

Transcript

Patricia Ceger

Good afternoon and welcome to the RECOVER Research Review or R3 seminar. My name is Patricia Ceger and I am a toxicologist with the RECOVER Administrative Coordinating Center, and I am the moderator of today's seminar. The goal of this seminar series is to catalyze a shared understanding of the research within the RECOVER consortium. I want to start by thanking everyone who submitted questions in advance. Please submit any questions that arise during today's presentation using the Q and A feature in Zoom. After the presentations, we will answer as many questions as possible. A Q and A document will be posted with the recording of the seminar on recovercovid.org. It will include the answers for submitted questions relevant to today's presentation. Questions about other scientific topics will be addressed in future seminars and answers to broader questions about RECOVER will be available in the FAQs at recovercovid.org. As a reminder, we cannot answer individual questions about clinical care. Next slide, please.

Next next slide. Thank you. Our presenters today are Dr. Timothy Henrich, Dr. Andrea Cox, and Dr. Akiko Iwasaki. They are joined by discussant Paul. J. Utz. Our first presenter today is Dr. Timothy Henrich, who is an associate professor of medicine at the University of California San Francisco School of Medicine. Dr. Henrich's research group is involved in the design and implementation of novel, nano and micro technologies and positron emission tomography-based imaging approaches to characterize viral reservoirs. He will be presenting on the immunopathology of PASC. Our second presenter is Dr. Andrea Cox, who is a professor of medicine and oncology at the Johns Hopkins University School of Medicine. Her laboratory investigates human immune response to SARS-CoV-2, including mechanisms through which SARS infections stimulate and it will aid immune response.

She is also the co-director of the Johns Hopkins SARS-CoV-2 Pathogenesis and Immunity Center with Dr. Sabra Klein. Dr. Cox will be presenting on clinical and immunological correlates of long COVID. Our final presenter is Dr. Akiko Iwasaki. Dr. Iwasaki is a sterling professor of immunobiology at the Yale University School of Medicine. Professor Iwasaki has been a leading scientific voice during the COVID-19 pandemic and is at the forefront of several long COVID investigations, including the Mount Sinai, Yale Long COVID study, Yale Listen study, and Yale Paxlovid trial. She will be presenting on the immunology of long COVID. Our discussant is Dr. Utz, who will help link the presentations together and lead off the discussion with our panelists.

Dr. Utz is a physician scientist and professor of medicine at Stanford University where he works towards characterizing immune responses to vaccines, infection with pulmonary microbes such as SARS-CoV-2 and long COVID. Professor Utz is co-PI of Stanford's RECOVER site, has previously served as vice chair of the Recovery Immunology and Hematopathology Committee and most recently joined the RECOVER Observational Cohort Steering Committee. Next slide please. The topic of today's seminar is understanding the role of the immune system in PASC. 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 will hand it over to Dr. Utz.

Dr. Paul. J. Utz: Thank you, Patricia, for that great introduction. The genesis of this specific seminar group really comes back to the Keystone Symposium that was held in August at Santa Fe on the topic of long COVID. And I can tell you it was one of the best symposia that I've attended in the last decade. Fantastic science. The keynote was delivered by Dr. Gary Gibbons, who's the director of NHLBI, and he really linked together what we heard throughout that three-day meeting. All three of our speakers today participated in that meeting actively, not just in giving great talks, but in integrating their work with other investigators who were attending the meeting. One of the central things that we talked about at the meeting was the multiple different mechanisms that could be involved in long COVID.

Some examples include persistent tissue damage, things like activation of the endothelium, which is the lining of the blood vessels that is persistent and could lead to cardiovascular and neurologic complications, reactivation of viruses such as Epstein-Barr virus and other herpes viruses, alterations in the endocrine system and also in metabolism. Finally, the microbiome was very actively discussed as well as a potential contributor to long COVID. The overarching theme today is to explore the role of the immune system in two of the most prominent hypotheses. One is persistent aberrant activation of the immune system, including both the innate and the adaptive arms of the immune system. And the second is viral persistence. Is there a reservoir for SARS-CoV-2, and if so, what triggers its reactivation and where does the immune system go wrong in that process? So let me stop there and turn it back over to Patricia for our next speaker.

Patricia Ceger:

Thank you. Next, we'll be hearing from Dr. Henrich.

Dr. Timothy Henrich:

Well, thank you again for the opportunity to talk at the R3 seminar series. This is a fantastic seminar series. I've always enjoyed the cutting-edge science that's been presented. And I think really as Dr. Utz was saying, really highlight some of the ongoing work and series that are now starting to bear fruit in terms of understanding the root causes of long COVID and post-acute sequelae of COVID-19. And also as Dr. Utz mentioned in his introduction, following acute infection and post-acute illness, many people go on to develop long COVID. This can be various different clinical phenotypes. But there has been several mechanistic hypotheses that are, I think, now starting to be understood. And actually all of these may play a role, at least in some individuals in ongoing post-acute sequelae. So for example, local tissue inflammation or immune cell infiltration following acute infection or potentially with persistent viral material in tissues, not necessarily replication, but viral RNA proteins, et cetera.

Could be human herpes virus such as EBV or CMV reactivation, pathogenic B-cell responses or autoreactive autoimmune response that dysregulated immunity and then microvascular thrombosis, fibrin clots, and potential endothelialitis as well. And so these are all, I think, now understood to play at least a partial role in many people that have ongoing symptoms. I just want to highlight quickly from our co-director of the RECOVER site here at UCSF, Dr. Michael Peluso, who will be speaking on this in a future R3 seminar series coming soon. Looking at a study along with David Walt and Jeff Martin that SARS-CoV-2 antigen whether a nucleocapsid or spike antigens can persist for a long time in the circulation following acute infection. This is a study looking at mostly non-hospitalized people that develop long COVID. You can see that three to six, 10 to 12 and even a year or so after infection, a not insignificant number of people will have circulating spike and or nucleocapsid proteins in the blood.

And this seems to correlate with certain phenotypic long COVID symptoms. But I'll let Dr. Peluso talk about that at a future R3 seminar. Just to show you the data that I'm going to present today is really through our LIINC program as you can see here with the LIINC principal investigators. And more specifically the pathogenesis long COVID team here and members of my laboratory which are shown, who have done all the work and produced the data that I'll be talking about today. So I want to mention first that I think as many of the scientists on the call today, as well as many others have shown now that there's persistent inflammation and immune dysregulation in long COVID. And this can happen months, but potentially years after initial infection. And so this is data from our group just recently on a medRxiv and now pending publication. But showing looking at circulating proteins in the blood that even eight months following initial infection.

So people in pink versus blue, pink being long COVID versus blue, non-long COVID in this heat map showing that there are different proteins that are expressed in people even eight months later that have experienced various long COVID symptoms. For example, olfaction or gene or gene products response to infection, humoral response or heme synthesis/regulation, et cetera. And what we also have seen as well is that looking at adaptive immune responses, we see that there's an increased amount of central memory CD4 T cells that usually develop in the response to antigen and long-lived response to viral infections and other infections. Follicular CD4 T cells which reside in tissue and B cell follicles and lymphoid tissue and likely coordinate responses to infection. But also seeing that CD4 T cells that are SARS-CoV-2 specific, so recognize SARS-CoV-2 antigen, tend to have more tissue homing receptors.

