Transcript

Claire Quiner:

Good afternoon everyone, and welcome to the RECOVER Research Review, or R3 seminar. My name is Claire Quiner and I'm an epidemiologist with the RECOVER Administrative Coordinating Center, and the moderator of today's seminar. The goal of today's seminar series is to catalyze a shared understanding of the research within the RECOVER consortium, and I want to start by thanking everyone who submitted questions in advance. Please submit any questions that arise during today's presentation in your 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 this seminar on recovercovid.org. It will include the answers for submitted questions today, 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 at recovercovid.org. As a reminder, we cannot answer individual questions about clinical care. Next slide, please. Our presenter... another couple of slides.

There we go. Our panelists. Our panelists today are doctor Catherine Blish, doctor Cliff Rosen, and doctor Avindra Nath, and our discussant today is doctor Sudha Seshadri.

Doctor Catherine Blish is a George E. and Lucy Becker professor of medicine at the Stanford University School of Medicine. She has a joint appointment in the Stanford Immunology Program, is the associate dean for Basic and Translational research, the co-director of the Stanford Medical Scientist Training Program, and is an investigator of the Chan Zuckerberg Biohub. Her research is dedicated to learning how to harness the immune system, and to prevent immune responses into a diverse array of pathogens, including HIV, Dengue-virus influenza, tuberculosis, and SARS-CoV-2.

Doctor Clifford Rosen is an endocrinologist and bone biologist. He currently runs an 11-person basic- and translational research laboratory at Maine Medical Center Research Institute. His focus is on understanding the relationship between bone cells and fat cells in the marrow niche, and the role of parathyroid hormone in defining lineage allocation.

Doctor Nath is the clinical director of the National Institute of Neurological Disorders and Stroke and NIH, where he's also chief of the section of infections of the nervous system, and director of the translational center for neurological sciences. He specializes in neuroimmunology and neurovirology. His research is focused on studying the clinical manifestations and pathophysiology in developing treatments of neurological infections, with the focus on HIV infections, and endogenous retroviruses.

Doctor Sudha Seshadri is a board certified neurologist, and the Robert R. Barker Distinguished University professor of neurology, psychiatry and cellular and integrative physiology at the University of Texas Health Sciences Center in San Antonio. [inaudible 00:03:36] and serves as the founding director of the Glenn Biggs Institute for Alzheimer's and Neurodegenerative Diseases. She leads a neurology working group, several of them, within several large consortia, such as CHARGE and RECOVER, and is PI for other large national and international consortial studies on genetics and vascular brain injury. Her research interests are in using clinical epidemiological genetic and multiomic approaches to identify the biology underlying healthy brain aging, stroke, VCID and ADRD, and to study possible preventive and therapeutic interventions.

The topic of today's seminar is slightly updated from when the meeting invite was first sent out. It's the impacts of SARS-CoV-2 infection on brain, immunity, and metabolism.

Today's speakers will share our current understanding, the gaps in our knowledge, and how RECOVER will contribute to filling these knowledge gaps.

With that, I'd like to hand it over to doctor Catherine Blish.

Dr. Catherine Blish:

Okay, and I assume I've got the right view going? I think I do.

Claire Quiner

Yes.

Dr. Catherine Blish:

I'm not seeing disagreements, so it must be okay. Let's [inaudible 00:04:55] just moving a few things so I can see.

So, it's really a pleasure to have the chance to talk to this large group about some of the work that my lab has done, looking at metabolic and immunologic basis of long COVID. And I'm going to start by very, very briefly mentioning some work that we've done in our interest in SARS-CoV-2 infection of adipose tissue.

And I'm going to mention this briefly because this is an ongoing study from which, unfortunately, we're still at the enrollment stage, and don't have a ton of new data. And the initial findings were actually published in this manuscript in Science Translational Medicine in late 2022, in which we found that SARS-CoV-2 can drive an inflammatory response in human adipose tissue by infecting both adipocytes and macrophages. And in this study, we were able to look at autopsy samples from about eight individuals, and we found evidence of SARS-CoV-2 infection in eight of them, within the adipose tissue. But we also did some in vitro work, looking at SARS-CoV-2 infection in a dish, and we found that we could identify infection both within mature adipocytes that was productive, as well as macrophages which was an abortive infection, but this combination drove a broad inflammatory response, which induced the secretion of multiple cytokines that are associated with symptoms of long COVID.

And this really led us to speculate that SARS-CoV-2 infection in adipose tissue could contribute to severe COVID-19 in obese individuals via two mechanism: one is the potential of the expanded viral reservoir, and the second is inflammation in peri-organ fat depots. And this is something that we've really been interested in following up on, but in particular, we're interested in whether this has any link to the post COVID condition, or long COVID. And, of course, there isn't, unfortunately, a set single case definition of long COVID, which I'll allude to again later, but, for the sake of this talk I'm going to focus on largely the WHO definition, which is the development of symptoms, or the persistence of symptoms, three months after initial SARS-CoV-2 infection, with symptoms lasting for at least two months, and no other explanation.

And, of course, we all know we're here because there's a massive burden of disease from long COVID. So, of course, the other we're all here is that we really want to understand what the mechanisms driving long COVID are, so that we can develop new therapeutics and treatments. And there've been innumerous really nice reviews on this topic. I'm just grabbing one picture from this nice review by Chen et al, in which the three primary mechanism that have been discussed are the idea of viral persistence, persistence of immune dysregulation, or direct tissue damage that's been persistent.

And, of course, when it comes to our adipose tissue, we're particularly interested in whether this viral persistence could be related to adipose tissue infection, and whether adipose tissue could be a reservoir for ongoing replication or inflammation, as has been seen in some other infectious diseases, including, for instance, HIV-1. And to this end, we're currently evaluating adipose tissue biopsies from long COVID patients to look for virus and inflammation, but unfortunately we're not quite at a point where we have the data all back yet from sequencing, to discuss.

So, I'm going to move on and talk about the second big area that we're interested in, which is immune dysregulation, and how that might contribute to long COVID.

So, again, same figure. This is the middle part, to some level of activation of the immune response or exhaustion. Is that contributing to these long COVID symptoms? And the work from our lab that I'm going to discuss has really been led by an infectious disease fellow in my group, Rebecca Hamlin, who generated all this data, and Shaun Pienkos, a very talented pulmonary critical care fellow, has really pitched in on much of the analysis of this as well.

And I want to begin with a very brief overview of what happens to the immune response during acute COVID-19. And, of course, many groups, mine, many others, evaluated this. This was our first foray into this, which was the single cell atlas of the peripheral immune response, which we published early in the pandemic, and made the data available in approximately May of 2020. And we followed with the multiomic profiling, and really, there's been great consensus as to what the changes are in the setting of acute COVID in terms of peripheral immune dysregulation.

And, for instance, what we found is that, monocytes and conventional dendritic cells take on a tolerogenic or immunosuppressive phenotype. We see hyperactivation of both neutrophils and NK cells, but, ironically, this hyperactivation is associated with dysfunction as well. And we see changes in hematopoiesis, leading to the development of immature neutrophils and a reduction in the number of lymphocytes.

And, of course, there are many, many other studies that have shown similar or slightly different or expanded upon results, but in general, this has been a very clear picture. And what's less clear is, do any of these changes persist or change? And, is that contributing to long COVID symptoms?

And, to that extent, I want to talk briefly about some of the data that has come out from other groups, and places in context for what I'm going to show. And, of course, and I think doctor Rosen is going to mention this as well, one of the most exciting findings recently has been the report that long COVID might be associated with diminished cortisol levels.

