this clinically. So if I saw someone in clinic that had these two values from visit to visit, I wouldn't think anything of that change between two values. So we did see a difference, but the clinical relevance of this is a bit unclear.

We also saw a difference in hemoglobin A1C, so a measure of average blood sugars over the last three months. This was slightly higher in those that had prior infection at 5.58 compared to those without prior infection at 5.46. And then we also saw a difference in the albumin creatinine ratio, which is looking at how much protein is spilling into the urine. And this we also saw was slightly higher in those that had prior infection compared to those that had no prior infection. Perhaps a bit more pronounced than some of the other measures in terms of that amount of difference.

To give you a sense of what this data looks like, these are the three values that I just mentioned. So platelets in the bottom here, hemoglobin A1C and urine creatinine ratio. And then you can see the range of values along this axis here going anywhere from three to 15 for these values. Those that it did not have infection are in the green and those with infection in this pink color. And this is not adjusted for the propensity score matching, but you can see the range of all of these values which each of these dots indicating one person. And there's quite a bit of overlap between all of these values. So if someone came in and had a hemoglobin A1C of six in this range here, we really wouldn't be able to distinguish them at all from someone who didn't have prior infection. However, there certainly are some values up here which may point more towards someone having that prior infection compared to those without.

We did do some exclusions or some subgroup analyses to further evaluate. We've continued to see a difference in A1C when we excluded people who had a known diagnosis of diabetes. We also saw a continued difference in platelets when we excluded people who had immune-compromising conditions. But really these differences were quite small between those that had prior infection or did not. We looked at this same group of laboratory values based on those that had a PASC score of 12 shown in the column in the center here versus those that had a PASC score of zero. And we interestingly found no clinically meaningful differences in these two groups. You can see some of these markers that we did see in the other group that platelets were quite identical between the two. These confidence

intervals overlapped between the groups, thus suggesting there's really not a big difference here. And then similarly with A1C and that albumin creatinine ratio, the groups were much more similar than what we saw in the other analysis.

Next we looked at some of the subgroups that had been previously defined, so the different clusters based on symptoms. These groups did become much smaller, but we wanted to see if there was any signal of a underlying pathology here that we might see with some of the different clusters. So the first cluster, cluster one looks at smell and taste impairments and we tended to see a higher average high sensitivity CRP in this cluster. In cluster two, which is really driven by post-exertional malaise and fatigue, we tended to see a slightly lower mean sodium and increase in the mean calcium. We saw no differences in that cluster three. And then in cluster four, driven by a high symptom burden, so fatigue, post-exertional malaise, dizziness, brain fog, GI symptoms and palpitations we also tended to see high sensitivity CRP.

Just to give you an example of what this data looks like. Again, not a super impressive differences when you're just looking at these tests overall by the data. This was the high sensitivity CRP in the cluster one versus the cluster four. So if someone came in and had a value in this range, it would be really hard to distinguish them from the other clusters. Similar results with the sodium and the calcium shown in the figure on the right. And so in summary, while clinicians should certainly obtain these routine clinical tests to rule out other treatable causes of past symptoms, we found no evidence that any of these 25 routine clinical laboratory values that we looked at provided a reliable biomarker that could really distinguish someone who had prior infection or who didn't or who had Long COVID by the PASC index or who didn't or even within these different phenotypes.

And so we did not feel that laboratory values really the definition of Long COVID from a research standpoint, which will go into the presentation that Dr. Geng will have after this. We did see some small differences among those with or without prior infection and among some of the PASC phenotypes, but these were of minimal clinical relevance in the laboratory values were within the normal range. So understanding the causes of PASC is going to require much more novel biomarkers that can really delve into the mechanism to find a biomarker that could clearly distinguish someone either with or without prior infection or with or without Long COVID symptomatology based on the PASC research index. With that, I'd like to acknowledge our co-authors on this paper as well as the participants, their families, different representatives, the funding agencies, government and scientific committees, and I will turn it over to Dr. Geng for the next presentation. Thank you for your time.