So these seem to be going to tissues, potentially sites of ongoing infection. And then lastly, looking at exhaustion or immune checkpoint in CD4 T cells, effector cells, again that are SARS-CoV-2 specific seem to be more exhausted over time. And all of this information, I think, together suggests that there may be ongoing viral replication that is driving immune dysregulation and inflammation. But I also want to mention that long COVID in PASC is a tissue-based disease. This is not a circulating blood disorder, this is a tissue-based disease. We know that for example, the cardiovascular system, the brain, the autonomic nervous system, spleen, liver, gastrointestinal tract, kidneys, pancreas, lungs, all of these may be involved and may be sources or potential sites of inflammation or other immune dysregulation in the setting of PASC. So today I'm going to talk specifically using some of our novel techniques to look at immune responses and viral persistence throughout tissue through the entire body, nuclear imaging.

And the hypothesis here is that again, SARS-CoV-2 may persist in tissues for extended periods of time in some individuals. This may lead to immune and inflammatory sequelae, but this again is tissue focused. That if you're going to have sources of ongoing inflammation, this is likely to happen in deeper tissues in the body

that we just don't normally or typically sample. Most studies have been limited again to the peripheral blood for this exact reason. Tissues is hard to get, it's expensive, it's uncomfortable for participants, it carries a certain amount of morbidity. So I think novel methods to identify abnormal immune responses and viral protein persistence across the whole body are urgently needed. So the first data I will show you today is using non-invasive nuclear imaging actually may play an important role in the identification characterization of long COVID.

So for those of you who are not aware, we have been using a technique called positron emission tomography. This is essentially a three-dimensional scan of the body that we do with either a CT or an MRI scan to look at three-dimensional tissue and anatomical location. And what we do is we can inject a tracer into the bloodstream which circulates through the body and gets trapped in its target. And what we're using is a molecule called F-AraG. And what this does is this is one of the analog that actually gets phosphorylated and trapped inside activated T-cells. So this is a marker that we inject into the vein, we let circulate for about 45 to 50 minutes and this molecule is selectively taken up by activated proliferating T-cells. And of course T-cells are an important part of our antiviral immune response.

It's quite sensitive, it's very specific to T-cells as well, much less uptake in B cells or other immune cells throughout the body. So we use this F-AraG PET CT imaging in long COVID specific marker again of activated T-cell falling antigen exposure. 24 participants for our initial study, each with prior documented COVID-19 and we imaged folks 27 to 900 days, up to two and a half years out from initial infection. We follow these folks very quickly. We make sure that there is no reinfection at least clinically or by antibody or other PCR data in our prospective cohort and actually, participants at up to 15 individual long COVID symptoms, about a median of six symptoms. So these folks are relatively sick with a lot of number of symptoms that actually also have interruptions to activities of daily life as well. We also had six people that reported no symptoms, so had rapid recovery after initial infection.

We also included, and I think this is really important, pre-COVID control participants. So these are people that were imaged for other studies that we were doing before the COVID-19 pandemic to understand how things are different post COVID and not just in the setting of long COVID itself. Again, this is a very busy slide, but just to give you an idea, we imaged folks that were infected. A vast majority were not hospitalized during initial infection. And it was a mix of folks infected during pre-Omicron and into the post Omicron era. Actually, many of our participants lost IgG, nucleocapsid SARS-CoV-2 nucleocapsid antibody detection, suggesting there wasn't recent reinfection in addition to our clinical questionnaires. And we were able to do gut biopsies and five of our participants for this initial study that had long COVID symptoms. I just want to show you, this is now three-dimensional images of the PET tracer.

This is the F-AraG uptake. Look again, T-cell activation over time. And you can see on the left this is a pre-COVID control participant as I spin around. And what you're seeing is just background uptake, so the liver here, some of the glandular tissues, the thyroid gland, the kidneys and also excreted through the bile. So this is background uptake. This is just normal tracer accumulation that we see in people before COVID-19 pandemic. Now here about two months after infection, someone with ongoing symptoms in the middle. I just want to take a look. As you can see that we're now seeing a lot more uptake in areas such as the bone marrow, in the nasopharyngeal tissues, probably a little bit more in the glandular tissues and lymphoid tissues in the wall of the gut and then even 663 days after initial infection. So we're now over a year out, there we go. We are now seeing that we also see in some individuals that there's persistence of this T-cell activation marker in areas of tissue that we saw more closer to early or later post convalescence.

Again, just to show you again, here is our pre-COVID control participant. Here is someone that was imaged with symptoms 63 days after infection. Again, notice all of this uptake that's different and then here as well in someone 663 days after imaging. So we're seeing this increases inactivated T cells and tissues many, many, many months after initial infection. So to put this a little bit more quantitatively, when we look at in the red bars here, these are people that are post COVID regardless of long COVID symptomology versus those image before COVID-19 using the same tracer and the same techniques. And you can see that overall, many different tissues including the spinal cord, the pons, which is a part of the brainstem. And we also see in, for example in some of the aortic arch or pulmonary artery wall and also potentially in the lower lung fields.

But also in the rectal of the colon wall, nasal turbinates, pharyngeal tonsils and a little bit less, but also in the parotid and submandibular glands, we're actually seeing increases in T-cell activation in these tissues across our entire cohort post COVID compared to those that were imaged before March of 2020 or

February of 2020. Just to show you what these images look like when we look at the PET data, which is in this heat map, so the redder or yellower and whiter it is the hotter it is, the more tracer uptake we see. We can just give an example of some of 246 days post COVID compared to one of our newer controls here showing that there is again, uptake in the nasopharyngeal space, some of the glandular tissues. But also you can see in the lung, parenchyma, hilar lymph node, these are the lymph nodes that drain the lungs, et cetera. So we're seeing increases in activated T-cells in these areas.

Well, the time for infection does impact tracer uptake. So if you look at people for example, less than three months, so without an image before kind of a technical diagnosis by WHO of long COVID versus those that were imaged later in time and there does seem to be a little bit of drop off in signal in some of the tissues. For example in the spinal cord also in potentially the vasculature in the rectal wall. But even with that, folks that were imaged later in time still had significantly higher or higher uptake in certain tissues compared to people imaged before COVID-19 pandemic. We actually saw a very, very modest association between post vaccine and uptake. So vaccination did not seem to influence the uptake of this tracer as much as natural infection from COVID. Well we also were very interested in learning about how uptake in these various tissues correlate with long COVID symptoms. I think this is the main part of the study and so we look at folks that have symptoms in the orange versus those that had full recovery in the lighter tan versus again our pre-COVID controls in gray.

