Transcript

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Christine Bevc:

Welcome everyone to today's RECOVER Research Review, or R3 Seminar. My name is Christine Bevc. I'm a senior analyst with the RECOVER Administrative Coordinating Center, and I'll be your moderator for today's session. If you're joining us for the first time, 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 and remind you that any questions that you submit during today's presentations, please use the Q&A feature in your Zoom menu. After today's panel, our speakers are going to answer as many questions as possible. A Q&A document will be posted along with the recording of the seminar on recoverCOVID.org. The document will include the answers for submitted questions relevant to today's presentations. Questions about other scientific topics will be addressed in future seminars, and answers to broader questions about RECOVER will be available in the FAQs [frequently asked questions] found on recoverCOVID.org. As a reminder, we cannot answer questions about individual clinical care.

Next slide. Today I am pleased to share our panelists and introduce them to you, including Dr. Steve Johnson, Dr. Carolyn Bramante, and Dr. Susan Vernon, and our discussant today will be Dr. Yu Chen. Dr. Steve Johnson is the Associate Director of the CTSI [Clinical and Translational Science Institute] Health Informatics Program at the University of Minnesota. He oversees the research data warehouse and supports researchers in their use of clinical genomic image and unstructured data. Dr. Johnson also manages the University's national research network collaborations, including RECOVER, the N3C [National COVID Cohort Collaborative], PCORnet [National Patient-Centered Clinical Research Network], and All of Us.

Joining Dr. Johnson today is Dr. Carolyn Bramante from the University of Minnesota. Dr. Bramante is board-certified in internal medicine, pediatrics, and obesity medicine. Her clinical work and research focus on the influence of adipose tissue on acute and chronic diseases. She focuses on decentralized clinical trials of remotely delivered interventions.

Dr. Susan Vernon is a biomedical scientist focused on infection-associated chronic conditions. She led the molecular epidemiology research at the CDC and advanced understanding of ME/CFS [myalgic encephalomyelitis/chronic fatigue syndrome]. As a scientific director of the Solve ME/CFS Initiative, she built an ME/CFS biobank and registry. Currently, she directs the research program at the Bateman Horne Center impacting national research on ME/CFS through clinical care and efficacy.

And our discussant today is Dr. Yu Chen, a chronic disease epidemiologist focusing on host and environmental factors in cancer and cardiovascular disease. She leads the NYU [New York University] Women's Health Study involving over 14,000 women since 1985. Collaborating with Columbia University and the University of Chicago, she studied arsenic exposure in Bangladesh through the HEALS [Health

Effects of Arsenic Longitudinal Study] program. Dr. Chen received the Outstanding New Environmental Scientist Award from the National Institute of Environmental Health Sciences, and she currently serves as the chair of the steering committee of the NCI [National Cancer Institute] Cancer Cohort Consortium.

The topic of today's seminar, based on, next slide, is "Understanding Metformin Use and Long COVID and ME/CFS Following COVID-19 Infection: Insights from Two Studies." Today's speakers will present findings about the association between metformin use and the incidence of Long COVID using the EHR [electronic health record]–based cohort study. The speakers will also present findings about the incidence and prevalence of myalgic encephalomyelitis/chronic fatigue syndrome, ME/CFS, following COVID-19 infection using the adult observational cohort study. Please take a moment to welcome all of our speakers.

And with that, I will turn things over to our first two speakers, Drs. Johnson and Bramante.

Dr. Carolyn Bramante:

Great. Thank you so much.

Christine Bevc:

While you're bringing those slides up, we have linked to the presentation associated with today's presentation in the chat there. And if you have any questions, please use the Q&A. Thank you.

Dr. Carolyn Bramante:

Are you seeing my PowerPoint?

Christine Bevc:

Yes, we are. It looks great.

Dr. Carolyn Bramante:

All right. I'm Carolyn Bramante, thanks so much for having us. Like she mentioned, Steve and I will be talking about metformin use and Long COVID. Specifically, we'll be talking about this analysis and manuscript that we did, *Prevalent Metformin Use in Adults with Diabetes and the Incidence of Long COVID: An EHR-Based Cohort from the RECOVER Program.* And one of the goals of RECOVER is to be able to understand how to prevent Long COVID, which is the main reason why we did that manuscript.

Another reason why we did that study [has to do with a] randomized clinical trial of metformin versus a placebo, [which] sent the participants surveys once a month asking "Has a medical provider told you that you have Long COVID?" But at the time this paper was published, all of us in the medical research/patient community, I think we were still trying to understand what was Long COVID. There were no clear definitions at the time, and so it was hard to sort of understand what were these medical providers, how were they defining Long COVID when the participants were out being diagnosed with their home physicians. So, it's important to be able to study questions in other data sources and see if things are similar.

That's why we undertook this retrospective cohort analysis in the EHR databases, and Steve will explain those databases. But this study was in adults with type 2 diabetes. Diabetes and metformin are generally synonymous, because the vast majority of diabetes is type 2 diabetes and metformin has been the first-line treatment for type 2 diabetes for a long time. So, individuals who have type 2 diabetes are

generally not eligible for a clinical trial of metformin because they're probably already taking it or they might've taken it and stopped because of a side effect.

So, with EHR analyses, we can study individuals who wouldn't be eligible for a clinical trial of metformin, so adults with type 2 diabetes who are taking medication. We excluded adults who were taking GLP-1 receptor agonists and SGLT-2 inhibitors, and we used a "prevalent user, active comparator" design. So, we compared those who were taking metformin alone or in combination with other diabetes medications, versus those who were taking other diabetes medications only and no metformin prescribed in the past year.

The index event where we started following them for outcomes was the earliest evidence of SARS-CoV-2 infection. And then to try to better capture any confounding variables, we required that to be in either cohort, the metformin or comparator cohorts, individuals had to have at least one office visit in the 0 to 12 and 12 to 24 months before their SARS-CoV-2 infection.

And Steve will talk more about the data.