And, in the study by Klein et al, from Akiko Iwasaki's lab, which we heard about, I think, last month, it was the most significant predictor of long COVID in their cohort.

Interestingly enough, this is one of the few signatures that's been seen in a completely separate study by another group in the Su et al study, led by Jim Heath's group and others, they also saw a decrease. But, importantly, it was a little more nuanced in this study. It was only decreased in those with respiratory symptoms or with four or more symptoms. And those with more mild disease, it was not decreased. So, maybe not quite a universal signature, but more work needs to be done.

Another recent study that's been very exciting is the idea that diminished serotonin levels could be contributing to long COVID symptoms, in this nice study by Wong et al, in which they found in a large Upenn cohort it decreased in long COVID patients compared to recovered patients. Now, this is important because comparing long COVID to recovered patient implies that this is associated with long COVID, and is not merely something that is associated with viral resolution in general. Now, they evaluated two validation cohorts in this study. In the first, and Irish cohort, they also found decreased serotonin levels, but importantly, they did not have a comparator group of recovered COVID patients. This was compared to healthy controls. So, it's not quite an apples to apples comparison. And then, their third validation cohort, samples taken from the UNCOVR cohort, from 2-24 months post infection, there was no significant difference in serotonin levels.

And the author speculates that different modes of patient recruitment, the number of symptoms, disease severity, any of these things could've contributed to these disparate results. But, this really raises many of the challenges that have plagued some of these immune studies of long COVID, which is there are differences in how we're defining long COVID, in the timing of the samples, in the populations in which we're studying them. And these have all made it very difficult to find a broad, generalizable signature, that we can really base a therapy on. So, with that in mind, I love the immune system, and I want to know, are there changes in specific cell types, or their function, that are contributing to long COVID? And, of course, we're only one of 20 or so labs who are evaluating this, and I thought it might be useful because Rebecca Hamlin, my fellow, has spent some time looking in detail at what else has been published, to summarize some of the studies that have come out first, before we talk about what we saw in our study, and place that in context.

And so, what I'm going to show is a painfully complicated table, and long table, looking first at the innate immune cells in long COVID, versus recovered patients. And just as an example, I've narrowed this down to one cell type, classical monocytes, and what I'm showing is several of the studies that have looked at long COVID, most of which are published, one of which is still in the pre-print era, and if the box is colored gray, that means this cell type was looked at, and there was no change between long COVID and a recovered patient. If there's a line through it, it means they didn't look at the cell type, so ignore that data. If it's red, that means this cell population was increased in long COVID, with sometimes some notes, maybe it was in a subset of long COVID. If it's blue, it was decreased.

And right away, you can see what my challenge has been in trying to find a unified signature, right? So, classical monocytes are the same in three studies, in different settings. In one setting, depending on whether it's pulmonary long COVID. There's a different subset that's increased, and one subset that's decreased. In another study they're decreased.

So, that's classical monocytes. If we look at other cell types in which we've been able to find more than one study that have looked, what we see is a bit of a smattering. There's not a consistence innate signature that we've been able to identify that has held across multiple studies in multiple populations.

Okay. So, maybe the adaptive immune response is the answer. Here are some of the major adaptive immune cell types that have been evaluated, and what we're seeing is the same thing. If you look at naïve B cells, they're decreased in this study, the same in this study, and increased in this study.

So, it's very hard to come up with a unified signature. I'm not going to go through all the different cell types.

But, of course, this is just phenotyping in terms of gross populations. What about their function? And there have been several studies that have looked, in particular at T cell responses, to stimulation with either PMA ionomycin, which is a generalized stimulation, or with SARS-CoV-2 peptides.

And again, once again, what we see is really a challenge in finding a consistent result. So, for instance, if we just look at these first two, both use PMA ionomycin as a stimulation, and one found an increase in function, one found no difference. And then, if we look at this Peluso study, again, a similar thing, a different stimulation though, a decrease in function, which then conflicts with this study.

So, with this setting, what we found, we were initially surprised and confused by, but maybe it's just that different cohorts are different. And it's going to be challenging, and we're going to need greater numbers to come up with the answers.

So, with this in mind, I'm now going to step back and talk about some very preliminary analyses. And keep in mind this is all very early analyses of a study that we developed early in the pandemic that we termed the Infection Recovery in SARS-CoV-2, or the IRIS study. And this was a study that was built in collaboration with many of my colleagues in infectious diseases; Phil Grant, Aruna Subramanian, Hector Bonilla, Upi Singh, Pras Jagannathan, and Bonnie Maldonado.

And we designed this study in June of 2020, began enrollment in August, before we even knew that long COVID was actually a thing, just to study the process of recovery from COVID-19 infection. And so, we enrolled 100 patients in August of 2020, between August and December, with the idea of following them for 12 months post diagnosis, with bio specimens collected at both 3 months and 12 months in clinical data at 6 months post diagnosis.

So, this is the battery of tests that we collected on these individuals. We had medical history and symptoms, a number of scores of anxiety, fatigue, work activity, and neurocognitive testing. But, importantly for my lab, serum plasma, PBMCs and PAXgene samples.

So, we had these bio specimens at these time points, and then we also, because many of these subjects were enrolled in our early out-patient trials of interventions for COVID-19, including the Peginterferon Lamda and the Favipiravir study, neither which showed any efficacy by the way, so we ended up grouping the placebos and the controls. We had some acute samples available.

So, with this cohort, we wanted to dove into are there any immunologic changes in those with persistent versus not persistent symptoms?

And so, what I'm showing here is just your classic demographic table, and I'm sorry, I know it's small. There's a lot going on here. We had, again, 100 total enrolled patients, 20 of whom were fully asymptomatic, and 80 of whom actually had some degree of persistent symptoms at that first three-month time point. If we look, they're relatively well-matched between the asymptomatic and persistence per age and gender, which is interesting because gender has actually been enriched in symptomatic in many other cohorts. We had a large percentage of Hispanic or Latino population given the demographics in our area. The BMIs were relatively well-matched, and a fairly low frequency of these subjects were hospitalized or in the intensive care unit, or intubated, and somewhat enriched in our long COVID patients in this.

So, what were the symptoms in this cohort? And they were pretty variable. So, first of all, I already mentioned, about 20% were asymptomatic, and by far, the most common symptoms, across all time points, and arguably even increasing when it comes to brain fog and memory impairment, were fatigue and brain fog, and memory impairment. These were followed by symptoms including myalgias and dyspnea, headaches, chest pain or tightness, arthralgias and sleep disturbance. And then, finally, we did see altered taste and smell, cough and hair loss, and these were the only symptoms that consistently declined over the 12-month follow-up.

So, with this framework in mind, we felt like we could address the three major research questions. The first is, what features of acute COVID predict recovery versus long COVID, using those acute samples we have? The second is, are there immunologic differences at 3 months post infection in those who recover versus those with long COVID? And, finally, what immune features correlate with persistent long COVID versus recovery at 12 months?

And this was our experimental study design. We used pre-pandemic controls as well, and we compared our acute COVID samples, for which we had 38, our 3-month post infection samples, for which we had 100, and

our 12-months post infection. And Rebecca generated a really nice massive dataset by stimulating these PBMCs with a generalized stimulation cocktail to do a CyTOF assessment of the function of multiple immune cell subsets. We froze some samples for single cell RNA-sequencing, and process that data to look at the transcript home, and then we used the plasma for proteomic study, using the Olink platform.

