

# Transcript

## Claire Quiner

Welcome everyone 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, Clinical Spectrum of PASC, Focus on Sleep. The goal of this Seminar Series is to catalyze a shared understanding of the research within the RECOVER consortium. This is not intended to provide clinical guidance. Rather, the presentations and conversations today pertain to the research realm. Our presenters today will be unable to answer clinical or treatment questions, but prepared to discuss and answer questions pertaining to research in their field. Before we move into introductions, I'd like to thank everyone who's submitted questions in advance, and please submit any questions that arise during today's presentation, using the Q&A feature in Zoom. During the presentation, we will answer as many questions as possible. Some questions may be answered within the Q&A feature on Zoom.

After the seminar, we will post on [recovercovid.org](https://recovercovid.org) a FAQ document with the recording of the seminar, as well as 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](https://recovercovid.org). We have a great set of presenters today, and I'd like to provide a brief bio of each of them, beginning with Dr. Monika Haack, who is an Associate Professor of Neurology at Harvard Medical School. And her research program is directed at understanding the mechanisms underlying the association between sleep deficiency and increased disease risk with a specific focus on inflammatory and neuroendocrine networks. Her most recent research focuses on sex differential effects of sleep disturbances on inflammatory networks, which may explain the pronounced sexual dimorphism of the many disease conditions that are comorbid with sleep disturbances, including PASC.

We also have Dr. Sairam Parthasarathy, who is a Marie and Clara Walker chair and Professor of Medicine, Chief Division of Pulmonary Allergy, Critical Care and Sleep Medicine, Director for the UAHS Center of Sleep and Circadian Science and Medical Director for the Center for Sleep Disorders at the University of Arizona, and is the current president of the Sleep Research Society Foundation. His research focuses on sleep and breathing in both ambulatory patients with sleep disorders, critically ill patients and survivors of critical illness, and investigates the role of community engagement to address COVID related health disparities along COVID and sleep. Next we have Dr. Susan Redline who is the Peter C Farrell professor of Sleep Medicine in Harvard Medical School, professor of Epidemiology at Harvard T.H. Chan School of Public Health and Director of Programs in Sleep and Cardiovascular Medicine and Sleep Medicine Epidemiology at Brigham and Women's Hospital.

Dr. Redline has led epidemiological studies and clinical trials designed to, one, elucidate the ideologies of sleep disorders in both adults and children, including the role of genetic and early life developmental factors. And

two, understand the cardiovascular and other health outcomes of sleep disorders and the role of sleep intervention and improving health and wellbeing. She has co-authored over 650 manuscripts and has served the sleep medicine community in many ways, including as passport member of the Sleep Research Society and American Academy of Sleep Science. In addition to our speakers today, we have a fabulous discussant, Dr. Janet Mullington. Who's a Professor of Neurology at the Beth Israel Deaconess Medical Center and Harvard Medical School. She also serves as the program director for the BIDMC's Clinical Research Center, where she is also Vice Chair for Research at the Department of Neurology. Her area of expertise in research is in sleep deficiency and is associated pathobiological consequences for systems including inflammatory autonomic state related neurophysiology, cognitive and subjective fatigue and mood.

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 Dr. Mullington.

## Dr. Janet Mullington

Thank you very, very much for your lovely introduction. So how much sleep do we need? The American Academy of Sleep Medicine and The Sleep Research Society had a panel of experts reviewing the data available for a number of health parameters and published a consensus that a minimum of seven to eight hours was necessary for the maintenance of health in adults for cardiovascular metabolic health, as well as mental health, immune function, human performance, pain and the AHA in 2022 added sleep as a component necessary for heart health. Not sure... That didn't advance. Oh, there we go.