Dr. Steve Johnson:

Yeah, thanks Carolyn. So, we were fortunate to have two very large observational data sets to use for this study. The first one is the N3C, the National Clinical Cohort Collaborative, which is sponsored by NCATS [the National Center for Advancing Translational Sciences at the National Institutes of Health] and consists of about 80 sites from across the United States that contributed COVID-related data. There're about 23 million patient records in that database. And then the PCORnet data set, which was funded by PCORI [the Patient-Centered Outcome Research Institute], consists of 37 institutions and they had about 30 million patient records in their data set. So, we had a very good databases to try to answer this question. Next slide.

So, I don't expect you to read this, but you can see that with all of the criteria that Carolyn talked about, they had to be diabetic patients, and they had to be on these certain diabetes medications. We start, like with on the N3C side, we see that there's 22 million patients at the start. But by the time we apply all of our inclusion and exclusion criteria, we end up with about 51,000 patients that meet all the criteria. Similarly, on the PCORnet side, we start with about 18 million patients in their data set that we used, and they ended up with about 37,000 that met the criteria. So, you can see when using observational data, you need really large databases to end up with a decent size cohort to do the analysis. Next slide.

So, the other decisions we had to make were how to define our outcome variable, which in our case was composite outcome. We were looking at the risk of death or Long COVID within 180 days after a SARS-CoV-2 infection. And so, there's no lab tests that you can take to know that you definitely have Long COVID, there's no biomarkers, at least that we know of right now. But there are multiple definitions, and we decided to use these multiple definitions to try to answer the question. The first is diagnosis code of U09.9, which became available for physicians to use starting in October 2021. And it's a determination by a physician that in fact this person, this patient may have Long COVID. We also had a computed or calculated definition, which we call a computable phenotype, and I'll explain what that is in the next slide.

So, we had two different definitions and the first is, I'll call it the N3C definition, where we looked at patients who had the U09.9 diagnosis codes and we looked at some other factors like whether they went to a Long COVID clinic and whatnot. And then we built a machine learning model that tried to

find similar people that looked like that, that may not have had the U09.9 or other characteristics. And so, the machine learning model tried to predict the likelihood that a patient in fact had Long COVID. And this would help us so that even before the diagnosis code became available, we're able to predict who might've had Long COVID, and also for people who didn't necessarily go to get a specific diagnosis.

And on the PCORnet side, they had a different way of defining likely Long COVID. They looked at some symptoms and conditions. In this case, there were 25 conditions that seemed to be most relevant. And they looked to see that if a person did not have any of those conditions before their SARS-CoV-2 infection, but they did have those clusters of conditions after their infection, that they may have Long COVID. So, these are the two different definitions that we use in addition to the U09.9 definition. Next slide.

So, our analytic approach was to try to adjust for the differences in the cohorts in those folks on metformin versus those on other diabetes medications. And we adjusted across dozens of variables. I won't go into huge detail, but it was important to adjust for differences in their hemoglobin A1C values, their age differences, what era they got COVID in. And you can see before, we've got a lot of pink dots which represent differences between the cohorts, and after balancing, the blue dots represent a very balanced approach. So next slide.

So, when we look at the unweighted frequency of the outcomes, we can see that for an outcome such as death, the N3C and the PCORnet are right around 4%, the U09.9 definition of Long COVID, 1.7 versus 1.3. So, they're similar in those definitions of the outcomes, but they're quite different in the computed definition, the N3C definition around 5%, and the PCORnet definition around 25%. So, keep that in mind as we go to the next slide. Next slide.

Dr. Carolyn Bramante:

Oh, sorry.

Dr. Steve Johnson:

Oh, you want to go?

Dr. Carolyn Bramante:

There you go. Okay. I forget when I'm taking back over.

Dr. Steve Johnson:

You can go for it.

Dr. Carolyn Bramante:

So, like Steve mentioned, the last slide was just overall frequencies of outcomes, metformin compared or together. So, this is the unweighted frequency in the N3C, death, U09.9, and then the calculated definition of Long COVID. And then PCORnet, same thing, death, U09.9, and then the calculated definition of Long COVID in their database. And then after the weighting, you can see the overall prevalence incidence of those outcomes went down. And the difference between metformin comparator also went down, U09.9, so the selected code definition and then the calculated definition in N3C and in PCORnet. So, these are the absolute differences between metformin and comparator.

And then the next slide is the relative differences. So again, N3C, the selected code for Long COVID and then the computed code for Long COVID, then PCORnet, the selected code, and the computed code. So taken together, all those different outcomes in general [tended] in the direction of benefit. With metformin though, some wide confidence intervals, and we can talk more about this definition later as well.

So how do these results compare with other observational data? So, one of the first slides I showed was from a randomized clinical trial. What about other electronic health record database analyses? This is an overview of several different studies looking at acute COVID outcomes. And these studies included adults who had diabetes, and they were already on metformin and then became infected with SARS-CoV-2. And so, these studies all looked at mortality or other severe outcomes in the first month or so after infection. And in general, dots on the left side of this line indicate benefit with metformin.

And then this is another observational analysis in acute outcomes, people already on metformin who then got SARS-CoV-2 infection. But this analysis excluded adults with type 2 diabetes. And I point that out only because in adults with type 2 diabetes, there might be exposure of metformin in your comparator group just because metformin has been prescribed for type 2 diabetes, whereas we don't think there's as much a risk of exposure to metformin in the comparator group in a cohort with prediabetes or PCOS—polycystic ovarian syndrome—just because metformin isn't automatically prescribed for those indications. But in general, these dots are in the same direction.

And then again, coming back to the paper that we just described, that's these top four lines, in adults who were already on metformin then got infected with SARS-CoV-2 and the outcome is Long COVID. These next two—Soff and Mateu—these are other observational analyses in adults who were already on metformin before they got infected. And the outcome with these is Long COVID, defined by natural language processing or by symptom surveys in a prospective cohort.

And then in the clinical trial I showed early on in these slides, these individuals got SARS-CoV-2 and then were prescribed or then randomized to metformin or placebo. And then this is a trial emulation analysis in adults who had no indication for metformin on- or off-label indication. They got SARS-CoV-2 and then they were prescribed metformin or another off-label medication, like fluticasone, for acute infection. But the main point here again is just sort of most of the dots are on the left side of 1, and 1 would be a value of no difference between metformin and the comparative group.