Dr. Linda Geng:

Thank you, Dr. Erlandson. Sharing the screen here in just a minute. Okay. Great. Can everybody see that? And please let me know if there's any issues with the viewing. So it's a privilege to be able to share this work about the 2024 update of the RECOVER Adult Long COVID Research Index. As you all know, this is a really collective village effort and we are grateful for all the contributors, in particular our patient and community representatives who contributed to this update of the research index. This is published in December of 2024 in JAMA and represents the work and of course efforts of the entire consortium and all of the participants who contributed to this. I also encourage you also to listen to a great podcast by one of our lead authors, Dr. Leora Horwitz. That is also posted on the JAMA website and it goes into some of what I'll be discussing as well, which is the rationale, the context and applications of this work.

And just as a reminder, as Dr. McComsey mentioned, this is based upon the working definition or the working index of the post-acute sequelae of SARS-CoV-2 infection or Long COVID. This was the

original JAMA paper in 2023 that published the working model based on the initial nearly 10,000 participants who had enrolled in the RECOVER observational study in the adult cohort. In that paper, in that initial model, which we will now refer to as the 2023 index, there were about a dozen symptoms that were identified shown here in this table that were the contributing symptoms to the index threshold that identified likely Long COVID or likely PASC. With this, it was then for those who had infections from SARS-CoV-2, you could then try to look and say what subtypes or clusters of symptoms might be derived from the heterogeneous mix of Long COVID based on this definition or this index?

And when we did that, we found the four clusters that Dr. McComsey had mentioned previously, and that was from the 2023 model. And again, something to emphasize here that you can see some of the most common symptoms that arose from these models including fatigue and post-exertional malaise. But these different clusters or different subtypes of symptom presentations can also demonstrate how different Long COVID can be in different people. From this, we learned a lot. We learned about some of the most differentiating symptoms. We also learned about some of the patterns of Long COVID. And so from that as Dr. Erlandson mentioned, we asked what else could help improve this model. And in particular, we knew that since that time we had additional data as well. So when the 2023 index was published, it was clear that that was a working model and that future refinement and update of the index is needed. And of course our understanding and new discoveries about Long COVID are constantly evolving in dynamic. And so this is an iterative update and it'll likely to be updated again in the future as well.

So since the 2023 index, nearly 4,000 additional participants had qualifying visits that then we could include additionally into the model and understanding from a data perspective how we can derive this index. As well as we had because of the great patient and community input, more data on symptoms of three overlapping syndromes were collected. These included myalgic encephalomyelitis, chronic fatigue syndrome, also known as ME-CFS. Dysautonomia such as POTS syndrome, as well as mast cell activation syndrome, also known as MCAS. These syndromes can occur in the infection associated chronic illness umbrella and often have features and symptoms that overlap with Long COVID. And so with these updates in terms of the additional data as well as additional symptoms that were added in June of 2023, our main research question is how do the updated data from nearly 4,000 additional participants informed the prior research classification for Long COVID?

And as a reminder, we took the similar methodology to the 2023 paper in which the first step is to identify what are the most distinguishing symptoms? And this was using a statistical approach called LASSO where it identifies the symptoms that are most likely to predict the distinction between those who were infected versus those who weren't. That doesn't mean that symptoms that aren't on this list can't be symptoms of Long COVID. It simply basically allows us to identify the ones that are the most distinguishing for infection status. If you compare this in the 2024 model, which is this column to the 2023 model, which is this column, you can see that a lot of the symptoms are very similar, including loss of taste or smell, post-exertion malaise, et cetera, brain fog, and many of the points that contribute to the ultimate index threshold to identify likely Long COVID are very similar as well. There are a couple of differences which I'll point out here. Shortness of breath and snoring or sleep apnea were new to the 2024 contributing index of the symptoms. And then a few of the symptoms that were in the 2023 model listed here, which actually had relatively low points previously were selected to be in this model.

And also I want to just describe that the population here similar to our previous index in terms of our overall cohort, the median age was 45 and about 73% were female, just to understand some of the social demographics that goes into the cohort of about 14,000 participants who contributed to this. Furthermore, if we compare the two models, we can see that overall the indices are aligned. You see a positive correlation here and each dot represents a participant and those participants who scored high

or have high points cumulative from the contributing symptoms in the '23 index as well as the '24 index overall, if you score high on that, you also score high on this. So there's a positive correlation. And many of the participants who met the threshold for the 2023 also met the threshold for the 2024. And there were about 20% of participants with known prior SARS-CoV-2 Infection who were classified by the 2024 index as having likely Long COVID, which is about a similar percent to the 2023 index.