So sleep timing is controlled. Sleep and wakefulness are controlled by brain mechanisms in the brain stem and hypothalamus. And sleep and wake are controlled also by hormonal signals, the circadian alerting signal as shown here. This slide is courtesy of Phyllis Zee. And you can see here, the rise of the alerting signal through the day and melatonin is the pineal hormone that is elevated through the dark phase in humans and suppressed by light. And you can see here it peaks in the middle of the dark phase. The homeostatic sleep drive, the drive for sleep increases through the day and dissipates at night across the night in association with slow wave activity. Sleep regulates hormones as well. And the pulsatile hormone, growth hormone is dramatically blunted in the absence of sleep. So if individuals stay awake through the night that peak in growth hormone disappears. Similarly, prolactin is inhibited without sleep.

Sleep deprivation also causes the hormones that are associated with hypothalamic, pituitary and sympathetic nervous system activity and stress to be elevated through the night. So you see here, small elevations in the circadian pattern of cortisol through the night and norepinephrine. On the left you'll see an example of EEG patterns associated with wakefulness, the alpha rhythms and beta rhythms. And these are also apparent during sleep. And in REM sleep, rapid eye movement sleep, the EEG is active, but the muscle tone is not. And there's a muscle atonia during REM sleep. Stage one, you can see the intrusion here of beta rhythms entering sleep and the

sleep spindles and K complexes are really the indicators or the hallmarks of stage two. N3, which used to be referred to as stage three and stage four really are showing the predominance of Delta wave activity.

Over here, you can see in panel A, the sleep wake pattern through the night, showing a dissent into slow wave sleep. And this cycle is repeated, rising out of slow wave into REM sleep back into slow wave, cycling through the night three to four times. And the second panel, you see the Delta power increasing and coordinating here. You see the slow wave in the histogram associated with this Delta activity in repeating through the night. Underneath you see heart rate variability, and this is the high frequency or parasympathetic indicator showing a correspondence to that Delta and the slow wave sleep. On the right we see the non-REM sleep across the night collapsed and the insomnia patient who frequently experiences sleep as non-refreshing or non-restorative has a higher elevation in the EEG across the night in the higher frequencies. This is, I think, relevant to this homeostatic process. Following sleep deprivation, a short period of sleep deprivation in twins who had chronic fatigue syndrome and the non-affected healthy sleeping twin, you can see here the dissipation in the slow wave of activity across these non-REM site bouts or bouts of slow wave and N3.

And what you see in the twin who has the chronic fatigue syndrome is a dampened pattern across the night. So they don't manage to dissipate that slow wave pressure across the night, potentially a marker of sleep deficiency, non-restorative sleep. Another example of non-restorative sleep is in chronic insomnia. What you see here is EEG sampled before sleep and after sleep. This is the beta frequency, this is fast frequency and gamma. Beta and gamma show very little change from pre-sleep to post-sleep across the night. And in the healthy sleeper, you see a very pronounced decrease in this gamma and beta frequency from pre-sleep to post-sleep. So sleep is reversing the beta and gamma amounts. And this I think, has led to some renewed interest in insomnia as an example, and the search for biomarker of deficient sleep.

So I think that the recovery sleep is a very interesting and important aspect. And we've talked about general sleep and we'll hear more about sleep in the immune system. And sleep in Shakespeare's words, chief nourisher in life's feast. But the question I hope we get some traction on is, how does it do that? Thank you.

Monika Haack: Yeah, thanks very much for inviting me and presenting our current knowledge on the association between sleep and immunity and with the potential consequences for long COVID. So I would like to start with the perception of the sleep immune connection in everyday life. And common belief or observation that have been passed down from generation to generation is that sleep loss renders us more susceptible to catch a cold or other infections or that a good night's sleep is the best medicine for an infectious disease. And the first scientific approach to the sleep immune relationship actually dates back to Aristotle, 350 BC. And in his book on Sleep and Sleepiness, he mentioned that the sleep response can be observed in feverish patients. So this slide shows you our current understanding of the sleep immune connection 2000 years later. And I will not go into details, but there is a bidirectional relationship between sleep and the immune system such that when you look to the very left, if an immune activation is too strong or too long, sleep is disrupted, we see fatigue.