So, taking a step back, this is a really nonintuitive use of a chronic diabetes medication to think about it being helpful in an acute setting, and especially to think about starting it in the setting of acute infection. But the earliest papers that I could find on metformin were actually in the setting of influenza, which was interesting in the 1940s and '50s and associated with some better outcomes. Then FDA [the Food and Drug Administration] approved metformin for diabetes, and then cohorts of people were on it for a long time and the anti-inflammatory actions were noticed. And then even in the 2010s, some antiviral studies were done with metformin in other RNA viruses.

As far as why would it make sense to look at metformin for SARS-CoV-2? Well, there were some papers published in 2020, like this one showing an association with lower IL-6 [interleukin-6], an inflammatory molecule, in those with metformin use compared to those without metformin use. Some in silico or computer modeling predicted that mTOR was a protein important for disrupting the viral life cycle, and metformin is a medication that inhibits mTOR. And then fairly early on in the pandemic, there

were a number of observational studies showing associations with potentially lower risk of mortality in metformin users compared to nonmetformin users.

I was a little skeptical of the antiviral actions, but what I knew about metformin pre-COVID is just that it has anti-inflammatory actions. And then when you map those onto the pathophysiology of SARS-CoV-2, or at least what we understood about the pathophysiology at the time, a lot of those actions were relevant to sort of tamping down the adipokine cascade that the virus can cause.

Other sorts of reasons why would it make sense to look at metformin, a group at Jackson Labs was using in silico modeling that I don't really understand, but they shared this that they had also predicted metformin, or metformin had ranked as a good drug to look at for repurposing in their modeling.

So, was there in vitro data against SARS-CoV-2? There were a few studies early on and there were some discrepancies, in vitro efficacy but not in a plaque assay. This study shows in vitro effect at 48 hours but not 24 hours. And then a recent paper published that there's a lot that we still don't understand about the pathways that metformin works on, and models to study it can be challenging because the ways that the body metabolizes the medication or not can affect outcomes. So just to sort of put this out there, I'm not a basic scientist, but there can be nuance in the type of delivery of metformin when you're looking at basic science and clinical studies.

As far as in vivo results of metformin against SARS-CoV-2, it did reduce the virus in two randomized placebo-controlled trials. This was a tiny trial, the DEMET-COV trial, and it had a lower viral load in that study. And then this is the same trial I showed at the beginning, that there was a lower viral load with metformin compared to placebo.

Beyond direct antiviral actions or potentially the actions are not direct against the virus, but sort of immune-supportive, which allows the immune system to better fight the virus, this study looked at the immunometabolic enhancement that's caused by the virus and that metformin sort of dampens that immunometabolic response, an inflammatory response.

So, everything that I've presented and Steve has presented has been in the setting of acute infection, either individuals already on metformin or who started it at the time of infection. And there may be a reason to think about it for after the acute infection for treating things like Long COVID. A paper just came out a few weeks ago with metformin improving pain results in adults with overweight, obesity, and osteoarthritis. And so, these medications that work against the inflammatory output of adipose tissue do benefit things like pain and function in some disease states.

As far as dosing and drug interactions, we had a few questions submitted ahead of time on that, so I'll briefly talk about that. Metformin generally has very few drug–drug interactions. It's listed as a medication that's safe to use in combination with I think most of the other acute treatments for SARS-CoV-2. And then in the clinical trial where we started people on metformin or placebo, we rapidly titrated the dose to 1,500 milligrams, much faster than you would typically do when prescribing it for chronic use. So, this is the dose titration used in the trial, and these are the side effects that have been published for that trial. So, there was a little bump in vomiting here after the increase to 1,500 milligrams. And then the difference in diarrhea was about 0.4 episodes per day more in the metformin group compared to the placebo group. So that's definitely different [although] 0.4 is not a large difference.

And then we also collected PROMIS GI surveys and there was a basically significant difference in diarrhea at day 7, but it did not reach the threshold that's considered clinically meaningful on those validated PROMIS surveys. So, there was more diarrhea, but it was not a clinically meaningful amount difference.

All right, so to wrap up the paper that we started with, the prevalent user analysis with the two EHR databases in adults with chronic metformin use for diabetes, this is not meant at all to change management of diabetes just to look at results in that population. There may be some metformin exposure in the comparator group which would bias towards the null. Individuals in either group may have started metformin or other medications after the index date. And in any observational analysis, we can look at prescriptions, but we don't know if individuals were actually taking any of the medications at all or during their acute infection.

But it's still important to try to study these comparisons in adults with type 2 diabetes. It was great that we had these two large databases where we could do this and show important similarities between the observational outcomes and the randomized clinical trial outcomes for metformin preventing Long COVID.

Thank you so much.

Christine Bevc:

All right. Thank you, Dr. Steven Johnson and Dr. Carolyn Bramante, for kicking off our panel today. As a reminder to our audience, if you have any questions, we'd like you to submit those using the Q&A feature in your Zoom toolbar.

Our next presentation will be by Dr. Vernon, who will take us further into the incidence and prevalence of post-COVID ME/CFS among adults. Dr. Vernon, I think we're almost there. We can see the slides. And if you want to move it into presentation mode, we should be all set.

Dr. Susan Vernon:

Ready?

Christine Bevc:

I think we're ready. Go for it.

Dr. Susan Vernon:

Perfect.

Christine Bevc:

Thanks.

Dr. Susan Vernon:

Hi everybody. I'm going to talk to you about a study we did in the RECOVER adult cohort to look at the incidence and prevalence of post-COVID-19 ME/CFS. I want to start with some acknowledgments, because RECOVER gave us such an incredible opportunity to study ME/CFS as a postinfectious sequela of SARS-CoV-2 infection and it wouldn't have been possible without these folks.