We did classify new category in the 2024 index model. It's clear that this is a spectrum and that although we identify likely PASC, it is also possible that somebody who does not meet the threshold may have Long COVID. I use PASC and Long COVID interchangeably, but Long COVID or LC. And this is where there is the possible Long COVID where you have a threshold score of one or index score of one to 11. 11 is the threshold for likely Long COVID and then one to 11. But it also means that if you have one symptom previously in the last model, you wouldn't have met likely Long COVID or likely PASC. In this case, I think it's clear that we are trying to define a spectrum and there is a wide range in heterogeneity to Long COVID presentations and we want to make sure that that's recognized as well.

And then the next step similar to the 2023 model is to understand now that we have identified and refined the model for the identifying some of the contributing symptoms to the index and the optimal threshold, then for those who were identified as having likely Long COVID, what are then also the subtypes or the symptom clusters that come out of this updated model? So the 2024 index symptom subtypes includes five different subtypes that occurred when we did the cluster analysis. Similar to the 2023, fatigue and post-exertional malaise are very common across all the different subtypes. So each column represents a different cluster subtype and you can see that fatigue as well as post-exertional malaise both are high. These are percentages in each subtype of the participants who have this symptom. And you can see, especially in post-exertional malaise, over 90% have it in at least four out of the five clusters and all except one, which seemed quite distinct from the other clusters.

And this one is dominated by the smell or taste changes. And then the other clusters including one that has a very dominant chronic cough, one that has dominant brain fog, one that has dominant palpitations in some other symptoms including dizziness, and one similar to prior, which was the cluster that had associated with the highest burden of symptoms. This cluster five or this subtype five also has symptoms really have a multi-system manifestation with high burden across all different types of symptomatology. And it is clear also that it is this group that tends to have worsened quality of life and function. And it was also interesting that we found that there were higher proportions of individuals with pre-Omicron era infections who were in this cluster or in this subtype. And also those who were not vaccinated, who tended to have this high burden subtype of symptoms. We think that this provides insight into potentially distinct pathophysiology that needs to be investigated further. And it raises the question of are there not only distinct phenotypes, which means the way that a patient may present and the symptoms they experience, but could they also represent underlying biology that are different that need to be targeted and tailored in terms of treatment and management that may differ and possibly also prognosis as we see here, correlation with tracking with functional status and quality of life.

The study has many strengths. Similar to the 2023 study, this is a very large sample size and we had a perspective collection of symptoms where we could follow people longitudinally in time, and we had the comparison groups with and without known SARS-CoV-2 Infection to be able to understand some of these symptoms may be common in the general population, but which ones are the ones that tend to be even higher in and more distinct in those who had SARS-CoV-2 Infection. There are of course many limitations as well to any study, but particularly for this one, we cannot distinguish time dependent effects. Again, as a reminder in terms of the study design, it was individuals who had at least a visit at the six-month visit, which was in the window of about 4.5 months or beyond, and that is a cross-sectional follow-up time point.

We don't know for the same individual or for across time what would've been like at three months versus three years, which we know now that people can be affected by this for years. And we know now that there's variabilities in people's trajectories and natural illness course. There's also the possibility of misclassification of some of the participants, particularly in the unaffected group. It's possible that although we did do nucleocapsid testing, that antibody response may not have been adequate in some people or it may have waned over time. There's also potential confounding from other conditions and medications. Post-viral syndromes or infection associated chronic illnesses is a very broad umbrella category with many overlapping syndromes and symptoms that are similar as well. And as well as similar to the prior study selection attrition biases, which means that the people who choose to be in the study or perhaps lost a follow-up and have not had data later on in the study may have affected cohort characteristics. And those who contribute to the data may skew the relative representation of different variants and different characteristics.