You can see that there may be some increased uptake, for example in the spinal cord in people that had symptoms versus when we compare that to the pre-COVID controls versus comparing the ones without symptoms to pre-COVID controls. Perhaps we see this also in some of the vasculature and in the colon and rectal wall as well. Interesting enough, pulmonary symptoms, so people that had pulmonary symptoms on the questionnaire at any time when they were surveyed or imaged, so this is contemporary with imaging. We see that there is a higher amount of uptake in the right hilum, this is the hilar lymph nodes, again, that drain the lung in the lower lung field, the [inaudible 00:20:28] itself. And so we're seeing that there is some lung uptake that correlates with symptoms in some of these patients. But interestingly enough, other symptoms like neurocognitive symptoms don't seem to correlate with central nervous system or spinal cord uptake of tracer, and I think that be for many reasons. First of all that the phenotypes of clinical long COVID can be very variable. They can come and go. It can be difficult to necessarily classify folks.

And also, that symptoms may be driven by inflammation in other tissues or other organs. So going forward, this is something that we're going to parse out with a much larger cohort to understand more fully the relationship between symptoms and inflammation and T-cell uptake in tissues. Well we also, if we use proteomics again to look at T-cell activation in some tissues is actually associated with systemic inflammation. So for example, here's high lung uptake. So if people had high lung uptake on the PET imaging scans that in pink here that they tended to have a higher levels of inflammatory circulating proteins. But other tissues did not correlate. So in other words, if we look at other tissues that we may not see an increase in circulating inflammatory proteins, even though we see T-cell activation or we see this tracer uptake in various tissues in certain systems. So sometimes it correlates with systemic inflammation and sometimes it does not. Again, this is why looking at directly at tissue is going to be absolutely key.

Well, next we looked at the five individuals that we biopsied for this preliminary study all having long COVID symptoms up to 676 days after initial infection without known reinfection. And as what we can see here in green, these are stains of the gut, so colorectal sampling. So we did sigmoidoscopies, collected tissue from pre-COVID control participants and also as well as those in our imaging study. And you can see that in our post COVID gut samples anywhere here showing 158 days to 676 days after initial infection, we see that there is SARS-CoV-2 spike RNA resident in the lamina propria. This is an area that is often macrophage and other immune cell rich and tends to actually have low expression of ACE2, yet we're seeing intracellular and cell associated SARS-CoV-2 spike RNA in all five of the participants with symptoms that we have biopsied. And so this is now ongoing. We are obviously trying to increase this and looking at digital spatial omics and host responses in C2 and how this may correlate with some of the imaging findings that we see using our noninvasive PET program.

So the last thing that we are now currently doing is we want to directly characterize viral protein persistence using PET imaging or noninvasive imaging techniques. And essentially what we are now doing, and we've just completed the preclinical work for this study, we've actually labeled two spike specific GMP quality SARS-CoV-2 monoclonal antibodies that are specific to the spike protein using a Zirconium-89 tag. And this is a metal that's radioactive with a half-life of about three days. So we can inject these monoclonal antibodies, we wait

for two to three days for it to go into tissue, get trapped or get stuck if it's bound to its spike receptor. And then we can image the same way that we look at F-AraG. We can actually detect throughout the entire body where SARS-CoV-2 proteins may be persisting. And so we're very close now to getting this into people.

We've done this for HIV protein persistence in the past successfully. And I think it should be very interesting because not only can we look throughout three dimensions, but we can look in tissues that we just can't biopsy in living people. So just some summary and also to set up some next steps for the other speakers. I think that inflammation and dysregulated immunity persists up potentially for years following SARS-CoV-2 infection. But long COVID is really a tissue-based illness and we need to do deep dives into this tissue and not just look at circulating markers, which are very helpful. Don't get me wrong, I think those are extremely important studies. But really looking directly at the source tissue of what's driving, over time, ongoing dysfunction is going to be absolutely critical. And I think that noninvasive imaging like we've been doing is certainly a way going forward.

We've also been implementing noninvasive imaging in interventional studies to RECOVER, again our clinical lead on these studies with Michael Peluso, who will be talking in a few weeks. But we actually are using these imaging techniques before and after long COVID interventions, for example, antiviral, anti-inflammatories, and we can actually look at whole body dynamics and how things change in addition to collecting detailed information about clinical symptoms, et cetera. And then my final concluding thoughts are that we are now in a new post COVID immune era. And I think that we've actually entered a new immune and inflammatory setpoint that there's a landscape now that is vastly different than it was before here in the US before March of 2020. And I think we need to account for this, the fact that we're seeing persistent antigen, the fact that we're seeing persistent T-cell dysfunction and dysregulation and that others are finding inflammation, metabolic dysfunction, autonomic dysfunction, et cetera, years after initial infection, really suggests that when we do these studies we have to take into account this new set point.

And obviously COVID-19 may influence outcomes and studies of other illnesses. For example, when we study chronic HIV-1 infection or other chronic viral infections, we're going to have to take this into account when we look at immune responses in the contemporary cohorts. And hopefully I'm wrong on this, I really hope so, but there may be longer term impact on human health if we do have viral persistence, if we do have immune dysregulation for a long period of time. Clearly this could potentially lead to longer term health impacts that we won't know until years down the road. And again, I hope I'm wrong on this one, but I think we need to keep a lookout and be very cognizant that this is a possibility and needs future study. With that, I just want to thank everybody here at UCSF, but also through our LIINC cohort and many, many of our internal external collaborators that have made all of this work possible.

Patricia Ceger:

Thank you Dr. Henrich. Our next speaker will be Dr. Andrea Cox.

Dr. Andrea Cox:

Thank you very much for the opportunity to present this fantastic seminar focused on what is increasingly really a problematic aspect of COVID, which is long COVID. I'm going to focus on some of the clinical and immunologic correlates of long COVID that our group has identified. So this really stems from the OutSMART cohort which was created by Yukari Manabe and Annie Antar to study individuals who were diagnosed in the acute phase of COVID, but not sick enough to be hospitalized and then followed with the sampling that you can see here over time. And this was basically people were consented very quickly after being diagnosed and they were enrolled between April of 2020 and January of 2022. And consistent with the Johns Hopkins location, which is where these participants were consented, the cohort was quite diverse as you can see in the bottom with nearly equal representation of Caucasians and other racial and ethnic groups.

So this really reveals that symptoms persist relatively commonly following non-severe COVID-19 because again, none of these individuals were hospitalized and that as other studies have shown these symptoms persist longer with this axis showing a return to usual health and the percentage of individuals who report returning to health over time is higher in men than in women. And so while there's resolution over time and a subset of individuals, that tends to occur more commonly in men than women. So this is a rather overwhelming slide and it's not meant to be shown so that people can focus on fine details. But just I'll note here that on the axis you can see a variety of symptoms plaguing individuals following COVID infection. But the primary point is that most of these actually grow more significant, oh, excuse me, more significant over time. So people complain of these more actually at month six than at month one following infection.

And want to give a shout-out to the Research Collaborative for Long COVID because this was very helpful for us, particularly Hannah Davis. This is a patient-led consortium helping us to know what are the tools that can help us to best assess quality of life and the variety of symptoms seen in people who are actually suffering from long COVID. So of the 70 participants who again, had non-severe COVID initially and were followed for 24 months, we were able to, this is really again Annie Antar and Yuka Manabe, validate tools to assess quality of life and symptoms and diverse patient outcomes, cohorts rather, which are very important I think for understanding long COVID. And we defined long COVID in the study as not returning to pre-COVID health status and having at least one symptom for more than 90 days after infection. And long COVID occurred in 33% over the 24 months following infection. So a third of the cohort described this, and this was more common in people who reported pre-COVID-19 obesity and hyperlipidemia.