Now, because these analyses are in the early days, and we don't have a ton of time, I'm going to focus just on some of the PARSE single cell RNA-sequencing data that we did, and for reasons of costs, this is extraordinarily expensive, we chose the 96 samples for this analysis, those with the most symptomatic versus the fully recovered patients.

And I'm going to focus just on one of our three questions in my remaining five minutes, which are, what are the immunologic differences at three months post infection in those who recover versus long COVID?
Okay, so what does this data look like at three months? First, here is a UMAP projection of our single cell RNA-sequencing data. So, each of these dots on this UMAP represents an individual cell. We've down sampled this because if I showed every cell that we looked at, it would be way too many and a giant blob. But, what you can see is that we can see all the major immune cell subsets, including some hematopoietic stem cells, as well as rare T cell types, like gamma deltas, and our major cell types.

So, to look at this data, one of the first things we did was, we wanted to know what the transcriptional profile was of these individual cell types, and we used these Blood Transcription Modules, which were described by my colleague, Bali Pulendran, in this nice 2014 paper, to describe the overall transcriptional network analysis of known genes that go together. And the advantage of these BTMs over some of the KEGG or other pathway analyses you might know, is that these were selected for Blood Transcriptional Modules, and are really focused broadly on immune function more so than on cancer, which is how many of the other ones were made. Importantly, these BTMs were named for the cell type in which they were most prevalent, but that doesn't mean that these transcriptional pathways can't be expressed in other cell types, which will become apparent why I'm bothering to say that in a moment.

So, what did we see when we looked at three months post diagnosis in our long COVID patients in red, compared to our recovered patients in blue? And if we look first at this particular Blood Transcriptional Module, we found that this was down-regulated, myeloid and dendritic cell activation via NRkB, one of the canonical transcription factors for immune cell activation, was down-regulated in three distinct cell types, CD14+ monocytes, conventional dendritic cell type 2s, and CD16+ monocytes.

So, if we then looked at additional transcription modules, we saw a transcription factor also associated with activation in a B cell transcription factor also down-regulated in CD14+ monocytes and cDC2s, and finally, multiple proinflammatory DC and myeloid cell responses that were down-regulated in our classical CD14+ monocytes.

So, we didn't see much outside this myeloid cell compartment, but we did see a down-regulation of a inflammatory module in natural killer cells as well.

In terms of modules that were up-regulated, or more proinflammatory in the setting of 3-month post infection, we didn't see much. We only saw these three modules: platelet activation in rich monocytes in our MAIT cells, as well as an integrin and cell adhesion molecule, down-regulated in conventional cDC2s.

So, overall this tells us that there seems to be some suppression of myeloid cells at 3-months post infection in our long COVID patients. But, the problem with the modules is, we really want to get to the genes and pathways that are involved. So, to do this, we performed a totally orthogonal analysis, using the bionet package to build networks of genes that were significantly differentially expressed between our long COVID patients and our recovered patients.

And so, what I'm showing here is a network of genes that are differentially expressed. Anything in green was significantly down-regulated in long COVID, anything in red was up-regulated in the CD14+ monocytes, where we saw the most changed. And, in fact, if we look, some of these genes were found in those same transcription modules, providing an orthogonal validation of this result. But, what we also see is networks that are interesting and involved inflammation. For instance, we see up-regulation of IL1b and CXCL8, which are involved in the proinflammatory response. So, both of these proinflammatory pathways are down-regulated in monocytes. Related to this, we see ANPEP, which is, interestingly enough, a receptor for one of the less pathogenic Corona viruses that is up-regulated. We see down-regulation also of RIPK2, which is involved in innate and adaptive signaling, and is though to induce apoptosis.

We see down-regulation of CD86, which is important for antigen presentation to CD4 T cells, as well as down-regulation of genes that are thought to be involved in viral infection, including EBV infection, interestingly enough. So, if we really put this all together, what we see is a number of genes that are interconnected, that are involved in innate immune activation, and also antigen presentation, that appear to be mildly suppressed at three months in those with symptomatic disease, three months following COVID-19.

So, now we followed this up with multiple analyses, including some cell-cell communication, but I'm running out of time so I'm going to skip that slide. And hopefully what I've shown you from our data, is that we've generated a multiomic transcriptional and proteomic datasets to investigate the basis of COVID-19. And we found several gene networks and modules that are perturbed in long COVID at 3 months post infection, which are in agreement with some of the prior published data, but inconsistent with others. And that, at the transcriptional level, we're really seeing a down-regulation of inflammatory pathways in innate immune cells, particularly CD14+ monocytes. And that genes in these pathways encode proteins implicated in human disease, inflammation, and viral infections.

But, of course, we're still at early stages, trying to figure out what this means. So, importantly, this is ongoing work that I'm presented here at preliminary stage. We're working to integrate our single cell RNA-seq, CyTOF, and plasma proteomic datasets. We're continuing to evaluate these communication pathways to see if we can interrupt some of these abhorrent pathways that might be inhibiting and inducing disease. And we're assessing the role of adipose tissue as a reservoir in long COVID.

So, with that, I really want to thank my lab, pictured here, minus Shaun, who I showed a picture of earlier, and really call out everyone who assisted with this, and this work would not have been possible without assistance from several people in my lab, including CASP Nato, for running the CyTOF studies.

And, with that, I will end.

Claire Quiner:

Wonderful. Thank you, doctor Blish. And next, we'll turn it over to doctor Rosen.

Dr. Clifford Rosen:

I'm going to represent the group of PROMIS PI investigators doctor Kirwan at Pennington, and doctor Kern at Kentucky as well as their co-investigators Jonathan Li at Harvard and Phil Scherer at the University of Texas Southwestern.

So, I'm going to talk about PROMIS, the path of biology and recover of metabolic and immune status, that is our clinical study, but for this talk, what I'd like to do first is give you a brief overview, and Catherine did a nice job summarizing some of the metabolic data that is consistent from published data, at least, that a couple of candidates are altered in metabolism, particularly serotonin and cortisol. And I'm going to talk and focus more on the local effects at the tissue level, and in particular dyslipidemia.

So, just to remind you, acute COVID is associated with marked hyperglycemia in a number of individuals, particularly those who are severely affected, and are in the ICU. And there's multiple pathogenic mechanisms for the hyperglycemia. But it's clearly worse with obesity, and is shown here that's a common theme during the course of my talk, that obesity not only exacerbates acute COVID but likely helps to predispose to some of the metabolic changes in chronic PASC.

But in addition to, and somewhat underrepresented in the literature, besides the hyperglycemia is the abnormal lipid profiles. HDL levels are profoundly suppressed in acute COVID, and this is also associated with an increase in triglyceride, and a increase in low density lipoproteins. And this is true also in PASC. So, with long COVID, the rate of incident diabetes is increased considerably. This is work from one of my colleagues, Ziyad Al-Aly, demonstrating the burden on our healthcare system for individuals who develop long COVID and consistently not only have glucose intolerance, but treatment with anti-hyperglycemics to control their disease.

And, in addition to the diabetes though, dyslipidemia again appears and is relatively common. And this is characterized by low HDL, high LDLs and an increase in triglycerides. So, these are very consistent in a more recent paper that I didn't show, Ziyad also showed that these datasets are persisted out to two years.