First and foremost, the Bateman Horne Center, where I'm research director, it's a nonprofit clinical research center dedicated to empowering patients, advancing research, and improving clinical care for all those impacted by ME/CFS and other infection-associated chronic conditions. Dr. Rachel Hess [is] the principal investigator of the Mountain States PASC [Post-Acute Sequelae of COVID-19] Consortium, which is part of RECOVER. And this is a coalition of investigators from a number of health systems in Utah, Colorado, and New Mexico, and includes the Horne Center. We're part of the Mountain Seeds PASC Consortium. Tianyu Zheng [is] a biostatistician who was just incredible to work with, working with this huge RECOVER dataset to help us understand everything that we could about ME/CFS in RECOVER. And last but not least, Dr. Leora Horwitz, who's the co-PI of RECOVER at NYU Langone.

So, RECOVER, as Carolyn said, has a goal to understand, diagnose, prevent, and treat Long COVID. Long COVID is a persistent viral illness characterized by persistent symptoms and health problems that can last for months to years after SARS-CoV-2 infection. When we began to recognize Long COVID, we quickly saw that there was an incredible overlap between the symptoms experienced by people with Long COVID and those with ME/CFS, or myalgic encephalomyelitis/chronic fatigue syndrome. And this is what I've studied all of my career, ME/CFS, and it is one of the best known and most extensively studied postinfectious illnesses there is. So, we really thought we had an incredible opportunity to tap into RECOVER in order to be able to understand what is going on in ME/CFS as well as in Long COVID.

So, ME/CFS is not new. As I said, it's one of the best known, widely studied postviral illnesses there is. And some of the first reported descriptions of ME/CFS in the medical literature actually occurred back in the 1930s, 1940s, and 1950s, with a series of reports about outbreaks of polio-like illnesses that were characterized by fatigue, muscle pain, neurologic symptoms, etc., that occurred in hospitals and in towns. And they really did have this infectious outbreak profile. Unfortunately, at the time, there was no pathogen detected, and this illness was labeled benign myalgic encephalomyelitis.

There continued to be a series of outbreaks. And then in 1980s, there were outbreaks that were associated or thought to be associated with Epstein-Barr virus and HHV-6 [human herpes virus 6]. And this led to the CDC doing some outbreak investigations and the name chronic fatigue syndrome being given to what we now call ME/CFS. That coincided with one of the first case definitions for ME/CFS, followed by a series of additional case definitions, both research and clinical case definitions, up until 2015 when the Institute of Medicine [IOM] published the latest case definition and clinical diagnostic criteria for ME/CFS. These will be the criteria that I will describe a little bit more in depth and that we used for this study, that is the IOM criteria or the Institute of Medicine ME/CFS clinical diagnostic criteria.

So, what is ME/CFS? ME/CFS is a chronic, complex, and debilitating illness that affects multiple body systems. It's characterized by a set of frequent and severe symptoms. This diagram shows the core symptoms that are captured by the Institute of Medicine clinical diagnostic criteria. First is profound fatigue that is disabling and not improved by rest, that persists for at least 6 months, and is accompanied by significant physical impairment. Also required is postexertional malaise, which is a worsening of symptoms after physical or mental exertion. These symptoms can start pretty quickly after physical or mental exertion and up to 24 hours later and can last 4 days to weeks. Also required is unrefreshing sleep. This is no matter how much you sleep, you wake, and you feel unrefreshed. So, sleep in ME/CFS is nonrestorative. And it requires cognitive impairment, which is problems with thinking and/or memory. Most of the research has shown a significant impairment of reaction time, and/or orthostatic intolerance, which are symptoms that worsen when an individual is upright.

Importantly, 80% to 90% of people who meet these criteria have not been diagnosed by a healthcare provider. And before the pandemic, we estimated that there were about 3 million people in the United States affected by ME/CFS. So, remember that number: prepandemic amounts were about 3 million people.

So, our premise for this study was the widespread occurrence of persistent symptoms following COVID-19 mirrors the core features of ME/CFS. And this created an unprecedented research opportunity to determine if ME/CFS is a postinfectious sequela of SARS-CoV-2 infection. Now remember, ME/CFS is not new, but ME/CFS has really not gotten traction as a chronic consequence of acute infection any time before. So, RECOVER provided this incredible opportunity because of the infrastructure, the longitudinal follow-up in the study, and the large, diverse patient populations necessary to really begin to understand ME/CFS in a way that was previously not possible.

So to cut to the chase, this is the paper that we got published back in January. And I'm showing this because the metrics on this paper are really pretty exciting. Remember, not too much attention has been paid to ME/CFS. Many of you may not have even heard of ME/CFS or may have heard of it but didn't know too much about it. And since it was published in the *Journal of General Internal Medicine* in January of this year, there's already been 34,000 accesses, downloads of the paper. It's been cited five times already and it has an Altmetric of 1092. Now what does that mean? It means that among the 350,000 papers of a similar age that are tracked, it's in the 99th percentile, which is really cool. And of the 140 tracked articles of similar age in the *Journal of General Internal Medicine,* it's ranked number one of a similar age.

So again, the opportunity to really study ME/CFS and understand that it is a postinfectious consequence of SARS-CoV-2 infection was great to really increase the awareness in general. So, this is just a very exciting opportunity all the way around. And of course, all the people that we work with in the RECOVER consortium—investigators, patients—I get goosebumps thinking about how important this paper is.

So, when we started the study, we hypothesized that within the RECOVER cohort, we would see the following types of people. So, people that get infected with SARS-CoV-2 and recover, we would also see a proportion of people that develop Long COVID. And within that Long COVID population, we'd also see ME/CFS, post-COVID ME/CFS. And again, we used the 2015 Institute of Medicine ME/CFS clinical diagnostic criteria; we applied these criteria to the people enrolled in the RECOVER cohort. So really quickly, what these diagnostic criteria do is identify people with profound fatigue that substantially decreases function and has persisted for at least 6 months and is accompanied by postexertional malaise and unrefreshing sleep, in addition to cognitive impairment and/or orthostatic intolerance. And remember, these five symptoms have to occur at least 50% of the time and be moderate to severe in intensity.