We did not find high sensitivity CRP or C-reactive protein, which is a measure of inflammation to be associated. Many of these more common symptoms as I pointed out, peak at six to 12 months after infection. And showing this graphically again, the most common symptom reported in red here is fatigue. But you can see that most of these symptoms really do insomnia, headache, fatigue being the most common increase over time from month one to month six in this cohort. And there's a subset of people who actually not a decrease between month six and 12, but unfortunately not all of them. So is this associated as was previously mentioned and hypothesized with persistence of virus? Well, I agree with Dr. Henrich that it's helpful to sample tissues other than those most readily accessible to us, oral fluid shown in the solid darker purple color, hair, nasal, and oropharyngeal samples. And these were basically taken over time with the PCR measurements and culture to determine if the virus was infectious.

And you can see the red dots basically indicate the time points at which virus was culture positive, meaning infectious virus was detectable. And that's actually a relatively short window after infection. But we do see that that is followed not far behind the nasal positive tests and/or pharyngeal positive tests for virus by RT-PCR, so detection of genomic material. In contrast to antibodies shown in oral fluid sampling here in the darker purple line as I mentioned, those antibodies increase over time in individuals following exposure to SARS-CoV-2. So we do not find evidence, at least in the nasal passages or oral pharyngeal space, of persistent viral antigen and certainly an even shorter course of culture positive virus. I want to say that our group has been working on mouse models, that includes Jennifer Liu and Sabra Klein. They have shown sex differences that mimic what we see in people, with more severe disease in the acute phase in male mice, but upon resolution of the acute phase of disease, persistence of symptoms in female mice.

And they've done an extensive characterization of the tissue to look for a virus in various mouse tissues. And will hopefully present those data at a later meeting. So we did find however, that there is an association between long COVID brain fog and muscle pain at least three months and a persistence of virus in the respiratory tract. So while a virus does disappear within several months, the longer the virus is detectable, the more that you see the subset of symptoms, brain fog and muscle pain associated with long COVID. So we basically looked at this cohort and said, what are the symptoms that are most commonly associated? And again, focusing on those that we see most commonly in the cohort, which are shown on the right-hand side, we then asked are there any immunologic correlates? We did note that some of these symptoms grouped together.

So using a technique called latent class analysis, we asked in people with low symptoms of long COVID and high, which of these symptoms were clustering together? So not only did we assess the most common symptoms, but which were clustering together and found that the top symptoms cluster in three main groups, anxiety, headache and weakness, body aches and joint pain segregate together and brain fog, memory problems and insomnia. So we then asked what immunologic signals correlate with these symptom clusters, looking at a variety of things that I'm showing here. But I'm just primarily going to focus on the cytokine network and the cellular networks today, this bottom right. So this is really work done by a phenomenal postdoc, now junior faculty member at Johns Hopkins' Elizabeth Thompson, who basically did extensive immunologic analysis combined with markers of immune cell metabolic activity or metabolic programming. And we included a T-cell panel as well as a myeloid and B-cell panel and looked at these markers of metabolic function in those cells as well.

And just to say that this method actually allowed us to subset cells into the groups that are shown here, CD4 T cells, CD8s, T regulatory cells, B cells, NK cells, myeloid cells, and granulocytes as well as all the subsets of them that you see. So important in this study are a subset of granulocytes shown third down, that's PMN, it stands for granulocytic or neutrophilic and MDSC is shown on the bottom, myeloid derived suppressor

cells. So these granulocytic or neutrophilic myeloid derived suppressor cells were associated, their presence was associated with increased reporting in multiple of the top symptom groups. So in the three groups I showed, anxiety, headache and weakness, body aches and joint pain in the middle and brain fog and memory problems or insomnia. Two of the three showed higher levels of these granulocytic MDSCs in the individuals with those symptoms.

In contrast, monocytic MDSCs and another type of myeloid derived suppressor cell were not different between the three groups based on the presence or absence of symptoms. So what are these cells? They're actually well-studied in cancer, they're known to be important regulators of the immune response in cancer. There is a receptor on the surface of them, it is typically used to characterize them as suppressor cells and that's the lectin type oxidized low density lipoprotein receptor or LDL receptor 1, LOX-1. It's undetectable in neutrophils in the blood of healthy people, but present in neutrophils in five to 15% of cancer patients, or sorry I should say present on five to 15% of the total neutrophils in cancer patients. And in neutrophils found in tumor tissues, up to half of them are positive for LOX-1. And this has been shown to be associated with gene signatures and immune suppressive activity and upregulation of ER stress signals and other biochemical characteristics of suppressive myeloid-derived suppressor cells.

So basically this has been shown in cancer to be a marker of immunosuppressive cells. And data I'm not going to show today we actually have shown that SARS-CoV-2 can directly induce normal neutrophils to become suppressor cells. But what has been previously shown in cancer is that induction of the ER stress neutrophils, sorry, in vitro and previously is that induction of ER stress in healthy neutrophil donors upregulates LOX expression and converts them to suppressive PMN MDSC. So numerous forms of sensing and neutrophils can drive them to become these suppressor cells. And we've actually shown in data I'm not showing today as I said that SARS-CoV-2 can as well. So we had previously shown these cells shown here to be associated with severe illness in the acute phase of COVID. And I won't go into great detail on this because it's been previously published. But that this was specific to severe COVID and not, for example, present in hospitalized influenza patients. And again, positively correlated with disease severity.

So in the acute phase these cells are highly associated with severe disease and that's really shown here from this paper. When we look at these granulocytic neutrophils, as I mentioned, they're not really present in patients hospitalized with influenza. They're present, present in the acute phase of COVID and then they, in pale blue at the far right, actually shown people who have recovered from COVID, they tend to disappear. On the middle and right panels, these are just further metabolic characterizations of these cells that I won't detail today. But again, this is a population of cells that seems to be unique to severe COVID in the acute phase. And while we show that they decrease in those who have resolution of disease, we do, as mentioned, see them in people with long COVID. And interestingly, they begin to increase over time in people who have increases in symptoms over time.

So if we look at this blue curve, these are groups of individuals complaining of anxiety, headache, and weakness. And this grows worse in those in blue over time. And what we see is that the percentage of these granulocytic MDSCs on the Y axis also increases over time. Not really a difference between people who do and do not have body aches and joint pain. But again, a difference in the far right column of people who have brain fog, memory problems and insomnia with increases of these granulocytic MDSCs present with symptoms and not increasing in those without symptoms over time. And so one other quick note in this analysis we also showed that memory B cells are associated with increased reports of anxiety, headache and weakness. And that a specific subset of them, the CD2 21 positive CD27 negative memory B cells are seen more commonly in those who are reporting anxiety, headache, and weakness, but not the other two groups of common symptoms in long COVID.