So, the high triglycerides, the low HDL and hyperglycemia are a bad formula combination for the develop of oxidized lipids, and we know that the first line of defense for oxidized lipids is HDL. So, many of you are familiar with HDL because it's a good lipid carrier in cardiovascular disease. But in addition to that, HDL is a large particle, and it carries a number of other enzymes which include paraoxinase-1 and cholesterol ester transfer protein. And both these are critical in their own ways, in making sure that lipids are handled properly and don't end up in

tissues where they're not supposed to be. And in particular, paraoxinase-1 is critical because it blocks the initial oxidation of lipids to lipid hydroperoxide.

And we know from our colleague Grace McComsey at CASE that oxidized lipids, particularly oxidized LDL is higher in those individuals that have symptom complex, and so are considered PASC high symptom related.
In addition to that, we know that oxidized lipids can generate reactive oxygen species, which are toxic to mitochondria. And in a recent paper, Guarnieri has shown that many of the core mitochondrial genes are down-regulated during SARS-CoV-2 infection, both in humans and in rodent models.

And so, the suppression in mitochondrial oxidation is potentially a key factor, not only in acute disease but in chronic disease as well. And some work from doctor Kirwan's lab has shown that in those individuals that did not recover from acute COVID, that oxidative phosphorylation of T cells is markedly suppressed compared to those that did recover, suggesting that, in fact, there is this chain of reactions that occurs that might trace back to what's happening with dyslipidemia.

So, in order to test that, we have a paper, it's on bio-archives in an iScience under revision, in which we worked with the group, Nference in Cambridge, Massachusetts using AI to look backwards and see if we could identify certain predictive factors that led to persistence of symptoms long term in COVID infected individuals. And so we did a retrospective case control study in which we found 1,000 individuals from nearly a million cases in the Mayo Clinic system that had symptoms at 42 days or later, and matched those to 1,000 individuals that were infected but did not have symptomatology. And there were three time points: time zero, which was before infection, and that is the amount that we had in the health records. Those that occurred at time one, which was during the time of acute infection. And those that occurred at time two. And we looked at specific, simple laboratory measures to assess whether we could predict, retrospectively, what changed between time zero and time one. And this is a very important point. We need longitudinal data and we need those cohorts that were filed before they got infected to really understand what metabolic changes are occurring.

And what we found was that, during time one, the time of infection, triglycerides was increased as you might predict, and there was a profound suppression in HDL. And it was HDL that was the best predictor of those individuals who went on to develop the long COVID symptoms. So, we were particularly interested in pursuing this. One problem with this study was, we were only out six to eight weeks, and so we wanted to see more long-term what was going on with HDL.

So, my colleague Ziyad Al-Aly, who's really been a pioneer in epidemiology of long COVID and its relationship to particular markers, was able to come back and take some of his datasets from the VA system, which he uses, and using BMI matching, he was able to show that HDL not only went down at a 120 days, or after the acute infection, but similarly, influenza, post-influenza individuals showed a decline in HDL. And it's been known that, even in HIV, HDL levels do decline and may stay down.

So, this was suggestive that there was high sensitivity but lack of specificity for a marker for SARS-CoV-2. And then we worked together with the group in Iran, at Kerman University, and they had a longitudinal series of individuals at different time points, out to 90 days, looking at a number of different biochemical markers, and they categorized them by the difference in their severity of acute illness, moderate, severe, and critical.
And you can see on the slide, on the left, that HDL levels do rebound by 90 days, but interestingly enough, they measured paraoxonase activity in the serum, and found that, that rebound did not occur as consistently as HDL did.

So, one thought is that, HDL is just the surrogate marker of paraoxonase activity, and that the persistence of high oxidized LDLs may be related to a persistent decline in the ability of the enzyme to prevent lipid peroxidation.

We investigated this a little further in the RECOVER cohort in Maine, we have a 120 subjects, and we looked at HDL levels as predictors of symptom complex, using the JAMA symptom complex from the RECOVER cohort. And interestingly enough, baseline HDL at the time they entered RECOVER in Maine was not predictive of subsequent symptom complex magnitude or frequency. But, the HDL levels at the time of their most recent response to their questionnaires was negatively related to symptoms.

So, the lower the HDL the greater the symptom complex. And we could not find additional parameters from the lipid studies, that we get in RECOVER, that actually could identify those individuals.

So, with that in mind as part one, just to remind you, insulin resistance, Type-2 diabetes, and incident diabetes are more common in PASC, and may reflect a number of different components, possibly including adipose

tissue dysfunction. Suppressed HDL, may be a marker of some lipid toxicity and probably has high sensitivity but low specificity, whether it's a surrogate marker for paraoxonase activity needs to be examined. And whether HDL may reflect a chronic metabolic inflammation still needs to be determined.

So, with that in mind, let me move to the second part of the talk, which is our investigation into the mechanisms that underline metabolic dysfunction in post-acute sequelae of COVID. And our hypothesis has been that PASC participants with a high symptom complex are characterized by insulin resistance, adipose tissue inflammation, high endotrophin levels, which I'll talk about, T cell bioenergetic fatigue, based on the work of doctor Kirwan, and that possibly this is related to viral persistence. And I should point out that we regularly meet with Catherine and Charles Roberts, and we're all in the same group, trying to identify whether virus persist in the adipose tissue. And, as Catherine mentioned, we're still in the early stages.

Okay, so, our design was a case control design of 60 participants from three rural states in the RECOVER program. So, we belong to the ISCORE Network, which is 11 individual sites in a hub network which is run out of West Virginia University, Sally Hodder, and we propose to get 20 RECOVER participants from each of three sites, Maine, Kentucky and Louisiana. And the idea was, one, rural individuals are generally under represented, so these were three rural states. And, two, we had a mix of ethnic and racial individuals that would be optimal for trying to understand the differences in PASC and recovery versus non-recovery.

So, it's a case control design, and it was based on a 2:1 ratio of 40 cases that had symptom scores of greater than 12. So, we developed the symptom score in collaboration with the DRC at RECOVER, that was similar although not absolutely identical to what was published in JAMA, but reflected very much the same eight most frequent symptoms, that Catherine also mentioned.

And our primary design was actually to look at T cell mitochondrial function using oxidative phosphorylation on the seahorse as a major outcome measure.

But, secondary outcome measures included T cell markers, glucose tolerance, DXA body composition, as well as the adipokines and cytokines and viral studies, including nasal swabs, blood and adipose tissue.
As well as some tertiary outcomes, which included spike protein analysis from David Walls, adipose tissue, secretome, histology and RNAscope and endotrophin.

So, where are we? Currently, we've had 17 fully consented. Almost all have been at the Maine site, we got started early, and early being in July. So, as Catherine said, we still have a ways to go in terms of getting full data sets, but they're matched by age, sex and body mass index through the data center at the Pennington Bio Research Center. Seven oGTTs have been completed, six adipose tissue biopsies, and body composition by DXA. And these are the pending studies. And we'll talk a little bit about endotrophin and its role as well as the studies on viral persistence.

So, I'm going show this data. It's very preliminary, but I want to give some idea of where we're going because a couple of these studies are unique to the patho-biology program, and they're also excellent for hypothesis testing and for the follow up. So, this is the first eight subjects who had DXA body composition studies. And although not statistically significant, there's a trend towards higher body fat in those that had the higher symptom complex. And, in addition, percent visceral fat was also trending in the higher range in those that had past positive symptoms. Now, this is interesting because the BMI are matched almost identical, and yet, we see these differences in visceral and total body fat distribution. And so, this is an important area that we need further investigation. It comes back, though, to the issue of whether obesity itself predisposes individuals to long COVID, and more symptomatology. And so, in a way, its reverse causation is, is the obesity actually causing the symptom complex, or is this symptom complex from viral persistence driving more metabolic fate? And that really needs to be determined by larger numbers of individuals.