And if sleep is disrupted, we see an increased risk for infections and variety of other diseases that involve immunopathology. And in the following, I would like to show you some examples of the consequences of disturbed sleep on infection risk, on vaccination responses, as well as on inflammatory homeostasis. So when we look at sleep disturbances in COVID, they are common prior to enduring SARS-CoV-2 infection, as well as following the onset of PASC. This are data from Davis, 2021, based on an international cohort of 6,500 individuals and symptoms were traced over seven months. So what you can see here prior to SARS-CoV-2 infection about 20%. And I should say, this is all self-report insomnia symptoms. Then following the acute infection we see an increase of insomnia to about 60%. And also after the onset of PASC 45% of individuals report insomnia symptoms.

And this is actually more than we see with other post-viral syndromes, for example, lung disease. So when we look at the prevalence of sleep disturbances in comparison to other PASC symptoms, so we see here insomnia with about, again, this is a study by Davis, 45%. And what is also very common are pain symptoms. And I mentioned this here because insomnia is known to lead to a hyper state and changes the way the brain processes pain. And of course at the very top, we see over 80% of patients reporting fatigue. So several potential mechanisms have been suggested and also tested that may lead to the development or persistence of PASC. These include auto immune factors, such as auto reactive T-cells, auto antibodies, persistent viral reservoirs, and tissues and monocytes have been reported, reactivation of latent viruses such as the EBV virus.

There are many reports on immune and inflammatory perturbations that all point to a persistent activation of the immune system following the acute infection and also impaired B and T cell memory development have been suggested. Risk factors that have been identified includes the severity of SARS-CoV-2 disease, age comorbidities such as type two diabetes, also female sex. And as we hear later, other racial and social factors. And given what we know about sleep disturbances, sleep disturbances may also be a risk factor that contribute to the development and persistent of PASC through dysregulation of innate and adaptive immune system responses. So first I would like to show you some data on sleep and infections in humans, and we'll first show you some data from natural occurring infections. So here it has been shown that sleeping less than five hours per night was associated with a 70% increase of ammonia risk, 80% increase of respiratory infections. And this are based on large cohort studies.

And also in the experimental setting, individuals reporting short sleep duration of less than seven hours... This was one of the studies first studies by conducted by Cohen, 2009 in the weeks before exposure to a cold virus was associated with an almost three times higher risk to develop a clinical cold. And this data could be replicated by using objective assessments of sleep using actigraphy. And what you can see here in this graph, sleeping less than six hours or those who sleep less than six hours are two times more likely to develop a cold.

So this association has been also found for SARS-CoV-2 infection. Just very shortly, the risk of mortality in COVID+ patient increases with a higher, poor sleep behavior burden. So this poor sleep behavior burden was based on a composite score based on sleep duration, daytime sleepiness, insomnia, and chronotype. And so this study

concluded that poor sleep is an independent risk factor. So after controlling for various other factors for hospitalization and mortality following COVID-19 infections. So now I would like to show you some data on sleep and aspects of the adaptive immune system. And for this, to study to better understand immune responses to infections, vaccination models have been used in humans because they mimic an infection without developing into or being associated with sickness syndromes, as we would see it with a real infection. And when we look at field studies, short habitual situations surrounding the time of hepatitis B vaccination has been associated with lower antibody responses, as you can see here.

And each additional hour of sleep in this study translated into a 50% increase in the secondary antibody response. And I think what's really a really important finding is that sleeping even fewer than six hours translated into a significant risk of being unprotected, as you can see here. So basically these individuals needed another booster because they slept less than six hours. There's also data on shorter sleep duration in the two nights prior to vaccination against influenza A strain predicted lower antigen-specific antibody levels. And very recently self-reported insomnia symptoms have been shown to be associated with lower levels of antibodies following SARS-CoV-2 infection.