So how do we use this going forward? The 2024 updated RECOVER Adult LC Research Index. This index builds upon the 2023 Index with additional data and symptoms and helps researchers classify symptomatic Long COVID and its symptom subtypes. Symptom subtypes can used to identify and recognize the different presentations in heterogeneity and the different patterns of Long COVID. This can be helpful in the clinical setting as well. But it's important to remember that this index is not designed for clinical diagnosis. So although we don't think that this should be used for clinical diagnosis, it can still be useful in the clinical setting in that it raises awareness and it raises again another way for people to recognize what Long COVID could present as. And we want to reemphasize and underscore here that having a single symptom may be sufficient to indicate Long COVID and as well as what I mentioned before is other symptoms not captured by this index may also indicate Long COVID. So this is a step towards helping research have a rigorous classification and reproducible classification, but there's much work also to be done for further refinement and classification to be able to aid in clinical applications as well.

So in terms of future directions, as I mentioned before, this is one update, but it's important to know that our understanding of Long COVID will continue to evolve and there are other studies and other investigations underway and deep dives in multiple domains, and it's really important to know that this index will need to be refined and our understanding will be refined as we continue on. And understanding trajectories and illness course patterns over time as people have relapsing permitting patterns or other trajectories would be important to understand how the symptoms and other features of the disease may change with time as a factor.

And as Dr. McComsey mentioned and Dr. Erlandson was mentioning that the investigation and potential incorporation of advanced testing not only routine clinical laboratory testing, but advanced and biomarker assay discovery would be really important as the next phase and as we try to better understand the underlying biology and the mechanisms. And in particular for potentially the distinct subtypes as well. And as we recognize these subtypes, it's important to then apply that and think about how we tailor treatment trials, for example, in RECOVER TLC is the next phase of testing interventions and thinking about would it be this subtype or these symptoms that may be best responsive to this type of therapy. And so there's much to be done next and it's an exciting time. And finally, with that, I would like to acknowledge of course all those who contribute to the RECOVER Consortium and all these efforts and in particular the participants and their families and caregivers and our community representatives and all the study sites and the RECOVER initiative as a whole. We're really grateful for this and there's much work to be done in continuation. Thank you all.

Dr. Grace McComsey:

Thank you Linda and Kristine. Very clear presentation. So what I first like to say is from reading all the questions that are coming, let me assure you, this is just scratching the surface. So we don't mean from the biomarker study that Kristine presented that this is all that RECOVER is doing. If you notice this on my slides, we already have a lot of mechanistic studies through ROA that are looking at a bunch of biomarkers. In addition, the clinical trial task force that I mentioned, I know there is a question, is that going to look at biomarkers or to be open to providing samples to biomarker studies? Absolutely, yes. So there's a lot still to happen. I just don't want people to feel after the session that, whoa, so we know nothing now in RECOVER. We do know a lot, but we have a lot more.

All I can tell you before we start the panel is that we have in RECOVER a lot of samples, a lot of data, but more importantly a lot of passionate scientists and community members who are working with us hand in hand. So we are committed to finding biomarkers and mechanistic pathways and to treat Long COVID.

So with that, I want to bring back Linda and Kristine. So looking at the questions we got, there are three questions that I would say are more clarification so I'm going to start with these. There was a question actually for myself was the arterial stiffness study that I alluded to on one slide, was if inclusive of pregnant women? Unfortunately 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. And there was another question. Does the mechanistic working group that I alluded to for providing samples from the clinical trials, does it cover biomarkers too? Absolutely, yes. So that is one of the key goals is the scientific studies that we're providing samples for from the six different clinical trials will be used for biomarkers as well. So there were a couple questions clarification for Kristine and Linda for the 2024 model. Linda, you mentioned including the three extra symptoms, was there an effort to exclude those syndromes not associated with COVID or were they just included in the model?

Dr. Linda Geng:

Yeah. That's a great question. And this could be a whole seminar and of itself just thinking about the overlap and the relationship among these infection-associated chronic illnesses and syndromes. It's very difficult to exclude these because actually a lot of the symptoms overlap with each other. So for example, the fatigue and the post-exertional malaise are also cardinal symptoms of ME-CFS or myalgic encephalomyelitis chronic fatigue syndrome. But I think the goal was to add these symptoms in response to patient and community feedback is to recognize that these are common symptoms indeed in Long COVID. What are the commonalities from these overlapping syndromes and 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 was one of them, was also one of the top of the list in the most common symptoms that we ended up seeing in those who identified as likely Long COVID.

So this raises of course 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, the 2023 recall that we use 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.