Similarly, the oral glucose tolerance test is performed on eight individuals, and again, those that had slightly greater visceral fat had more symptomatology had a higher area under the curve mean glucose. So, very consistent.

What was a little surprising to us, and it's picked up because of the way we do DXA body composition studies, bone density was lower, both total body area BMD, spine bone density, and femoral hip bone density were all lower in the past positive individuals. And this was consistent with some biochemical studies we did on their plasma, which showed that urinary CTX, which is a marker of bone resorption, was increased.

We see in chronic inflammatory states, some reduction in bone mass. We also see reduction in bone that can occur from large amounts of visceral fat, with secretion of cytokines, that are detrimental to the skeleton. So,

this is an area that hasn't been further investigated, but an important for us to try to understand whether long-term complications, including osteoporosis, may occur.

And then, blindly evaluated were initial measurements of surface markers, including CD26 and CD3 and CD8, which suggested that these individuals do have some inflammatory component. And again, this is very preliminary and may or may not be consistent with some of the literature.

So, one of the things we've done in our study is do wedge biopsies on fat tissue, and we've been so impressed... and I have to have a shout-out to RECOVER participants, they've been fantastic. They want to participate in this study, and they're willing to go ahead and do these, including individuals that are fully recovered from long COVID. So, on the far left is the biopsy done by a dermatologist, here is the two grams of fat tissue that we get, and then Sam Costa, which is a very talented PhD student in my lab, has been extracting fat tissue, culturing them, and then looking at the results.

And Charlie Roberts has helped us in addition by doing RNA scope. This is just the first subject that we sent him fat tissue on to look for virus, and unable to find it. But obviously this is very early. This is a single participant.

One of the interesting things is that, these individuals, when their fat biopsies were analyzed by targeted qRT-PCRs, we were very interested in what this fat tissue was doing at 48-hours in culture, and what we found was an up-regulation in those that had symptom complex of some of the classic proinflammatory markers, TNF, IL-6, and CRP. In addition, Col6a3.

And I want to just briefly divert to mention Col6a3. So, Col6a3 is induced by hypoxia, and HIF1a, and it's a major extracellular matrix that is secreted from cells. And once it's secreted, it's broken down by a proteases to form the compound called endotrophin. And endotrophin is a major inflammatory marker that can actually persist around fat cells, and particularly in fat cells that are inflamed from obesity. And you can measure endotrophin in the plasma.

So, endotropine's major activity is the recruitment of macrophages. Macrophages can also make endotrophin. But endotrophin levels have reported to be high in end-stage renal disease, and in Type-2 diabetes in particular. And so, we wanted to know whether or not this was the case. It's also present in lung tissue from individuals with pulmonary fibrosis, and those with chronic pulmonary disease from COVID.

So, we set out to see, using different cohorts, whether endotrophin could predict markers of severity of symptoms. And again, using similar symptom complex, we were not able to show any relationship between endotrophin and the severity of post acute sequelae symptoms. We were able to see, in those that had lung disease, and we either had respiratory arrest or those that had low-flow oxygen, there was an increase in endotrophin, which was significant.

Claire Quiner:

Excuse me, doctor Rosen, we're a bit over time, are you able to finish up in about-

Dr. Clifford Rosen:

Yes.

Claire Quiner:

Two minutes? Thank you.

Dr. Clifford Rosen:

Yeah. I can.

So, endotrophin levels, we were unable to identify a change in individuals in the UAB cohort we used. And I just want to show and finish with this slide, which shows some of the immunohistochemistry of endotrophin around fat cells, right around the margin of fat cells. And so, we're pursuing that now.

And I want to finish with a slide that just suggest that SARS-CoV-2 infection can indeed result in hyperglycemia, increased oxidized lipids, and this could be a vicious cycle that enhances metabolic dysfunction. So, our future directions are, we're going to Seven Bridges to test the predictive value of HDL. We'll expand our participant number, and we'll examine our secretome analysis. And this is the group of individuals that we've been working with.

So, thank you very much. Sorry to be a little over.

Claire Quiner:

Wonderful. Thank you, doctor Rosen. And with that, I'd like to hand it over to doctor Nath.

Dr. Avindra Nath:

So, I'm going to talk about clinical phenotypes of long COVID, pathophysiological mechanisms and opportunities for intervention, 20 minutes or less.

Okay. So, I have no disclosures. That's the one thing I have when you work for the government, you usually have no disclosures. Sometimes I wish I actually had some.

Okay. So, first thing is, we don't even know what long COVID is. So, there're a lot of definitions of long COVID, and there are three. The term long COVID was actually coined by patients. And so, what they found was, it was pretty simple. They got COVID, and then they weren't getting any better, "Well, I think I got long COVID." And interestingly, that's the term that has stuck, and NIH came around, they came up with this term of post-acute sequelae of COVID-19, and people do use the term PASC, but really it's [inaudible 00:51:04]. And then, the WHO came up with post-COVID-19. So, the definition that WHO has, is the one that most people are still using, and that is, "The continuation of development of new symptoms three months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least two months with no other explanation."

But, in reality, none of these definitions are good, because what you have is, you have two buckets of individuals. You have the first set of individuals who developed COVID, they were admitted into hospital, and they got all kinds of end-organ damage. They come out, and they're not healthy, it takes them a while to recover, and sometimes they don't recover completely. But, it's not because there's something ongoing that's new, but rather, it's the end-organ damage that occurred during the acute illness.

Then you have a second set of individuals, and these are the individuals that I think we need to worry more about, and these are individuals that had probably mild infection. They never got admitted to the hospital, they recovered or almost recovered. And then they say a few days, weeks later, they develop these new symptoms and they relate it to COVID. So, the pathophysiology of that has to be really different than the first one.

Okay, how many people developed this thing? So, this is the CDC data, and what they claim is that, you just look at the entire U.S. population, about six percent of the U.S. population has long COVID. That's huge. If you look at those individuals who had COVID and now complain of long COVID, they claim that's about 11% of it [inaudible 00:52:47] currently. So, that's still a huge population.

And it affects those in the age group where you're most productive of your life. So the socio-economic consequences are huge.

If you look at the symptoms that people have, over a period of time, you'll see that the neurological symptoms seem to persist. This is the lower box [inaudible 00:53:08]. They seem to persist six months to a year later. A lot of these symptoms are anosmia, respiratory stuff. This all disappears for a period of time. So, the neurological consequences are really a major problem with the studies.

So, if you look at the second group of individuals, and they need to come up with a better term for them, but over here, I just used long COVID for them, you can divide them into four buckets.

And so, one is, they have predominantly exercise intolerance. They can take a flight of stairs and then they are totally fatigued. You have those that have cognitive problems. They may not even be aware of it until they actually go back to work or are challenged, then they are like, "Oops, I'm not thinking they way I used to." And all they can do [inaudible 00:53:56] episode of major depression, sleep disorders. And there's another group that developed dysautonomia or POTS. And that's a separate category by themselves. And then there're individuals who have pain syndromes. But there's overlap between all of them. So, you can have predominantly one or the other, but you can have multiple symptoms.

So, we wanted to study the neurological manifestations. So, what we did is we booked in cohort of 172 patients, and we had all kinds of criteria, and we ultimately studied 12. Brought them over here.