So I also would like to show you some experimental studies. And to summarize experimental acute sleep restriction or deprivation studies prior to a following vaccination against influenza has been tested, hepatitis A hepatitis B, H1N1, reduced B cell antibody responses, as well as antigen-specific CD4 T cell responses. As you can see here in this graph, this is a study by Tanya Lange from 2011. And even after one year, we see a reduction in these T cell responses. So when we summarize all these studies, the magnitude of the effect across studies is about a 50% reduction in vaccination responses under conditions of acute sleep loss. Excuse me. So this means improving sleep around the time of vaccination may serve as a natural adjuvant to optimize B and T cell responses to vaccinations. So now I would like to talk about aspects of the innate immune system. And first we would like to briefly talk about the concept of inflammation. They're excellent reviews by Medzhitov Science, 2021, also on Nature Medicine and Cell.

So inflammation is conceptualized on a spectrum of inflammatory responses. So on the one end we have the typical acute inflammatory response caused by infection and tissue damage and that leads to the cardinal signs of inflammation. As you can see here in this picture, this is heat, so fever, wetness, swelling, pain and loss of function. But on the other side we have inflammation induced without infection or tissue damage, but that goes back to physiological perturbations by molecular cues of cell stress, for example. Mediators of inflammation are involved in almost every human disease and also in a wide range of biological processes, including metabolism functions of the nervous system. And nowadays chronic low-grade inflammation can be seen as a consequence of ongoing perturbation and an effort of the body to reinstate homeostasis.

So when we look at poor sleep, poor sleep has been shown to affect a wide area of immune markers in function. I will not go into details. There are about 150 studies that have been conducted within the last 40 years.

And with a focus on inflammation, there are multiple pro and counter inflammatory pathways involved in inflammatory regulation. That's not only the nuclear factor kappa B pathway with the production of cytokines, but also the cyclooxygenase pathway, which is the target of non-steroid anti-inflammatory drugs, such as ibuprofen or aspirin. And there are various counter-inflammatory pathways that control inflammation, including the hypothalamus pituitary adrenal axis with effect to hormone cortisol, inflammatory resolution pathways, the so-called SPMs, as well as endocannabinoids. And what I would like to show you now are some examples that sleep disturbances affect all of these pathways that are involved in inflammatory regulation. And to study the impact of sleep, and to really understand the causal impact of sleep on inflammation, we and others mimic common sleep-wake patterns in the laboratory setting.

And for example, we can model short sleep duration or common patterns of sleeping very little during the week days catching up on sleep on the weekend days, or we can model insomnia-like sleep disturbances. And I will show you some data from this model. This is a 19 day model where participants start with regular amounts of sleep and then sleep is disrupted. So they sleep 40 minutes and then they're 20 minutes awake, 40 minutes sleep... So it's a pattern that we, for example, see in chronic pain population, they have intermittent recovery sleeps. And we are also very interested in how do biological processes recover from these challenges. So in the very end in this protocol we had four recovery nights. So first I would like to show you how individuals respond with respect to their feelings of fatigue. We ask them every four hours to rate their fatigue levels. And here you can see the fatigue response.

So here day four is the baseline. And in red, then we see the fatigue response during sleep disruption, of course, fatigue increases, decreases with intermittent recovery sleep. And what I would like to point out that even after, oops, two nights of recovery sleep at end of protocol, fatigue responses are still elevated, and this is mainly driven by females. So on the left here, you can see the fatigue responses in females, and it's the females who even after three nights of recovery sleep have still significantly elevated fatigue. Now, I think this is a very important finding because fatigue is a huge problem in many diseases for which females are overrepresented.

So I would like to now show you some data on inflammatory COX pathway. As I mentioned before, the COX-1, COX-2 enzymes are the target of non-steroid anti-inflammatory drugs. They inhibit these enzymes and then prevent the synthesis of prostaglandins and their biological or physiological consequences such as fever, pain, vasodilation and so on. What we do see is sleep disturbances dysregulate COX-2 expression. Here we see baseline max sleep disturbances. And then here we see the recovery. What I would like to point out, we do see even these responses continue during the recovery. So there's not a quick normalization during the recovery period. And again, we have sex differences. There's a strong inflammatory COX response in males. And I think that's important because sleep disturbances may contribute to the hyper-inflammatory state in COVID-19 disease that have been specifically reported in males. And what's also important is these COX pathways recently have been shown still

unregulated in the post-COVID period after resolution of clinical symptoms. So it may be that sleep disturbances contribute to these regulations in these pathways.