And again, basically we see using either memory B cells or the subset of memory B cells that we see increases over time in individuals with increasing symptoms over time. Specific again here to the anxiety, headache and weakness group and not the other two. So these cells have been associated with high B-cell receptor avidity in the production of neutralizing antibodies after SARS-CoV-2 infection. And so these intermediate B cells do correlate with each other but not with SARS-CoV-2 specific antibodies. And we had hypothesized they might because again of this association of these intermediate B cells with antibodies, but they do not correlate. They do again correlate with each other. And so again, not clear that this is directly driving antibodies or autoantibodies in association with this. But they do correlate with each other. And this is just again showing this

correlation, not just of the atypical or sorry, not the atypical, the intermediate memory B cells, the 21 positive 27 negative memory B cells. There is a good correlation with a granulocytic MDSC presence as well.

And again this is actually associated with in the cytokine analysis we performed eotaxin three and interferon, not all types of interferon but type I interferon and type III interferon high levels are associated with long COVID and also with the presence of these cells. In contrast, gamma interferon type II interferon is not associated with these cells or long COVID in our study. And this is shown in further detail here. Interestingly, this is something we've seen in the acute phase of COVID on the top row. This is a paper by Andrew Karaba and are all from our group showing that interferon alpha and interferon beta actually are present at higher levels. The panel here on the far right shows the key, but present here in patients more with severe flu than with severe COVID. So in contrast, you can see what I said, which is that on the bottom panel from left to right are the interferons shown on the top, interferon alpha and beta on the left and then gamma and lambda interferon on the right.

And you can see in long COVID that we have either increasing or persistence of high level in the case of interferon lambda interferon levels over time while interferon gamma tends to go down with time in long COVID. So in conclusion, we found that long COVID occurred in a third of people with non-severe COVID-19 in the acute phase over the two years following infection, that predictors of that included obesity and hyperlipidemia and that validated tools to assess the quality of life and symptoms as well as diverse patient cohorts are important for understanding long COVID. Most of the common symptoms we saw peak at six to 12 months. It must involve some altered immune function or something that causes virus to be recognized differently over time if it is a persistent viral reservoir.

In both granulocytic MDSCs and intermediate memory B cells are associated with anxiety, headache and weakness over time. But the PMDCs were also PMN MDSCs were also associated with brain fog memory problems and insomnia over time. We're doing a more in-depth phenotyping of the intermediate memory B cells and the granulocytic MDSCs with our single cell RNA-seq data analysis that's ongoing right now. But we already know by flow cytometry that the CD21 positive memory B cells are less metabolically active. And I'd just like to acknowledge my team, really a long list of individuals who helped tremendously. But a particular shout out to Elizabeth Thompson, who did the flow cytometry and Annie Antar who was instrumental in creation of the cohort and defining the symptoms of long COVID. Thank you very much.

Patricia Ceger:

Thank you Dr. Cox. Next we'll hear from Dr. Iwasaki.

Dr. Akiko Iwasaki:

Hi, everyone. Thank you very much for having me on this really great panel. So today I'd like to add on to the theme of looking at immunology of long COVID with our own studies on people with long COVID compared to those who recovered from COVID. So as we've already heard, there are many, many consequences of having had COVID and there is a significant proportion of people who suffer from post-acute phase of this infection. And this is just to remind us that there are multiple other pathogens that can also lead to similar kinds of long-term outcomes, such as those that happen after Ebola infection, dengue polio, SARS, chikungunya and many others. And there are also non-viral pathogens, bacterial and parasitic pathogens that are known to cause these medically unexplained post-acute infection syndromes. And this was a review that I was fortunate to co-author with Jan Choutka, who himself has me MECFS and has done an enormous amount of research in this area. That's been a theme for us during the pandemic, is that we learned so much from listening to patients.

So as already talked about there are several potential root causes of long COVID. And in this study we focused on four root causes that could be triggering long COVID symptom, including the viral reservoir hypothesis that we have already heard about. This can result in the persistent antigen as well as viral RNA presence that can trigger both adaptive and innate immune responses. Secondly, it's possible that autoimmune responses can be triggered as a result of acute COVID. It's also possible that there is a microbiome dysbiosis or reactivation of latent biases. And there's also evidence for tissue damage, that if unrepaired will cause chronic symptoms. And this is just one highlight for a collaboration study that we did with Professor Michelle Monje's group at Stanford where we demonstrated that acute mild COVID in the respiratory tract alone in an animal model led to chronic changes in the brain, including reactive microglia, oligodendrocyte loss, as well as demyelination of axons, which could lead to a lot of the neurocognitive symptoms that we are seeing in patients with long COVID.

So today I'd like to focus my talk on this wonderful collaboration we have with Dr. David Putrino's team at Mount Sinai School of Medicine where he runs a large long COVID clinic. He and his team see thousands of

patients with long COVID and they helped recruit the participants of this study. So David is the lead for the Mount Sinai team who also include Jamie, Laura and Dana. And from the Yale side we have six trainees, John, Rahul, Peiwen, Jill, Jeff and Sasha, who contributed equally to this study as well as two senior corresponding authors, Aaron Ring, who developed the REAP strategy to map out autoantibodies as well as viral specific antibodies. And David van Dijk who did the machine learning approach to understand the immunological correlate of long COVID.

So this study recruited participants from both Mount Sinai site as well as Yale long COVID sites. So we had 99 participants with long COVID, 39 convalescent control. These are the people who acquired acute COVID around the same time as those who developed long COVID, but they recovered from COVID. We have 39 healthy controls who never had COVID and 37 healthcare workers from Yale who contributed blood samples for immunophenotyping as healthy controls. And we also had 53 external long COVID. This is the Yale Respiratory Long COVID Clinic patients who also donated their blood for our analysis. And then we did all kinds of different immunological analysis on the peripheral blood cells, including flow cytometry as well as looking at these human exoproteome specific autoantibodies, SARS-CoV-2 specific antibodies, peptide display library that basically allowed us to look at antibody reactivity to any peptides and plasma proteomics and symptom survey and EMR.

The median age for the long COVID participants were about 45 years of age, control, slightly younger. But these really represent the bulk of the long COVID patients that are seen in the Mount Sinai Clinic and they're dominantly female, which is also typical of long COVID patients. And also, we focused on patients who were not hospitalized during the acute phase of COVID. In order to really understand the vast majority of long COVID patients who develop long COVID after a mild non hospitalized disease. Days from acute COVID hovered around 400 plus or minus days. So this is a later time point for people who've had COVID for extended time period. By looking at the flow cytometry data, we did notice significant changes in a certain cell subset such as non-conventional monocytes. They were increased in circulation, conventional dendritic cell type 1. These are the cell type that are responsible for stimulating T-cell immunity, which was reduced in circulation of people with long COVID. We saw increased activated B cells as well as double negative B cells, which is consistent with what Dr. Cox just told us.

We also saw some changes in the memory T-cell population, including reduction in the circulating TCM population as well as increase in exhausted CD4, CD8 T-cells, which you also heard about from Dr. Henrich's talk. We also looked at different cytokines that are secreted by T-cells and what we noticed is that people with long COVID had elevated levels of cytokines secretion capacity including interleukin-2, 4 and 6. And we also identified T-cells that secrete both IL-4 and IL-6. These are kind of strange cell type that we only saw in participants with long COVID, but not in the other groups. This was also seen for the CD8 T-cell subset as well. Looking at the plasma factors that we collected from these participants, what we noticed was that the most significantly altered factor in circulation was cortisol, which is shown here on the left top here. The cortisol levels were reduced in people with long COVID. There were some inflammatory factors that were elevated in patients with long COVID, including the complement C4B as well as many chemokines and cytokines listed here.