This is the schedule of assessments for the RECOVER observational adult cohort. And as you can see across the top here, there are assessments every 3 months from the index date. From the time someone enrolls, there's assessment every 3 months. Using these assessments, we were able to pull out and operationalize those clinical diagnostic criteria, which I'll show you in the next slide. I also wanted to point out that the people enrolled in RECOVER included acute-infected people, which are those that enrolled within 30 days of their SARS-CoV infection, so acute-infected individuals. Post–acute-infected individuals, which were people that enrolled in RECOVER but had been infected greater than 30 days from their acute infection. So, somebody might've gotten sick in 2020, and RECOVER didn't start until

the end of 2020 or the beginning of 2021, so they're greater than 30 days past their acute infection. So those people were also eligible to enroll, as well as a number of uninfected people.

Now what we did was, we assessed fatigue and physical impairment using the PROMIS Global Health 10 [questionnaire], which assesses severity of fatigue over the past 7 days and inability to carry out daily physical activities. Unrefreshing sleep was captured with the PROMIS sleep disturbance tool, which asked the individual, is your sleep refreshing? And they had answered either not at all or a little bit in order to be considered moderate to severe for unrefreshing sleep. Postexertional malaise was a yes or no question. And this is, they [were] asked [did] symptoms worsen even after minor physical or mental effort. And their answer had to be, yes, I have it now or yes, I still have it. And then the individual also had to have cognitive impairment assessed by the Neuro-QoL [Quality of Life in Neurological Disorders measurement system], and/or orthostatic intolerance, which was a question that asked, are you feeling faint, dizzy, "goofy," difficulty thinking soon after standing from a sitting or lying position? And the answer had to be, yes, I have it now or yes, I still have it.

So, ME/CFS for this study was defined as moderate to very severe fatigue over the past 7 days, plus moderate to complete interference with ability to carry out everyday physical activities, and the presence of postexertional malaise, and unrefreshing sleep, plus orthostatic intolerance and/or cognitive impairment. We also assessed "ME/CFS-like," which was at least one of these ME/CFS symptoms, but not meeting all of the above criteria. So, they were considered ME/CFS-like.

And here's a CONSORT [Consolidated Standards of Reporting Trials] diagram of what we studied. Hopefully, you can see this. So, there were 15,000 adults enrolled in RECOVER. We excluded hospitalized, we excluded individuals with preexisting ME/CFS, and individuals that never answered any survey. So about 1,000 people were excluded. In the infected group, we removed anyone who didn't have a 6-month visit. Remember, you had to have those symptoms at least 6 months in order to qualify as ME/CFS. That left us with 11,785 infected, which we then sorted into post-COVID ME/CFS; 531, or 4.5%, met the criteria that I just described. 4,692, or almost 40%, were ME/CFS-like, had one or more but not meeting all the criteria for ME/CFS. And 55, [or] 56%, had no ME/CFS symptoms.

In this post-COVID ME/CFS group, we looked at the amount of the acute-infected individuals. Remember those that enrolled within 30 days of their acute infection, 73 or 1.6% of them [with] post-COVID ME/CFS were acute-infected, and 6.3% post-COVID ME/CFS were from the post–acute-infected or those that enrolled greater than 30 days from their acute infection were post-COVID ME/CFS. So, 4.5% prevalence of ME/CFS within the infected group, compared to the uninfected group where there was a prevalence of about 0.6% ME/CFS.

Now this is interesting, and this was not published in the paper. We actually did this analysis for ILLInet [Illinois RECOVER hub], the group up at University of Illinois. And why it's interesting is because I think a lot of times when we think of CFS, chronic fatigue syndrome, we tend to think of the *Newsweek* article back in the '80s where they talked about "yuppie flu" and that it's a bunch of Caucasian yuppies that have this weird thing that they labeled as yuppie flu. And what you can see in this table is that the demographics of the acute-infected individuals actually are represented in multiple races and ethnicities, and the highest rate is actually in the multiracial ethnic group compared to in the White non-Hispanic 1.7%. And interestingly, this holds true for also Long COVID and also ME/CFS-like. So, it's not just somehow affecting Caucasians, it affects all races and ethnicities. So, everyone is at risk, not just yuppies. So, this was a very interesting analysis that we did.

We also had the opportunity to look at incidence. Because of the acute-infected group, remember these are people that enroll within 30 days of their infection, this is a really an unbiased group. And so, it gave us the chance to determine the rate of new incident ME/CFS, people who develop ME/CFS because of the SARS-CoV-2 infection. And what we found was that 201 participants in RECOVER developed new ME/CFS compared to 98 propensity score matched uninfected individuals. This is an incident rate among the acute-infected of 2.66, a significantly different rate than the 0.93 in the uninfected participants with an attributable risk, which is the excess ME/CFS incidence in infected individuals of 1.74. And the hazard ratio, which is the rate of ME/CFS in infected versus uninfected of 4.93. So, the SARS-CoV-2 infection significantly increases the risk of ME/CFS development.

We also had the question of... How does ME/CFS and Long COVID... Are they the same? Do they overlap? Are they different? And we did this comparison by looking at the clusters that were defined in one of the original *JAMA [Journal of the American Medical Association]* papers for the case definition of Long COVID, which created a PASC research index score and grouped Long COVID people into one of these four clusters. There's since been an updated Long COVID research index, which now has five clusters. We hypothesized that most of the ME/CFS would occur in this fourth cluster, which is the darkest cluster, which is the most symptomatic cluster. And also has all of the symptoms, the highest rate of symptoms that are consistent with or required for ME/CFS criteria.

What we found in this graph here, the post-COVID ME/CFS group, [was] that 90% of the post-COVID ME/CFS fit into any one of these four clusters, so they overlapped with Long COVID in that way, and that 45% of post-COVID ME/CFS fit into the most severely ill or the most symptomatic fourth cluster of Long COVID. This was flipped around for the ME/CFS-like group where most of the ME/CFS-like, again, that's one or more symptoms of ME/CFS criteria, but not meeting all, they were actually indeterminate. So, they did not meet Long COVID criteria.