Dr. Grace McComsey:

Yeah. Another question, is there a rationale or evidence, which is an interesting question actually for why using in the definition and I think that alludes them to the NASUM definition as well as when anybody talk about Long COVID now we say three months plus. Is there any evidence for the three months or why isn't that shorter like the initial WHO definition of four weeks?

Dr. Linda Geng:

Yeah. I can start. And Dr.Erlandson and Dr. McComsey please feel free to chime in. This is a great point and one of great debate and of course the NASUM definition comes from the work of many stakeholder input from researchers to clinicians to patients of course policymakers, researchers, et cetera. And this is of great discussion. It was in one of the workshops. And 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 two months and 15 days that you're not suffering from a post-acute sequelae. And in fact, some people even propose should we call medium to Long COVID. I think it all is just 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.

Now it does look like ... And the reason why three months seems to be selected or favored as a consensus, although again, it's a spectrum because some people get better at two months and people don't get better for months to years. So it's a spectrum. But 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 three months. So the likelihood of somebody improving past three months and the likelihood of somebody staying in the chronic illness state is higher within the three-month period. So that's part of the rationale. But again, I want to emphasize it's a spectrum.

Dr. Grace McComsey:

That's correct. So there's a question, how did we control in RECOVER for those without prior infection? It's a great question since most of us by now, right, the vast majority of people did have COVID. Is it just self-reported no prior infection?

Dr. Kristine Erlandson:

Yeah. That's a great question. Most of this time of enrollment was from a few years ago, and so I think we did have 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 didn't have an 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 did have people who came in with acute infection, but that was a different group of people. So self-report confirmed by antibody testing.

Dr. Grace McComsey:

Great. And before we start talking about other biomarkers ... And I know people have actually great ideas, I could tell the audience has read every paper that came out on biomarkers. So there was a question. Are we including post-vaccination long haulers? And I just want to answer that because 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, individual who reached out to me, I was skeptical actually 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 some of them were tested T-cells all kind of testing to ensure they never had COVID infection. So it does happen. I think now it's more accepted.

In RECOVIER we have not added post-vaccination induced long. 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 who's 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 long hauler or Long COVID after vaccination is way more rare. The prevalence incidence 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. So I'm going to leave it there.

So we had a bunch of questions, Linda and Kristine, very good questions about different biomarkers that are in the literature. Now, some of them I have to say there's like one study here and there a couple of studies. There are some conflicted data out there. Let's start with inflammation markers. So Kristine, the study that you had had only CRP. So people are asking like, yeah, of course you're not going to find with these clinical tests anything. So what else RECOVER is doing? To clarify again, I know that I put a long list of things that we are planning to do it at least in the ROA and the small studies that are funded by NIH, but do you ... And not to pick on Kristine. But Kristine or Linda, what are your thoughts about other inflammation markers that seemed to correlate with Long COVID, at least in most studies? Do you have any thoughts into that?

Dr. Kristine Erlandson:

I think IL-6 has always been of interest in some of their early studies, really targeted IL-6 and acute COVID as we know that it has an important role. I think some of these pathways looking at the inflammatory pathways, I know 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. And I think looking at how these biomarkers all interact with each other is probably more relevant than just focusing in on one biomarker.

Dr. Grace McComsey:

Yeah. That's a great answer, Kristine. You can tell that we do a lot of inflammation studies in HIV. So one question, which actually I had on my list to mention relate to kynurenine, which is a metabolite of tryptophan. And Kristine, and 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. So I am aware of one group doing a lot of work on it, and I don't know if the person who asked the question belongs to that group, but it's definitely something that require further testing. So I don't know that we have definite plans yet within RECOVER, but that will be something to look into if we do not yet so that's one good potential for biomarkers.

Another one I want to mention is oxidative stress. So I know my group had 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. So oxidized LDL is very different. This is only one marker. There are a lot of other markers of oxidative stress. So I think that is another pathway that's definitely worth investigating in Long COVID. And we do have plans within RECOVER, including the role I received in RECOVER to look at more oxidative stress. There's a great question that came on micro clotting. So Kristine, in your study D-dimer was included, and D-dimer, which is a product of clots degraded, did not pan out. What do you think about this question? How can we look at micro clotting even more?

Dr. Kristine 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 it may have been related to timing post-infection, and we did have a variety of individuals, some that had had infection within the last six months and some closer to six 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 absolutely think it's worth further investigation looking at the activity of platelets well beyond just the total platelet count.