So because we saw this significant reduction in the cortisol level, we wanted to ensure that this observation is not just confined to the Mount Sinai Long COVID Clinic. So by looking at the participants from the Yale Long COVID Clinic, we also saw a significant reduction in the circulating level of cortisol in that participant group. We also wanted to ensure that the time of collection was not significantly different between the groups, which is important because cortisol is a diurnal hormone that is elevated during the early morning and that declines throughout the day. So the fact that cortisol levels are significantly lower in long COVID participants prompted us to look upstream of the cortisol, which is regulated by the hypothalamus and pituitary axis. The CRH that's secreted by the hypothalamus acts on the pituitary to induce ACTH, which then act on the adrenal gland to produce cortisol.

By looking at the ACTH levels in these patients, we did not see an elevation in the level of ACTH in the participants with long COVID, suggesting that there is a dysregulation of the cortisol level potentially based on the non-improper function of the hypothalamus pituitary signals that control the cortisol levels. We also examined reactivation of latent viruses, using the rapid extracellular antigen profiling that Aaron Ring has developed. Now he included 100, no, 200 plus viral antigens in this library. And we were able to demonstrate that indeed, antibodies against EBV, Epstein-Barr virus protein 23 and GP 42 were elevated in those with long COVID compared to the controls. And then Varicella-Zoster Virus gE specific antibody was also elevated, suggesting that there may have been a recent reactivation of EBV or VZV in participants with long COVID. And this was not because

the seropositivity differed between these participant cohorts. In fact, the latent viral seropositivity was similar between those with and without long COVID.

And we did two other orthogonal methods to measure EBV specific antibodies and including the Serimmune panel as well as the ELIZA and we again saw increases in antibody levels against these lytic proteins of EBV and VZV. And here in the purple you're seeing the levels of the REAP score for P23, which is higher in long COVID compared to the controls. And we also see that looking at a Serimmune score, which enabled us to pinpoint the peptides that are specifically recognized by the antibodies in patients with long COVID. Again, that level is increased in those with long COVID compared to the controls. And what's intriguing is that this level of EBV reactive antibodies actually correlated with the level of the strange cell type that I mentioned before, which is the IL-4, IL-6 double positive CD4 T-cells. So there may even be a functional link between EBV reactivation and the existence or elevation of the cytokine secreting T-cells.

So all in all, when we applied the machine learning strategies to look at the key immunologic factors that can distinguish people with long COVID, what we came up with is a handful of factors that enabled us to do so with high accuracy, which included the lower levels of cortisol, lower levels of CDC1 and TCMs, as well as higher levels of EBV reactive antibodies and some other cytokines and T-cell factors. So taking this same dataset, now we are analyzing sex differences in long COVID and this is an effort led by Julio and Takehiro. Here what we did is to take the same set of data from my long COVID study, but divide them into female versus male and control versus long COVID and then we do the analysis in sex disaggregated manner. When we did this, first of all, we noticed that females, which are indicated in blue color here, have increased symptom burden as well as increased organ system involvement based on their health survey results compared to male patients with long COVID.

Another very interesting thing that came out of this symptom analysis is that while some certain symptoms such as sleep disturbance and fatigue and nausea were similarly frequent between male and female patients, we saw certain other symptoms that are more predominant in female, which are down in the bottom here, including dizziness, body temperature issues, cough and so on. And the most sexually discordant symptom turned out to be for the male, it was the sexual dysfunction. And for the female it was the hair loss, which is interesting because it tells us that this disease affects women and men very differently. And immunologically, when we looked at the different cell subsets, female participants with long COVID had higher levels of exhausted CD4 T-cells, interferon gamma secreting CD4s, and then this double positive CD8 T-cell, whereas the male patients really didn't have much of a difference compared to the controls. And also very interestingly, this EBV reactive antibody that I just told you about, that's predominantly happening in the female long COVID patients.

We also saw that the autoantibody levels numbers were elevated in female patients with long COVID and these antibodies tended to target regions such as the basal ganglia and gonadal tissues. Conversely, long COVID males had elevated levels of NK cells, TCF beta in April. So again, there's very different immune phenotypes associated with male versus female long COVID. And looking at all the different circulating hormones, what we noticed is that the female patients with long COVID had lower levels of testosterone in circulation, whereas the male patients had no differences in their levels. And of course males have a lot higher levels of testosterone than females. But when we looked at the odds ratio of predicting long COVID based on the hormonal levels, testosterone turned out to be the best predictive factor. The lower levels correlating with the long COVID status. And conversely estradiol levels in males turned out to be the top predictive factor for long COVID status.

So taking this all together, what we now think is that there's a dysregulation of the hypothalamus pituitary organ axis, starting with the cortisol level dysregulation and now sex hormone dysregulation. And if we extend this to other possible organs that might be affected by this dysregulation, we could even start to study other tissues like adipose tissue, bone marrow, muscles, and fibro glands and mammary glands. So coming back to the original root causes, we now see evidence, direct or indirect evidence for all of these four root cause hypotheses. And based on the viral reservoir hypothesis, we are doing a Paxlovid long COVID trial by giving a 15-day course of Paxlovid and measuring biomarkers prior to and after the Paxlovid treatment to see what is correlating with benefit from treatment with Paxlovid. And we're also developing animal models to probe each of these four root cause hypotheses. I want to end here by thanking the amazing people that I have the opportunity to work with every day as well as the funding agencies that enabled us to do this.

Dr. Paul. J. Utz:

I want to thank all three speakers for giving absolutely phenomenal talks. These are very thought-provoking, they integrate really well. And what I thought I would do would be to just start off with some questions

for each of our speakers, just starting at the beginning and moving on down and then end with the same question for each of the speakers. So we'll start with Dr. Heinrich. So one is a specific question. You showed beautiful data on involvement of presence of virus in colon biopsies, were those participants, were they symptomatic? Was there a reason that you selected them? Meaning symptomatic from a GI standpoint.

Dr. Timothy Heinrich:

So they were not specifically symptomatic from a GI standpoint, but they were symptomatic from a long COVID standpoint. But I think that's an important question because it suggests that viral persistence is a main driver or a potential main driver of a lot of downstream immune dysregulation, inflammation, nervous system dysfunction, things like that. It's possible that you can have tissue sources of persistence that don't necessarily correlate with symptoms. So for example, when we ask about gastrointestinal symptoms, whether someone has diarrhea or stomach pain or lack of appetite, would that necessarily correlate with viral persistence, specific gut tissue? It might, but we're not seeing this tight correlation.

I think that's probably because that inflammation in the gut can cause systemic inflammation, systemic response, et cetera. So it could lead to other symptoms as well. Where we did see the correlation was with lung uptake. So what we saw PET lung uptake correlated with pulmonary symptoms. So certainly there could be bowel persistence in that area or even other locations that are causing more systemic symptoms, especially with the neurologic phenotypes as well. But yeah, that's an excellent question. Going forward, we're actually now doing folks with full recovery, no known prior COVID and different clinical phenotypes to do deep tissue dives, not just in the gut but also in bone marrow, lymphoid tissues, et cetera. So that's now ongoing as we go forward.