We also took a step back and looked at the rate of the symptoms in all of the infected population and compared it to the uninfected. So, in the acute-infected individuals, postexertional malaise is the most common symptom. Also, postexertional malaise is the most common symptom in the post–acute-infected group. And then we have a much lower rate of symptoms, with fatigue being the most common symptom in the uninfected group.

So, in summary, the key finding for our study is that there is an increased risk of ME/CFS after SARS-CoV-2 infection. We found the prevalence rate of 4.5% equates to approximately 15 million people in the US. Remember, the prevalence rate that we started out with in the beginning, prepandemic, was 3 million. So, we have a fivefold increase in ME/CFS prevalence following the pandemic. The incidence rate of 2 per 100 person years is almost 10 times greater than the prepandemic incidence rate that was determined by the CDC, I think that was back in a 2005 study, of 0.18 per 100 person years. So, the pandemic has resulted in an increased prevalence and incidence rate in ME/CFS.

Post-COVID ME/CFS patients experience frequent and severe fatigue that impacts physical function, causes postexertional malaise, unrefreshing sleep, orthostatic intolerance, and cognitive impairment. And ME/CFS patients are a severely ill subset of Long COVID patients, with the hallmark symptom of postexertional malaise being the most frequently reported symptom.

So, ME/CFS is diagnosable and it's a consequence of SARS-CoV-2 infection. It can be diagnosed and should be diagnosed. The RECOVER cohort presents a unique opportunity to explore the biological mechanisms and the natural history of post-COVID ME/CFS, an opportunity that as tragic as the pandemic is, an opportunity that is really an unprecedented opportunity to understand and potentially

solve ME/CFS. And it also gives us an incredible opportunity to develop objective biomarkers for diagnosis and severity.

And with that, I'll wrap up. Thank you for your time.

Christine Bevc:

Thank you to all of our presenters. And I'd like to invite our discussant Dr. Yu Chen to help lead off our discussion.

Dr. Yu Chen:

Thank you. Thank you Dr. Johnson, Dr. Bramante, and Dr. Vernon for the excellent presentation on this impactful research. So, I have a few questions for the metformin presentation. There seem to be different results between the two cohorts, PCORnet and N3C. Specifically, there's a known association, known finding in PCORnet using the computable outcome. The hazard ratio is 1.04. So, what are the potential explanations for this finding? I think you mentioned that these two cohorts are very different, the computable outcomes are very different, but could you please elucidate?

Dr. Carolyn Bramante:

Yeah, and I am not a computable phenotype expert, but I will try to put these in an analogy with a clinical trial. So just looking here, this is what was published in the paper, the unweighted frequencies are similar between the databases except for the computable phenotype. So, there's just a lot more people who met the computable phenotype definition with PCORnet's computed definition versus N3C's. And I think that PCORnet actually does have a new definition now that might be a little bit more specific. But when you have an outcome that is so common, you can have outcome misclassification.

And while you were talking, I tried to put this together. This was from a *JAMA* podcast I listened to a few years ago. But let's say you hypothesize that this intervention is a professional archer, what does a professional archer do? They hit a target at a professional archer distance. Your *P*-value is the size of the target, your number of arrows is the number of analyses you do on that same pile of data. But what if you define the outcome just incorrectly? You make it too easy to hit the target, you put the target too close. So basically, it's very easy to hit the target, you don't have to be a professional archer to hit the target.

And I did this in the acute outcome of the COVID-OUT [Outpatient Treatment for SARS-CoV-2 Infection] trial. We defined severe COVID as one low reading on the home oximeter, an ER [emergency room] visit, hospitalization, or death. But one low reading is not severe COVID. We know that now, but at the time we didn't know that. And so many people, we made it too easy to hit the outcome target by just having one low oxygen reading. And you can see that in the results here. So, this is the same clinical trial I've shown earlier on the acute outcomes. In the first 28 days, 23%, 25% of people had the composite outcome. When you include the one low oxygen reading, when you exclude that part of the composite outcome definition, it's 4% to 7%, 1% to 2%.

So basically, a bunch of people we think were misclassified as having the outcome, but they didn't actually have the outcome. They didn't actually have severe COVID just because they had one low reading on a home oximeter. And then when you look at the a priori sort of subgroups that look at a dose effect and the validity of the outcome, hospitalization and death, you can see how this outcome misclassification affected the results here.

And I think the same thing might be happening in the PCORnet computable phenotype where you have 25% as the incidence, so just a really high incidence. So, it might be very sensitive, but not specific. And then when you have more specific results, you get rid of people who don't actually have the outcome.

Dr. Yu Chen:

That makes sense. In epidemiology, we also say this could be nondifferential misclassification leading to a bias towards the null.

So, my next question is, it's very interesting that even clinical trials now show that metformin is effective even in those without diabetes. So, the evidence seems to be accumulating. It is now justifiable that metformin can be prescribed even in, I think in the online question there is a question asking about what about thin people? So, my question is that, is it justifiable to prescribe metformin? And also in your analysis, have you done any exploratory analysis to identify subgroups that may have a greater benefit from metformin?

Dr. Carolyn Bramante:

Yeah, so a few quick things I'll point out. So, this clinical trial, the 1,300 people, they all had a BMI [body mass index] greater than or equal to 25, and so it was overweight or obesity. And I'll come back to the second part of the question in a minute. As far as exploratory subgroups, we really haven't looked at just a BMI of 26 and below, but these subgroups were prespecified, and you can just see a little bit of a dose effect. Those who started this study drug early, so within less than 4 days of symptom onset, and we chose that subgroup because that was what the primary analytic sample was for the new antivirals being studied. But just logistically, we didn't think we could enroll an entire trial that quickly after finding people and consenting them. You can see this is for ER visit, hospitalization, death, when you started earlier, that point estimate moves to the left and that same sort of dose response was there in the Long COVID outcomes too. So earlier initiation, smaller point estimate.