So, as I mentioned before, there are various counter-inflammatory pathways that restore, maintain inflammation. We have cortisol, resolvins and cannabinoids. When we look at cortisol, we do see dis-regulations increases during sleep disturbances. And this now is driven mainly by females. It's the females who have a stronger counter inflammatory response to those patterns. And in addition to looking at the amount of cortisol production, it's also important to look at the effectiveness of cortisol glucocorticoids in controlling inflammation in response to sleep disturbances. And we can do this by exposing cells, in this case monocytes to synthetic glucocorticoids, so dexamethasone, for example. And this are data from an ongoing protocol. So what you see here is there is an increase in COX during five days of sleep restriction that continues again into the recovery period.

And then when the cells are exposed to dexamethasone, what we can see here, so the blue is the control condition. Dexamethasone is less effective in inhibiting COX when sleep is deficient. So sleep disturbances may compromise effectiveness of glucocorticoid treatment in COVID-19 disease and other diseases where glucocorticoid treatments are used. So now I would like to show you some new data on the inflammatory resolution pathway, the so-called specialized pro-resolving mediators. They are based on omega-3 fatty acids that are contained in various foods, salmon, avocado, flax seeds. And there is also lots of omega-3 supplements that many people take to control inflammation. And activation of inflammatory pathways in general leads to these initiation of the resolution pathways. And failure to mount resolution mechanisms has been shown to prevent the return to homeostasis and contributes to the development of inflammatory diseases. So when we look at COVID-19 disease, in my COVID-19 disease it has been shown that the resolution mechanisms are activated, but in severe COVID-19 disease they fail, they're not activated. And I think what's important is also that these still remain disrupted 12-25 days after resolution of clinical symptoms.

So what I would like to show you now is that sleep disturbances may contribute to the dysregulation of the resolution pathways. So here I show you the D-series resolvins, this is the precursor. At baseline, we see a reduction with sleep disturbances that continues into the recovery period. And again, there are resolvins D3 and D4 that are still reduced. So this may mean that sleep disturbance contribute to the persistence of inflammatory dysregulation by down regulating these inflammatory solution pathways. So in summary, I wanted to make the point that sleep disturbances can dysregulate multiple pro and counter-inflammatory pathways, and some of them have been also shown to be dysregulated in long COVID. I think it's important to notice that these dysregulation persists following recovery sleep, sleep disturbance increased infection risk.

I haven't talked about autoimmune disorders, but there's more and more data out now showing that insomnia perspective is associated with increased autoimmune disorders, which I think is important to know because autoimmune mechanisms have been suggested in long COVID. And when looking at the vaccination outcomes, B and T cell responses are reduced by on average 50%. And most importantly, the clinical protection is

reduced by approximately 20% when sleep duration is less than six hours. So what are the clinical implications and the research opportunities? Of course, we have to, and we can manage sleep disturbances to reduce inflammatory dysregulation, but also the many other consequences of sleep disturbances. I think what has been mentioned in the beginning of this session is to understand the role of these micro-structural sleep indices in promoting inflammatory homeostasis, there's data out now that show correlations between slow wave sleep and a better antibody response to vaccinations. We have to be careful with sleep disturbing medications in the management of long COVID in particular opioids that have sleep disrupting effects in the long term.

I think we need to better understand the role of sleep in the persistence of other common long COVID symptoms, such as fatigue and pain. And it's important to also better understand sex differences, the reasons for these sex differences, to better treat both females and male in long COVID. And I close here and thank you very much.