Dr. Paul. J. Utz:

Thanks. You made a really important point also during your talk about the long-term impact of this and this new set point. I think that is really important for all of us to think about, that each of us have either had COVID or been vaccinated, the whole population, it's a completely different population than existed in 2019. And so almost certainly will be the same thing with the tissues. One other comment I would make is that this is a direction that I know NIH and foundations are going for autoimmune diseases, which is the primary area in which I worked before the pandemic in, I wouldn't say abandoning studying blood cells because you can see the beautiful results you get from studying blood cells as well as circulating factors.

But there is a push now to obtaining tissues to really go at the root cause. And that can be challenging for things like lupus where you're getting kidney predominantly. But here, if you can get skin or colon biopsies, which are much more palatable for patients, that's very important. A second follow-up question for you. Is there anything in the literature about other viruses, for example, RSV or influenza? Have people done such studies to see if there might be reservoirs there as well?

Dr. Timothy Heinrich:

That's a fantastic question. We do know that certain RNA viruses or other viruses that we don't typically consider as chronic viral infections, for example, Ebola or other, what we would consider acute infections can certainly lead to viral persistence. I guess the question is are more routine standard annual respiratory viruses like RSV, influenza, things like that, the set-up shop. I think part of the reason we actually haven't looked very deeply for that, I think SARS-CoV-2 is essentially paradigm shifting in how we think about acute viral respiratory illnesses. And it's possible.

That said, we also don't see the same burden of postviral. We do see postviral syndromes after these, don't get me wrong, but in terms of the burden, seems to be much higher in the setting of SARS-CoV-2. So I think there is something unique about this particular Coronavirus or related group of Coronaviruses that may lead to more chronic infection in deeper tissues. But that said, we really haven't done a deep dive. And so when we're looking at tissue, that's certainly something we're also looking at now, is other persistent viral either RNA, DNA or proteins from virus in those deeper tissues.

Dr. Paul. J. Utz:

Great. Thank you. All right, Dr. Cox, let me ask you a few questions. Your talk was phenomenal. I'm glad that I didn't see the Krebs cycle shown on a slide because that brings back all of these terrible memories from undergraduate and from medical school to have to memorize the Krebs cycle. But I will ask you a question about that. The PMN MDSC cells that you have shown are upregulated in long COVID and in patients with SARS-CoV-2 infection, they have a suppressive function and I think that's pretty well established. So one might think that

maybe these cells would be good cells to have if they're suppressing an immune response and downregulating inflammation. Did you want to comment a little bit about the function of these cells?

Dr. Andrea Cox:

Yeah, so they have been shown in cancer to be suppressive. They're less well studied in infectious diseases. Sort of as a precursor, we had a similar question and we actually defined both. And data didn't show today that the virus can directly upregulate within an hour of exposure LOX-1. So it causes degranulation of neutrophils and upregulation of LOX-1. Interestingly, prolonged exposure to H1N1 influenza did not. So I do think with regards to the question, do all these respiratory viruses do the same thing? Pretty clearly not. And that's really consistent with our in vivo data, or ex vivo I should say, showing that human beings with severe acute influenza did not have these granulocytic MDSCs.

We have actually, and again, I'm about to submit this paper, we have actually shown that the virus can directly induce these cells to be suppressive and the mechanism through which they are suppressive. I will say that they are suppressing T-cell proliferation and cytokine elaboration, which in the acute phase is probably not ideal and they are very strongly associated, positively correlated with severe disease. And they actually proceed in the blood, the development of severe disease. Those who go on to acquire severe disease may not... Sorry, they actually appear concomitant with not before. So it seems more like they may be responding. But the problem is we can't look in the lungs and people with mild illness for comparison. We do see them in the lungs of people with severe disease. Hard for us to know what's happening in the lungs of people without severe disease because we don't routinely access the lungs.

We don't see them in the normal healthy people undergoing intubation for gastroenterological procedures, for example, like colonoscopies. So we have looked in normal healthy people, so they're clearly not present in the lungs of people who are not ill. Whether they're present in the lungs of people with mild illnesses, hard for us to know. But they do appear in the peripheral blood about the time of severe disease. So cause and effect, very hard to sort out. And you're right, we could say if it's a hyper inflammatory immune response, their production could be beneficial. But they are very strongly associated with a severe disease and also correlate with later death from SARS-CoV-2. So they don't seem, in the acute phase, to be helpful. In the long COVID, that's much more difficult to assess. But we do see that they go down with resolution of symptoms, they go up with increases in symptoms. So again, hard to know cause and effect.

Dr. Paul. J. Utz:

Great, thank you. One of the really nice things about your cohort is that it's very diverse. And so one thing I didn't hear in your talk was about any differences that you've identified in race or ethnicity. Did you find this across different groups?

Dr. Andrea Cox:

Interestingly, we did. So we did see that these cells are increasing with increases in severity across racial and ethnic groups.

Dr. Paul. J. Utz:

Okay. And a final question I have to ask about just metabolism in general. In Santa Fe, you gave a wonderful talk that included a lot about that and oxidative stress and so forth, ER stress. Can you just elaborate a little bit more about what role that might play in long COVID and how?

Dr. Andrea Cox:

Yeah, so we do see some markers. I did briefly show a little bit about this. We have characterized the metabolic markers on these MDSCs and where we really see metabolic mitochondrial membrane dysfunction in severe COVID, in COVID rather, is in the acute phase in severe COVID in T lymphocytes. The MDSCs that are present in these patients with long COVID don't seem to have profound evidence of mitochondrial membrane dysfunction. But ER stress absolutely induces MDSC formation. And so certainly, it could be that in long COVID that tissue damage is also associated with the development of MDSCs. That's a distinct possibility as well.

Dr. Paul. J. Utz:

Thank you so much Dr. Iwasaki, I have a few questions for you. First, it's really clear there are alterations in the endocrine system more broadly in your studies and could you just expand a little bit? And if someone was a clinician or a patient going in to see their doctor, how might this impact their treatment? What would you

recommend there? And I recognize you're not a physician, but your findings are so striking that we have to look at this.

Dr. Akiko Iwasaki:

Thank you Dr. Utz. So what this suggests is first of all, we don't know how universally applicable this finding is. We've done it in two different cohorts, but it may depend on the types of symptoms that people are experiencing and the kinds of severity of long COVID. So I don't want to say it's a universal fact, but it's what we've observed in two different cohorts. Secondly, this is actually not a novel observation. If you look at the MACFS literature, there's been many studies that looked at cortisol levels and there has been studies demonstrating hypercortisolism in people with MACFS.

And there've been several clinical trials, randomized clinical trials that were done to look at the impact of supplementing cortisone in patients with these low levels of cortisol. They haven't been able to identify a dose that is well tolerated and is effective yet. It doesn't mean that it doesn't exist. But I feel that there should be future studies to really look at this more carefully. Can we supplement cortisol level in people with low cortisol to a physiological level, not superseding the physiological level in order to help those with this particular condition? So that's something that I would love for physicians to be thinking about, measuring morning cortisol levels and seeing if supplementation might impact their patient care.