So those results are there. We haven't done exploratory things, but the ACTIV-6 [Accelerating COVID-19 Therapeutic Interventions and Vaccines-6] clinical trial, that primary paper will be published soon. And that trial did not exclude people with a BMI under 25, so it included people with a BMI under 25. There were no safety events, there were no episodes of healthcare-measured hypoglycemia. And so, when that paper's published, it would probably make providers feel comfortable prescribing below a BMI of 25, but I think they could already feel comfortable and safe prescribing above a BMI of 25.

Dr. Yu Chen:

Very interesting. Thank you so much. And for the second presentation, I only have one question. So, the ME/CFS seems to be a very important phenotype that may be related to a lot of viral infection. So, are there treatments for ME/CFS? Is there similarity between risk factors for ME/CFS and Long COVID besides the multiracial background that you mentioned? Perhaps, I don't know, metformin can be used to treat the ME/CFS, just to connect the two presentations. So, Dr. Vernon?

Dr. Susan Vernon:

So, I'm not a clinician, but I work with Dr. Lucinda Bateman who is an ME/CFS clinician and also part of RECOVER. And we spoke a little bit about metformin use in ME/CFS and Dr. Bateman has used metformin for some of her patients, and there does seem to be a particular ME/CFS phenotype that

benefits more so. This is not published. And I think this is a dilemma that the whole ME/CFS field has had because again, more than around 90% of the people with ME/CFS are not diagnosed. So, there's only a handful of clinicians in the US that actually treat ME/CFS patients. There are no FDA-approved treatments. So hopefully RECOVER will help solve that. By understanding Long COVID, we will also gain a significant understanding of the pathophysiology as well as possible treatments for ME/CFS.

Dr. Yu Chen:

Thank you so much. Yes, Dr. Bramante.

Dr. Carolyn Bramante:

Just to confirm, none of the data I presented was about treating Long COVID. It was just about metformin, either started or already in place at the time of infection, and then the prevention of Long COVID. So yeah, we don't know if it would treat Long COVID.

Dr. Yu Chen:

Yes, I think I misspoke. I mean in preventing Long COVID. So, Christine, I think there are a lot of online questions, so we can move on to your session.

Christine Bevc:

All right, that sounds great. And thank you and feel free to add to this based off of your experience working on RECOVER as well. It's incredibly valuable to have you here with our panel today and appreciate all of our presenters.

We do have quite a number of questions. Some of them have already been answered, and you can click on the answer tab to be able to see those. One of the first questions I have is just in general, and we saw this both in advance submitted earlier, and then also in our live Q&A submissions, other than publications: Can you speak to what's been done to disseminate information to providers on metformin's role in reducing the risk of Long COVID? Just the experience from both individuals that submitted this question is that primary care physicians aren't aware of this and it's surprising, but also, they're pleased to know about it. So, if you could speak a little bit to that dissemination piece.

Dr. Carolyn Bramante:

So, this question, I know we were asked this, this could be its own lecture in and of itself I think. A few points that I'll make, it's very nonintuitive. It doesn't make sense at face value. So, it makes sense that even if someone saw it, it wouldn't land with them. That's one issue.

A second issue is, I was pretty inexperienced when our clinical trial results came out and I thought it was not at all my role to disseminate, just do the work and it'll shake out in the literature. But I think I have been pushed since then to do more to disseminate. And in looking at the first randomized trial of metformin versus placebo for treating acute COVID, there were a lot of mistakes and some sort of aspects of that inclusion criteria that were not clear and that do dramatically affect the results. And we know that because the authors have been cooperative in sharing those corrections and what happens when those issues are corrected.

But when the first rounds of treatment guidelines were being developed, that was the only clinical trial available. And so, two treatment guidelines that I know of that mentioned metformin are

sort of grounded in that clinical trial, which has an expression of concern published about it now, which means you can't use it for guidelines or decision-making. But those guidelines don't necessarily know about that update to the paper. I think the paper's going to be formally updated probably. And then the other guidelines just don't mention metformin. And that has been confusing to us, why it didn't reach the reviews in those guidelines.

But I do have an IND [Investigational New Drug application] for the COVID-OUT clinical trial and the FDA does review all data submitted with an IND. So, I have submitted for an EUA [Emergency Use Authorization] application as a way to sort of help make sure we review all this data carefully and disseminate it. But that's really hard to do when you're not a manufacturer. But that process is moving forward. It will be slow and guidelines and individual clinicians don't have to wait for FDA action to prescribe something off-label.

Christine Bevc:

And the data that you presented, were you able to capture or reflect any of the information for off-label use in there?

Dr. Carolyn Bramante:

Well, there's only one FDA indication for metformin and that's for type 2 diabetes. So, anything else is off-label. So, all the prediabetes, all the PCOS, it's often used for weight loss in kids and adults, so that's all off-label. And then of course the clinical trial was off-label, but we got an IND [approval] for it. And then the new user trial and relation analysis, those were the few people who saw the early papers on metformin and who asked their clinician for a prescription when they got infected. That's who we presume, because they had no other indications for metformin, and that would also be off-label.

Christine Bevc:

All right, thank you. The next question is for Dr. Vernon. Were there any social determinants of health associated with having ME/CFS, particularly in the racial and ethnic minority groups?

Dr. Susan Vernon:

We did not specifically look at that. But I have another question in there that asked whether or not we will dig deeper into those demographic characteristics and SDOHs [social determinants of health]? And we are. So hopefully we'll be able to answer that soon.

Christine Bevc:

Great. Going back, great set of questions coming in. An additional follow-up question for you, Dr. Vernon. Was postexertional malaise the predominant symptom which contributed to the ME/CFS-like symptoms or ME/CFS-like illness among those who did not meet the full diagnostic criteria?

Dr. Susan Vernon:

I didn't look at that in particular. They could have any one of the five, but that's a good thing to look at and easy enough to do.

Christine Bevc:

Okay, great.

Dr. Susan Vernon:

I would suspect that postexertional malaise is interesting because not many people know what it is. And even when they experience it, they don't know what they're experiencing. So much so that when we're studying it, we have to explain what it is in order to be able for people to say, oh geez, that's what I'm doing. Because I think there's a tendency to, when you start feeling better, to go ahead and resume normal activities, and that's actually an exertion that could then cause you to quickly relapse. It's just not something that kind of clicks in our brain.