Dr. Grace McComsey:

Yeah. Correct. So it's not just number, it could be the platelet hyperactivity. And I think there is a group actually from ... I don't remember from where. But I know that the scientists presented at a meeting and I listened to her and she had this wonderful studies actually. Basically because the clots have inside of them different platelet hyperactivity, different amyloid. So there is a way to stain them, and she was able to show that in less than a hundred, but still a significant sample size people with Long COVID, all their blood had micro clots that she was able to see under the fluorescent microscope. So I think it is an interesting ... The whole thing 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. So I think it is getting actually more and more sophisticated test rather than just do a regular blood test that's going to give us the answer. But going to another one that came out is on autoantibodies. So we all hear about different autoantibodies that are seen after COVID. Anybody wants to ... And I'm happy to if you don't feel comfortable taking it. But talk about autoantibodies after COVID. Are they linked to Long COVID? What do you think?

Dr. Kristine Erlandson:

I'll defer that one to you, Grace. It's more in your area I think.

Dr. Grace McComsey:

Yeah. No. No. It's fine. I wanted to mention, because somebody asked that, and honestly it's a great question. Yes. There are a bunch of autoantibodies to anything. Autoantibodies to different immune system, autoantibodies to specific organs, specific system. So it's unclear actually which one is meaningful. However, we did a study in Cleveland where we looked at new onset autoimmune disease. So 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 the

group 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. So 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. So we have to do more of these autoantibodies for sure. Beth, you want to take it from here?

Beth Linas:

Yep. I got it. Thank you so much. We're just going to have a couple more Q&A and everyone can participate, which would be great. The first question I noted is for Kristine. It says a slide showed the difference or higher rate in aluminum creatinine, for example, did you see a rise or decline over time from the start of Long COVID over time? Did you monitor this and what was any change attributed to any treatment during this change?

Dr. Kristine Erlandson:

Yeah. I think this was really just the first laboratory assessment or that initial time point, just a cross-sectional look at laboratory, at the laboratory measures with either COVID or with prior COVID infection or with the Long COVID index. For the way that laboratory measures are done is there's a certain number of people that if they have an abnormal test, then they'll have a repeat of that. And so we will have these longitudinal trajectories for participants who had abnormal tests over time. We did not look at it in this current manuscript, but it certainly is planned to be looked at as how these measures change over time. I think there was a similar question related to platelets or another lab measure. And so certainly of interest still look at how these measures are changing over time. For this paper, because those tests were re-triggered and people based on initial abnormal, we didn't want to bias the results just by looking at the abnormal. So it was just the first assessment, but definitely of interest to look at over time.

Beth Linas:

Thank you. I have a question for you, Linda. I have read and heard about people having long influenza and other prolonged symptoms post-viral infections. Is this accurate and similar to Long COVID?

Dr. Linda Geng:

Yeah. That's a great question. And it goes back to that umbrella term of infection-associated chronic conditions on which Long COVID falls. And there are many viruses, many infections, bacterial, viral that can induce long-term sequelae or long-term consequences and symptoms and syndromes. And so influenza is also one of those. And there had actually been great EHR studies looking and comparing during a period of time when they looked at 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 I think this is, again, goes back to these overlap syndromes and that viruses and other infections can trigger whatever the downstream pathways are that often manifests in very similar ways, be it fatigue or be it cardiovascular or pulmonary or multi-system. So I think this is a great area where we really need to do deeper biological investigations about all the different post-viral syndromes of post-infectious illnesses.

Beth Linas:

Great. Thank you. And Grace, a question for you, 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 carbon monoxide poisoning survivors, I'm wondering why these studies did not measure CO. My question is whether any tests of CO in blood, breath or skin were considered and excluded for some reason or was just not considered?

Dr. Grace McComsey:

Yeah. That's a great question. I'm aware of the studies during acute COVID, mostly in the ICU people, very sick, but I'm not aware anybody's looking at it in Long COVID so that's a great question actually. We should look at it.

Beth Linas:

Thank you. Kristine, again, for you, it says from one of your slides. Goals one and two will not be proven by routine clinical laboratory tests. We should focus on specific cellular processes, stress looking at acute COVID to H2S levels and in PASC to ... I think kynurenine pathway biomarkers. Excuse me. I wasn't sure how to say that. Literature supporting this.