What we found was that 100% of them had cognitive difficulties and fatigue. And then they had a variety of other neurological symptoms.

Okay, what about age? So, here, this is an interesting study. What they did was, they looked at individuals who develop any kind of respiratory infection, and those who developed COVID. So, the blue line is COVID, and the red is other respiratory infections. At every age group you can see that the chances of developing COVID is much greater than with any respiratory infection except when you turn 70 years of age [inaudible 00:55:07]. Okay?

But, at that age group, it's toast. It doesn't matter what infection you get, the news is bad.

Now, these guys did some PET scanning, and they said, "Okay, let's look at the brain, and see what happens in these individuals." So, on the left-hand side is a composite scan of normal individuals. You can see where the fluorodeoxyglucose, all these bright colors, means there's increased uptake. It's a nice uptake even.

You look at the brain from a COVID patient, then you can see there's decreased uptake in the front, and in the basal ganglia. The posterior parts of the brain are [inaudible 00:55:46].

And another study looked at it a little bit more closely, and they claimed that, if you look at the bottom of the brain, actually there is more dysfunction than other parts of it.

So, what are the possibilities? The possibilities are, one, that you reactivated some kind of virus that was there. Second is that maybe you have a persistent viral infection, you never got rid of it. Or thirdly, maybe there's immune dysregulation and that's driving the pathology. Or, there could be interrelationship between all three of them.

So, here's evidence of persistent activation, or reactivation of Epstein-Barr virus. This is a study from Yale, and they looked at patient with long COVID, and you can see that the antibody [inaudible 00:56:32] to Epstein-Barr virus are greatest in these long COVID patients, and they looked at two different antigens here.

Okay. What about persistent infection? So, we thought that the infection is in the nose, and infects the nasal mucosa, so I'll just go up cribriform plate and it'll infect the brain. That's what our original thinking was. It turns out, it doesn't occur that way. In fact, if you look at the nasal mucosa, there's a lot of virus, in fact, you can see it here, in this electron micrograph. There are these black dots here, each one of them is a bio particle.

But, if you go into the olfactory bulb or cubicle, you don't find the virus at all. They rarely do find some individual who has a little bit of virus. And that's because the virus infects the sustentacular cells and not the olfactory nerve. If it infected the olfactory nerve, it would go in, no problem. But if it infects the support cells, then it sticks around, it'll cause edema, and that's why you got the loss of smell.

We looked for virus of the brain at autopsy, they didn't find it. They looked very hard with all kinds of different techniques, we couldn't do any [inaudible 00:57:38]. There are others who have reported that they can find virus, but, when they do find it in small quantities, or they find a Brian injury.

So, this is a beautiful paper from some of my colleagues here at NIH [inaudible 00:57:49], and what they found was that, yes, you can find a lot of virus in the respiratory tract, less than 14 days. But even if you go there further than a month, you can't find anything. You can look at the brain here, less than 14 days, only 32 copies compared to what they found there. And then, if you go 31 days or later, can't find that.

So, I don't think the virus itself is driving the neurological symptoms. There's got to be more to it.

Now, this is a interesting case report of two children who they claim had trans-placental spread of the virus, and you can see this massive atrophy of the brain here, and the found viral antigens here, in the brain, at autopsy. The immuno stain [inaudible 00:58:36] that's one [inaudible 00:58:40].

And this is a study by doctor Egan who is a national [inaudible 00:58:45], and what she did was, she looked at the papillae of the tongue, and she claimed that she also found some evidence of viral antigen in some of these patients, and they were 40 weeks out of the original infection. So, some of the loss of taste would be related to [inaudible 00:59:01].

And this is another group from Boston, and they find that they can find some spike protein in the serum of patients with COVID, suggesting that there is persistent antigen.

But just the presence of antigen alone doesn't necessarily mean that that's the cause of the symptoms. A number of viruses you can have persistent antigen [inaudible 00:59:23] actually do nothing. But the fact that you can find something I think one needs to explore what the consequences [inaudible 00:59:32].

So, what about immune dysregulation? I'm going to show you data on both antibodies and macrophages.

So, this is a group, and they looked at patients who had depression and cognitive problems, and they used a ligand called TSPO, which binds to activated microglial in the brain, and they find that those individuals who had these cognitive problems have decreased uptake, so they think there's more microglial cell activation.

And at autopsy, we looked at these brains, and we found the same thing. There is a lot of microglial cell activation, both in the gray matter and white matter. And this is a group from the Netherlands, again showing a great activation of the brain, using this ligand that is very similar to TSPO. It combines to activated microglial.

We looked at brains of individuals who had died of COVID. They had died acutely, however, we got them from the New York Medical Examiner's office, and these were individuals who died early in the pandemic, either at home or in the subway. They were just found dead. So, they never went to the hospital, they weren't seen,

because their respiratory symptoms are very, very mild, if any at all. So, had they not died, their symptoms would've persisted, they would've developed symptoms of long COVID I believe.

So, anyhow, we looked at their brains, because the brains were highly abnormal, and you can see this blood vessel here. This is a high-resolution MRI scan, the 7 Tesla scanner. You can see thickening of the blood vessel, a small blood clot over here, and in another cross section, you can see a little micro hemorrhage here.

So, three different types of pathology in the same blood vessel.

This is an 11 Tesla scanner, now you can see the [inaudible 01:01:21] and you can see the olfactory bulb, and the immuno stain fibrinogen, which is a very large protein, and should never enter into the brain. So all this brown staining is fibrinogen, and you can see that, around the blood vessels there's huge amounts of staining, and then peters out. Even in the olfactory bulb, there's lots of fibrinogen. So, they're leaky blood vessels, and I think that's a major problem.

We looked at substantia nigra, and you can see here a high resolution MRI scan, you can see loss of a signal here in the substantial nigra. And there is fibrinogen leakage in the substantia nigra as well.

Histologically we found a lot of macrophages in the perivascular region. That's no surprise, because if you have all these proteins leaking from the blood, you need the macrophages to remove them. But, once the macrophages get in there, they're bad actors. They don't leap. And these activated macrophages can cause a lot of damage.

Found activated astrocytes... what we did not find is T cells, okay? Very few T cells around the blood vessels, none in the brain. If this was a viral encephalitis, you would find a lot of T cells. There are no T cells. But we found a lot of activated platelets, sticking to the blood vessels. Some of them are clogging blood vessels.

And, when we quantified all the histopathology, we find that most of it has... and, at least there's more in the posterior parts of the brain, the hind parts of the brain as compared to the other parts of the brain.

We looked at the blood vessels more closely, and what we found was, there's activation of adhesion molecules. You can see that over here. We found deposition of compliment, and we found deposition of some IgG, but predominantly IgM.

So, we think that's an antibody [inaudible 01:03:10] damage to the endothelial cells, and that's what's making it leaking. But why would the antibodies attack the endothelial cells?

Okay, now, going back to the same cohort of patients that I told you earlier we brought to NIH, 100% of them, when we looked at the CSR, they had activated B cells in their CSR. And their antibodies secreted B cells here. And there were other kinds of immune abnormalities as well in these patients.

Now, this is a group from Hopkins, and they looked at antibodies to ACE2, okay? And what they found was that, in these COVID patients you could find IgM antibodies to ACE2 out several days. I mean, [inaudible 01:03:59] three weeks, but they don't switch. They should get a class switching from IgM to IgG, but it didn't occur. So the IgM persists. And that, it correlates with the findings I just showed you.