So yeah, postexertional malaise is super serious, it is something that we all need to better understand and recognize. So, I would guess that it actually was not maybe one of the more frequent ones, because a lot of people just don't know what it is.

Christine Bevc:

All right. Shifting back over to the metformin study, just kind of taking a step back, and this is a question, Dr. Johnson, that you had provided a written response to, but I think it's helpful to put this into context around just kind of what prompted the work these studies? What was the trigger for looking more closely at metformin of all the medications and the diagnoses and everything that was happening with Long COVID? So why metformin?

Dr. Steve Johnson:

I mean, I'll let Carolyn weigh in a little bit. But the COVID-OUT study she talked about showed there was some benefit, but they asked the question, "Did the doctor say you had Long COVID or something?" It wasn't as precise as we'd like. And I would also say that, Carolyn talked about, I call them the mechanistic, the simulation studies that looked at different compounds that might be effective against the molecular ways that the virus worked. I think that's what prompted some of these initial thoughts that metformin might be good to look at. Carolyn?

Dr. Carolyn Bramante:

Yeah, exactly like Steve said, I think I went through why we did the trial and why those initial studies were done. And then as far as why we did this RECOVER EHR paper, was really to just understand the outcome from the clinical trial. And in the clinical trial, this was ongoing real time, and we didn't know how to define it, so we said, let's just ask, "Have you been told by a medical provider that you have Long COVID?" Yes [or] no. Because while the definition would continue to change, that was the reality that those providers had at the time, their knowledge at the time. And it was something that at least drove the patient, the participant in the trial to go see their doctor. So, it was important to be able to look at that in the EHR, and I think the U09.9 is exactly the code for choosing this patient in front of me has Long COVID.

Christine Bevc:

Okay, we have just a few more minutes left today. So, we are going to turn to another question, which is a little bit more forward-thinking around the interest in preventing or mitigating the effects of Long COVID with metformin. And then also to Dr. Vernon, around ME/CFS and what are the next steps for the research that we've heard about today? So, I'll start with Dr. Vernon around ME/CFS and where the research goes next with this.

Dr. Susan Vernon:

Again, RECOVER has just provided an unprecedented opportunity to understand post-COVID ME/CFS. If you think back to that assessment table, included in that assessment table, all those dots across there all across time, was also the sampling framework that was used during the RECOVER observational study. And there are samples that were taken in the first tier—blood samples, fecal samples, saliva samples—that are ripe for testing and digging deeper into ME/CFS. Because now all the people that we have identified as ME/CFS in RECOVER, those samples are associated with those individuals. So that provides a crazy cool opportunity to dig deeper into biomarker discovery and pathogenesis. And then of course there are the tier 2 and the tier 3 assessments that are going on in RECOVER, which dig a little bit deeper in each tier into the various aspects of the pathophysiology.

So, my hope is that now that we've identified this particular subset of post-COVID ME/CFS within RECOVER, and the data and the samples that have been collected and the additional assessments that are being done, will just really pull back the curtain on ME/CFS and show us a lot of what's going on.

Christine Bevc:

Right. Well, we're looking forward to being able to hopefully share that work in the future. Dr. Bramante and Johnson, next steps. Are there efforts to look at children? Since we saw that the response there that this was looking at individuals who were 21 and up, are there plans to look at younger patients?

Dr. Carolyn Bramante:

Yeah, I mean that is something that I have not seen as far as a randomized trial in pediatrics, metformin versus placebo during acute infection, nor metformin versus placebo for treating Long COVID. So, I think that is something that probably should be done, unless we think results from adults do extrapolate down to children. We could look at children in EHR analyses; however, metformin prescription in kids is pretty rare. But you're right, we could design a study to look at a prevalent user analysis in people under age 21.

Christine Bevc:

And more broadly, the directions for this work here?

Dr. Carolyn Bramante:

Broadly for all of us, not just me. I have biosamples from the COVID-OUT trial, microbiome samples that I would like to analyze and do some mediation analyses. And then of course for Long COVID treatment, I think the data support—and the mechanisms support—looking at metformin as a treatment for Long COVID. And so, I know I and others collaborated on putting together a proposal to do that. There are many future directions, but the EHR analysis in kids is a good point. We could try to do that.

Christine Bevc:

Dr. Johnson, anything to help close us out?

Dr. Steve Johnson:

Yeah, I would just say that we're mostly using EHR data in the cohorts that we're looking at. It'd be difficult to do a treatment study using it, because why would people just randomly start using metformin? It's hard to see that in the observational data itself.

Dr. Carolyn Bramante:

And it's hard to look at Long COVID improvement in observational data. So, it's hard to understand treatments, whether it's metformin or anything, just because it's hard to quantify Long COVID resolution.

Christine Bevc:

All right. Well, thank you to all our panelists today and everyone for just such a rich discussion. It's been invaluable for our audience. So, we're going to wrap up our Q&A session for today. And thank you also to our audience for attending today and engaging with the Q&A. There are so many questions that we saw go through. 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 that we received today, including some of those that we didn't have time to address.

All right, let's see. And then before we conclude, just a reminder to the researchers, both within and beyond the RECOVER Initiative, can now apply to use RECOVER data for ancillary studies. So, the EHR data presented here today and some of the cohort data are available. The data for all three cohort studies—adults, including pregnant adults, pediatric, [and] autopsy and biospecimens collected from cohort study participants—are available. Interested researchers must submit an ancillary study proposal and receive approval, but they must have independent funding support to conduct the proposed study. And you can learn more by visiting recoverCOVID.org/ancillary.

All right, next slide. Thanks, Cameron. Now, and with that, we do hope that you join us again. So please keep an eye on recoverCOVID.org for updates and a list of the future seminar topics. And also, you're going to see a short survey come up on your screen that's going to ask for your feedback about today's webinar. So, if you can take a minute to fill that out, we would appreciate it. And this also includes topics that you'd like to see covered in future seminars. Thank you and have a great day.