Dr. Paul. J. Utz:

Great, thank you. And another important finding from your work is the potential role of EBV reactivation. As you know, there are a number of studies now very strongly linking EBV to diseases like lupus and more recently multiple sclerosis. So maybe do you want to speculate about whether you think this EBV reactivation is the driving thing and whether these people will go on to develop autoimmunity?

Dr. Akiko Iwasaki:

Yeah, that's a really striking link there. There's a lot of viral infections that have been linked to autoimmune diseases, but the EBV is at the top of that list, as you say. And we think that there may be a link here because when we do the sex desegregated analysis, as I mentioned, there's the EBV reactivation and autoantibodies predominantly seen in female patients with long COVID and there may be a link between these things when we correlate their levels. So I don't want to say one is causing the other, but there is definitely a close association of these two observations. And because EBV is latent in B cells and epithelial cells, it's possible that the reactivation may induce activation of those B cells, that may be reactive to self antigens or it could induce de novo activation of B cells that are surrounding or that are being infected by the reactive virus. So there's a lot of hypothesis and links that we can make. We don't have a cause and effect relationship though yet.

Dr. Paul. J. Utz:

Okay, thank you. And for those who are on the call who may not be aware of this, there have been three very large epidemiologic studies published recently, two in Asia, one in Germany, linking previous SARS-CoV-2 infection with the development of later incident autoimmune disease. So we're almost at the point where we want to ask for audience questions to come to our speakers. But I wanted to ask one question of all three of you and maybe we'll start with you Dr. Iwasaki. So I note that all three of you have beautiful cohorts that were developed very early in the pandemic before RECOVER got going. But RECOVER now has this beautiful cohort of over 20,000 individuals with serial samples. Maybe just in like 30 seconds to 40 seconds each, can you just let us know what you would be thinking about doing with these RECOVER samples, since it's such a great resource?

Dr. Akiko Iwasaki:

Yes, absolutely. I'm very excited to be able to collaborate with investigators of RECOVER to be able to look at so many different things. But one thing I think Dr. Henrich mentioned that's very important is the tissue level analysis. And so for example, the autopsy biobank that's being developed by RECOVER. And that's going to be an incredible resource for many people, including our own team, to be able to look at what is this viral presence? Is this replication competent virus that's persisting? If so can Paxlovid or monoclonal antibodies, target that? And where are these reservoirs? Can we look at the RNA? So that's something that my team would love to engage in when these things become made available.

Dr. Paul. J. Utz:

Great, thank you. Dr. Cox?

Dr. Andrea Cox:

Yeah, I completely agree. In addition, Donna Farber's really excellent collection of living organ donors because some things are harder to assess in autopsy specimens where people are no longer alive. So I do think that is another possible situation in which we can assess tissue reservoirs. So I think tissue reservoirs remain an important outstanding question. One should contemplate how they would become associated with more symptoms over time. Is the viral reservoir growing in size? It seems more likely to me that it may be a combination of immune responses to a viral reservoir that's persistent and possibly changing over time.

Dr. Paul. J. Utz:

Great, thanks. And then finally Dr. Henrich, and then we'll turn it back over to Patricia for audience questions.

Dr. Timothy Henrich:

Well, I agree obviously with the tissue work, so this is where we've driven all of our recent efforts to design panels, to look at post responses in C2 in these various different cohorts, and would love to see in autopsy cohorts, in organ donor cohorts. But also from specimens prospectively from otherwise healthy folks with and without long COVID symptoms because I think that's going to be important going forward. I think the problem with some of the autopsies that people a lot of times tissues are from people that are in the hospital are chronically ill have other inflammatory makes actually host viral responses difficult to study. But RECOVER is an amazing resource and with just the sheer volume and the types of samples and the type of clinical correlative data that is being collected is unprecedented. So we're very excited to certainly be part of RECOVER and it's a great resource.

Dr. Paul. J. Utz:

Great, thank you. Let me turn it over now to Patricia.

Patricia Ceger:

Thank you. We have some questions from the audience. The first ones for Dr. Henrich. Is the proteomics data that you were displaying during your presentation from patient plasma?

Dr. Timothy Henrich:

So I showed two data, I apologize for the confusion. The first slide I showed looking at persistent inflammation eight months after in people that were not for majority of people not hospitalized, was actually RNA seq data. So gene expression data in peripheral blood monocyte cells, so essentially immune cells that are circulating in the blood and showing that there was a dramatically altered profile of those. The slide I showed showing inflammation that correlates with lung uptake on the PET scan is proteins in the plasma. And so that certainly is looking at circulating proteins that may be produced elsewhere, but are circulating in the plasma itself.

Patricia Ceger:

Do you think that immune biomarkers might be useful for long COVID surveillance? That's for anyone.

Dr. Andrea Cox:

Yeah. I think this again highlights the power of RECOVER with the huge numbers of people participating. I think that's critical because there is a really a diverse array of symptoms. There are people who have restrictive lung disease, cardiac dysfunction, autonomic dysfunction. So I do think having large numbers of people so that we can make sure. And I doubt there's one biomarker of every one of those manifestations, for example. But I do think if we have large numbers of people which RECOVER affords us that opportunity, we might identify some biomarkers that are associated with different patterns of symptoms.

Patricia Ceger:

Anyone else?

Dr. Timothy Henrich:

Yeah, absolutely. I think there's certainly to be correlations between certain immune biomarkers and symptoms. But I would also warn that I don't think there's going to be a simple single test or something that is clinically validated that is going to really predict either particular symptoms. This is going to be an iterative approach that looking at larger populations, and I don't want to be pessimistic, but may have some limited utility in terms of clinical management in the clinical side itself. But certainly, looking at some of the machine learning techniques and looking at how to integrate this data, that would make sense.

And again, many of the studies that are being done are really research only focused and really can't be done in the clinical setting. And so we would love to have a specific clinical marker. Certainly Dr. Iwasaki

has been talking about cortisol levels or other things that we can use that are available to the clinician and in our hands immediately. So although there's certainly potential and understanding the pathogenesis and potentially looking at how we could combine different biomarkers to become a clinically effective test. We just lack that capability, I think right now unfortunately, in the clinical sphere. And some of these changes are subtle and may vary within the population. So on the individual basis may be difficult.

Patricia Ceger:

Okay. We have a lot more questions, but unfortunately we do not have a lot of time. So I will read our closing remarks and remind everyone that we will be providing answers to these Q and A sessions online, including questions that we did not get to during today's presentation. So thank you so much to our presenters and thank you to the audience for attending this seminar and engaging with the Q and A. As a reminder, a recording of today's seminar will be available on recovercovid.org within a few weeks.

We will also be posting a Q and A document that has responses to the questions we received today, including those we did not have time to address. Our next seminar will be on December 12th from 12:00 to 1:30 PM Eastern Time. We will have some exciting topics coming up in 2024 and we hope to see you at future sessions. At the end when you are logging out, you will see a short survey come up on your screen which will ask for your feedback on this seminar, we would very much appreciate it if you could take a minute to fill out this survey. Thank you and have a great day.