But, why ACE2, is the question? So, one possibility is that what the patients are producing are anti-idiotypic antibodies. So you have the antibody against spike protein. If you produce an antibody against that, antibody is going to mimic the spike protein, and bind to ACE2 receptor.

But, if you look at the vasculature in the brain, you can see the blood vessels everywhere, right? So, if you damage the blood vessels, it can attack almost any part of the brain, at any [inaudible 01:04:44].

So, we looked at the neurons in the brain, and you can see here, in the cerebellum, there's loss of stria, and that's because there's loss of Purkinje cells. And so, there is neural damage that is occurring because of this.

We were very interested in looking at the brainstem, and particularly I was interested in the pre-Botzinger complex, because, remember, these patients had died in their sleep, and their hearts, there was no pathology there, so the question is, could they have died from a central cause? And the Botzinger nucleus complex controls respiration.

And we found that there was a profect neurons, activated microglia, and there's a phenomena called neuronophagia, and that's what's killing these [inaudible 01:05:26]. So, I suspect that they died from central hypoventilation, which is also called Ondine's Curse, okay. It's a nice fairy tale, but I think you should look up. I'm not going to tell you the entire story.

Now, there are some interesting other abnormalities that people have described. This is an interesting paper, and what they claim is that, it all has to do with tryptophan uptake. And the tryptophan uptake from the gut is impaired, so you're not getting serotonin, and that's the reason for it. I mean, it's an interesting observation, and

they've done a lot of work, but I doubt that one single metabolizer is going to explain the whole complicated disease syndrome. And patients who are on SSR-... a lot of these patients are on SSRI, and it doesn't really too much for them.

Now, this is a paper from Yale that just got published, and they claim that the cortisol levels are decreased in patients who had COVID. And it's the same one that also showed [inaudible 01:06:31] antibodies.

Okay, what about acceleration on neurodegenerative disease? So, the UK Biobank looked at these individuals, and they showed that there's atrophy of the brain in those individuals who got COVID, compared to their counterparts who did not develop COVID. And, if you look at blood markers, you will see that neurofilament levels are increased, and amyloid levels are decreased, which coincides as biomarkers for Alzheimer's disease.

And some patients developed POTS or dysautonomia. So, when they stand up, their blood pressure can go down, and heart rate can go up, and then multiple other types of dysautonomia that can occur in this population. And some can show up as small fiber neuropathy as well.

And then the variety of pain syndromes, headache being a common one. Quite common. And the causes are sometimes difficult to figure out.

So, the potential for loss of symptomatic treatment that one can do. You can intervene. There's a number of things you can do to help patients. And certainly, there are a lot of different immunomodulatory bloods that can potentially be used, depending on what you find in these patients that is dysregulated, and we have an ongoing [inaudible 01:07:48].

Another thing you can do is prevent long COVID. So, vaccinations do help. If you get one vaccine, and in this meta analysis you can see, going to the left, doesn't do much. You get two dosages, you start going to the left. Three, it really favors it. And I have had five.

All right. So- what we're doing here is-

Claire Quiner:

... I apologize, doctor Nath. If you're able to wrap up in a minute or so, that would be great-

Dr. Avindra Nath:

Okay.

Claire Quiner:

... so we can get on to the Q&A. Oh, wonderful. Thank you.

Dr. Avindra Nath:

So, and I will conclude by saying that, direct invasion of the brain by SARS-CoV-2 is rare, and may not explain the neurological complications.

Neuroimmune dysfunction is driven by innate immunity, immune exhaustion and antibody mediated phenomenon.

And clinical trials with immunotherapy should be considered in these patient.

These are all my acknowledgements. I'll stop here.

Dr. Sudha Seshadri:

Thank you, doctor Blish, doctor Rosen, and doctor Nath, for these wonderful descriptions of the ongoing work, the ways in which RECOVER is highlighting this.

It is clear from your discussions that one of the challenges we face is that both the definition of long COVID itself, as well as components of long COVID, like brain fog, are largely patient driven, as is appropriate in many ways, but something that where we are describing it as we go along, trying to understand it.

It is clear that there are responses to this infection in the form of activation of T cells, changes in antibody responses, changes in hormonal responses, as well as metabolomic changes that appear to play a role in, or, at least, are associated, whether they are causal or not is not clear, but they are associated with the presence of symptomatic long COVID.

There've been a number of very interesting questions, and one of them from a sufferer is, what is the average duration of the symptoms? And is there for people who have persistent symptoms as long as a year, does then the subsequent expected duration, what would that be? And I know this is an area where the pandemic itself is only about three years old now, so we may not have much by way of answers, but I would just like to pose it to each of you, as to what in your judgment is the course of the long COVID and resolution of the biology, the pathobiology, as well as the symptoms?

Doctor Blish, do you want to take a...

Dr. Catherine Blish:

Sure. And, Catherine's fine. I mean, that's... I think, intrinsically and unanswerable question, because I don't... I mean, my hope is that this is what RECOVER, as a consortium, is going to be able to do. In trying to come up with a consistent even definition, or a consistent description or a consistent biomarker. I mean, it's been very, very difficult. And I think many of us believe that is because there is heterogeneity.

Long COVID isn't one thing. And, I mean, both doctors Rosen and Nath alluded to this. Doctor Nath had his four kinds. Maybe there are four kinds, maybe there are eight kinds, maybe there are ten kinds. And I think that what is likely is that the underlying pathophysiology, driving each of those, is going to be slightly different, and the resolution therein is going to be different.

I mean, in our data, we do note that altered taste and smell and cough actually resolved, almost universally. But, brain fog seem to actually be getting worse. So, if you lost your smell, hooray. If you have brain fog, we don't know what the natural history of that is going to be. And that's where we're going to need these numbers, and these people looking across different cohorts, with different BMIs, different ethnicity, different other risk factors, to try to figure out, are there unifying groups that we can find, where we can say, really the air of personalized medicine, for this subset, that might help resolution, and for this subset...

But, I just don't think that we have a good enough grasp. Or, at lest, I don't feel like I have a good enough grasp, to say respiratory COVID is caused by X, and it's going to get better in three months. I don't... I mean, I would give my eye teeth to find that, but at least, in our data, and in my re-analysis of other people's data, I can't find a unified definitely.

Dr. Sudha Seshadri:

Just one follow-up, there have been a number of questions asking about, say, pediatric age groups, young adult age groups. Doctor Nath showed that, for instance, if you're over 70, perhaps the risk is greater. Have you seen differences in these immune responses across the lifespan? Would we design different kinds of trials for different age groups or age cohorts, of people with long COVID?

Dr. Catherine Blish:

I mean, I think that's a great question, and I think there was even one in the chat about differences in sex. And the problem with that is, our cohort is 100 people, and we don't have every analysis on every person. And by the time we get into our subgroups, we're not seeing many differences by sex or by age. But, the finer we dice that, the more power we lose to find significant differences.

So, we haven't seen a dramatic difference in our cohort, but we haven't looked that hard because we're under powered.

Dr. Sudha Seshadri:

Thank you. Doctor Rosen, I think one of the questions that you alluded to as well is that, some of the people with hormonal metabolomic changes, like low HDL, insulin resistance, Type 2 diabetes, are at a higher risk anyway of COVID, and of long COVID. And so, the question was whether this is pre-existing, whether this may be unmasking syndromes in people with a propensity, or whether this was driven by a reaction to either a persistent viral infection, a reservoir, like fat, we know it's not persistent in the brain.