Dr. Kristine Erlandson:

Yeah. Absolutely. And those were some of the pathways we talked about that Dr. McComsey mentioned earlier. 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 market 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 ROAs that Dr. mentioned as well as in some of the mechanistic sub-studies or mechanistic working groups within the RECOVER clinical trials so absolutely agree.

Dr. Linda Geng:

Can I just add to that.

Dr. Kristine Erlandson:

For sure.

Dr. Linda Geng:

This is from my just be a clinician hat I think. Because it is really important that we actually ... Because 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 themselves see, well, it's all 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 ... There are many people suffering from Long COVID, that these aren't necessarily the signals that we need to look for. We just haven't developed or haven't validated a clinically available test yet as a biomarker for Long COVID. But

it's important then to educate a lot of our clinicians out there in practice who are running these tests, not to dismiss then that there may be other underlying biology pathways. We just haven't established those yet for Long COVID. But that just because somebody has negative findings in these list of common routine laboratory tests, it 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. Grace McComsey:

So if I could add one thing. I couldn't agree more. I think Linda, 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. I agree with you. This is a very important negative study and clinicians should not dismiss people just because their labs are normal. I totally agree. 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 PBMCs or in the blood of people or in the muscles. I think definitely mitochondrial dysfunction is one of the potential mechanisms we are looking at RECOVER. We do have even muscle biopsies that will be done within RECOVER as well as 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 at the back of our mind. We didn't forget about it.

Beth Linas:

Great. Thank you. And the next couple of questions are for everyone, so feel free to jump in. Are there other tests that the team is brainstorming considering after seeing that the basic tests are not showing clear differences between Long COVID and non-Long COVID patients?

Dr. Grace McComsey:

Yeah. I would say everything we mentioned. From the oxidative markers to the tryptophan metabolism to the inflammation. We have an exhaustive list really 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. So even 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 antigen. The symptomatic people, it was like 80%. So there was a relationship between having the circulating antigen and having symptoms. So that's another, I would say 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. Gut is very important in Long COVID. So there are a lot of different issues that will be looking at some of them in RECOVER to try to decipher if there is really a reservoir of the virus hiding somewhere.

Beth Linas:

Great. Thank you. Next question. Has RECOVER taken a look at the damage done to NMDA receptors from Long COVID?

Dr. Grace 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. I think we would love it if people submit thoughts and collaborate with one of the investigators in RECOVER to be able to learn more. We did a lot of work. We want to learn as much as possible from RECOVER.

Beth Linas:

Great. 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?

Dr. Grace McComsey:

Linda, you want to take that or you want me to?

Dr. Linda Geng:

Sure. Yeah. I can start. Yeah. I think the whole vaccination discussion is such a really important discussion both in terms of what Dr. McComsey already talked about in terms of post-COVID vaccination syndrome, but also the impacts in a lot of literature that suggests that it's also, of course protective against developing Long COVID as well. And what we found in the index research is, as I mentioned, there's that hybrid and subtype of subtype five where individuals in that group really have a significant number of symptoms and that seems to correlate also 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. But 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 there's the 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 a worsening effects as well. That all requires teasing apart from a biological level of understanding what is the immune pathways likely that are being triggered or dysregulated.

Beth Linas:

Okay. I'll just give one more question then I'll wrap it up. Is it thought or suspected post-infection with individuals with Long COVID become more susceptible to inflammatory processes than say someone with a different viral infection initially?

Dr. Grace McComsey:

Yeah. I think obviously it will be just postulating. But I think that is the concern. Having enhanced inflammation for a long time 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 is the concern that most researchers have. How much of this inflammation is going to end up with a lot of comorbidities? So not only cardiovascular and diabetes, but even cancer, even premature aging. So there's a lot of concern about this chronic inflammation and what 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? That is to me, a huge concern as well.

Beth Linas:

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

Before we conclude. A reminder that 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, pediatric 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 to support the conduct of the proposed study. To learn more, simply apply at recovercovid.org/ancillary. This slide lists the 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 the seminar. We would appreciate if you take a minute to fill out the survey. Thank you so much and have a great day.