So, the Lancet article, with Aly in the VA seemed to suggest that, if you have a persons that cases and controls, pre-infection seem to be fairly similar. So, I just welcome your thoughts on how much you think this is a reaction to the virus, which is resulting in some metabolomic changes, whether it's direct or the immune-mediated changes. And I know you talked about this, but there have been questions in the chat about it as well.

Dr. Clifford Rosen:

Yeah. So, that's a really difficult question, and we don't know the answer. Certainly, when you compare those non-infected versus those that are infected, their increase in incident diabetes in long COVID patients is higher.

The question really becomes whether or not that is exacerbated by body or host metabolism? That is obesity, which predisposes individuals to a more chronic inflammatory type of adipose tissue, which could then lead to either viral persistence or more chronic, inflammatory markers. And we don't know yet, because we need longitudinal data. We need individuals who... and this is where RECOVER comes in so much, because it not only tells us more about the long-term duration of the disease, in answer to your first question, but it also has a sub-

cohort of individuals who were not infected, and then got infected during the last couple of epics. And those individuals are probably the most valuable group of individuals because they're non infected, they're being followed, they get infected, and then they have a post COVID symptomatology.

I do want to emphasize that, some people do get better, and it's really important. Although, we don't know why they get better, if this is the natural history of long COVID, or if their taking agents offline, through the internet, through any friends, colleagues, that may be ameliorating their symptomatology. A lot of my people are starting to take metformin based on the randomized control trial.

Do these interventions or Vitamin D or any of these, [inaudible 01:17:52] are being tested by individual patients. Whether that helps we don't know, and we need a registry to really help us understand what those agents are, and if they're any better.

RECOVER's the first step, and it really is extremely valuable, because I think we're still early, and we still need that natural history. And finally, it's heterogeneous, so everybody's going to be a little different. And the host response is going to be very, very important to this virus.

Dr. Sudha Seshadri:

And, the longitudinal study of all of these changes as well. I think there was one question about, if I had a bad long COVID response, but have recovered, and I'm infected again, am I more likely to have long COVID? And I don't know if you have an answer for that?

Dr. Clifford Rosen:

Yeah. Well, there's... multiple infections lead to a greater risk of long COVID. But whether you're fully recovered, and then you get another infection, whether that predispose... I don't think anybody knows.

Dr. Sudha Seshadri:

Doctor Nath, you had clearly discussed many immune related ways in which the changes in the brain might be explained. Do you think the hormonal and metabolomic changes that doctor Rosen was speaking about might have a role as well? Is there a way... many of the autopsy information we have right now is clearly from more of an acute or subacute setting. So, how relevant may these different streams be to the brain fog of long COVID?

Dr. Avindra Nath:

As I'm pretty sure hormonal and metabolic abnormalities will be found in this patient population, and there's some work doctor Iwasaki from Yale presented and the Keystone meeting, and some of it is included in our paper that just came out in Nature, but not all of it.

And there she did show both types of abnormalities. She showed that cortisol levels were decreased. And if you look at women, their testosterone levels were decreased. And she's claiming that some of those things probably make a difference.

And another parallel is, ME/CFS, this chronic fatigue syndrome, where people have looked at these kinds of things, [inaudible 01:20:27] again. You can find all kinds of metabolic abnormalities there too. She looked at low metabolomics of the CSR, or blood. So, I think there are a lot of parallels there, and you'll find all these things. You look for them, you'll find them.

Dr. Sudha Seshadri:

There have been a lot of questions about subjectivity versus objectivity, and whether there could be serological biomarkers. You had shown some data on NFL, and we know that NFL crosses the blood-brain barrier, and circulating levels do reflect what's in the CSF. What are your thoughts on that as a marker of... acute injury, yes, we have this information. What about persistent neurological impacts from post-COVID.

Dr. Avindra Nath:

Yes, I mean, this is an area that's very near and dear to you, I know. And you know that better than anybody, that, now, you have NFL in a number of things you can measure, and there are a lot of literature now on these things. They are not showing that in progressive neurological disease, no matter what the cause is. If there's ongoing neural damage, neurofilament levels, light, heavy [inaudible 01:21:47], NTFAB, these things are becoming really good biomarkers.

They don't quite tell you what the underlying disease is, but at least it tells you there's progressive disease.

And now this test has become so sensitive that you can measure them in serum. You don't need spinal taps on all of them. So I think you're absolutely right, these patients should be monitored, and the presence of that indicates ongoing [inaudible 01:22:12].

Dr. Sudha Seshadri:

There's another question, I'll let any of you answer that, about studies underway, looking at persistent or other decline mitochondrial function, as an explanation. We could also decide that some of these are for a subsequent R3 seminar because these are whole areas that we could explore. But in case any of you want to take that?

Dr. Clifford Rosen:

Well, there was that paper...

Dr. Sudha Seshadri:

Yes.

Dr. Clifford Rosen:

That I illustrated that suggested that mitochondrial genes were down-regulated. And I think there's a bit of evidence that was shown at Keystone, from a group in the Netherlands, that showed decreased mitochondrial function in muscle tissue, from muscle biopsies of long COVID people.

But-

Dr. Avindra Nath:

No, it's a tricky thing to study, right? Because if you get muscle deconditioning, and you're not moving around, you're not doing anything, your muscle's going to show a decreased metabolic function, and the mitochondrial and everything. But that just could be all deconditioning.

One has to be careful how to interpret mitochondria. We did a lot of mitochondrial DNA sequencing, gene sequencing, out of the muscle and chronic fatigue patients, [inaudible 01:23:26].

But if you do the seahorse experiment, you'll find abnormalities, but if you look at it, it's all deconditioning.

Dr. Sudha Seshadri:

Thank you. Those are clearly questions still to be answered.

There was a lot of interest also on ongoing treatment trial information, and what the panel might think the most promising interventions to prioritize in studies, focusing here, I guess, on brain immune and metabolomic or metabolic dysfunction.

There were, for instance, suggestions about transcranial direct current stimulation for brain fog, about LDN and [inaudible 01:24:16]. So, I just wondered if one of you wanted to address...

As far as I know, these are not ongoing trials. I didn't fine anything on clinicaltrials.gov, but if perhaps the panel is aware of efforts to set up something, or what they would think would be most promising interventions?

Yes. Go ahead.

Dr. Clifford Rosen:

Yeah, I was just going to say, the metformin study's really interesting. The previously published paper, a number of my people have been put on the metformin now as one way. It may actually improve mitochondrial function as well as all the other things that metformin does.

And I'll only say that, providers are desperate for interventions. So, it's really important that these clinical trials be done, and done well.

Dr. Sudha Seshadri:

What about-

Claire Quiner:

I'm sorry to interrupt. We just have about two minutes left. Would it be okay if I shared the closing remarks?

Dr. Sudha Seshadri:

Right.

Claire Quiner:

Great. So, thank you so much to all of our presenters today, and thank you for our audience for attending the seminar, and for engaging with the Q&A. We recognize we didn't have the time specifically for some of those questions.

So, as a reminder, both the recording of today's seminar will be available on recovercovid.org within a few weeks. And we'll also be posting a Q&A document that has responses to the questions that we received today, including the ones we didn't have time to address.

We have some upcoming seminars, although I believe we don't yet have a defined date, but we're working on some additional ones for the future, and we hope you return.

Finally, you'll see a short survey come up on your screen, asking for your feedback on this seminar. We'd appreciate if you could take a minute just to fill out this brief survey.

With that, thank you all, and have a great day.