## **Dr. Sairam Parthasarathy**

Thank you, Monica. Both you and Janet really dive deep and I'm going to continue along with regards to sleep and circadian dysregulation and PASC. Just wanted to start off with the slide to underscore the fact that the burden of sleep related issues is high in individuals with PASC and set the stage with regards to the four overlapping domains of how sleep is affected. A, you have sleep disorder breathing such as obstructive sleep apnea, which Susan Redline will be talking more in depth about after my talk. Excessive sleepiness or hypersomnia, and then shortened sleep due to either short sleep duration or due to insomnia as well as circadian rhythm disorders. And so these are the four overlapping domains that underscore sleep health, where you can see that it may not be a sleep disorder, but just less of sleeping excessively may be a problem. And as Monica pointed out, fatigue is overlapping in all of these four domains. When you have any one of these sleep conditions, it can manifest with a symptomatology of fatigue. So ties the sleep problem to probably the most dominant symptoms in past patients, which is fatigue.

And unlike certain other neurological involvement, the treatment of sleep conditions that influence both disorders and sleep state and timing could probably favorably influence the prevalence and severity of fatigue. So this is actually a meta-analysis of 1.7 million participant in studies involving 50 research studies, which is very recent in June of this year. And you can see that they lined up the symptomatology that were most prevalent. And you can see that the top five, you start out with fatigue, memory problems, dyspnea and sleep problems ranks fourth, that is in this meta analysis. However, I would caution you that sleep may actually be more prevalent, A, you need to compare it with a control group, which is the career study is the only study that's going to comprehensively look at that. But also that a lot of individuals with fatigue may have concomitant sleep problems. And also there's a certain bias in the sense that many of these studies did not really look for a sleep problem. And

again, the required study is going to be unique in actually studying the presence or absence of sleep problem in all participants using validated questionnaires.

And so we'll be talking a little bit more about that in the next few slides, with regards to how these questionnaires are going to address various domains. In another meta-analysis you can see that sleep difficulties including insomnia, which is shown on the bottom ranks third in the list, following fatigue and weakness or dyspnea, breathlessness. You have insomnia and sleep difficulties at 12%, at least in this earlier meta-analysis in February, as opposed to the previous one which was done in June, showed that the body of literature points to sleep difficulties being third on the list. So the study again is going to be the only comprehensive evaluation that meticulously assesses sleep problems in all participants, because these are pooled effects only in studies that actually looked at the sleep problem and may be underestimating. So what is the downside of insomnia? Insomnia has been associated not only with the patient-centered outcome of not feeling well and adverse effects on health related quality of life, but more importantly, insomnia is associated with greater risk for all-cause mortality.

And we see this all-cause mortality... This is essentially from the Tucson Epidemiological Study of Area Obstructive Disease, which is a community based cohort, which is looking at the origins and prevalence and incidents of obstruct air disease. And with the collaborators of the Tuscon cohort, which has been going on since 1971, we were able to see over a 20 year period and subsequently we have data on over 40 years because the cohort started 1971, that the presence of insomnia, especially chronic insomnia or persistent insomnia is associated with greater hazard ratio for death. And so you can see that intermittent insomnia is not as much associated with such a mortality risk. And with bank samples dating back in 1971, in this cohort, we were able to show that in these individuals' persistent insomnia, there was actually a pro-inflammatory milieu with the increase in serum C rector protein by the insomnia category.

So when you compare with never versus intermittent insomnia versus persistent insomnia, you can see there is a market elevation in these individuals over a 20 year follow-up period of the C rector protein, which is not how it was when it started, suggesting that there's a pro-inflammatory milieu in these patients with persistent insomnia. And notably in this particular study, the all cost mortality was driven by cardiovascular events as opposed to cancer related mortality, which connects the C rector protein with the mechanistic pathway for why it should be driving plaque disruption, and cardiovascular events. So coming now to the present, with regards to sleep a duration, which can be a manifestation of insomnia where there could be individuals within insomnia and short sleep duration, which has also been shown to be associated with increased mortality. But also this sleep duration could be a process of habit and work related issues or socioeconomic setting. So in this case, the slide is showing you that sleep duration is subject to health disparities during the pandemic.

So this is essentially data from about 28,000 individuals in Arizona. And the University of Arizona Antibody Surveillance Study was funded by both the governor, as well as the University of Arizona. And you see on the left panel here by ethnicity, that when you look at self-reported duration, Hispanics sleep significantly less compared

to non-Hispanics. So these are sizeable numbers, and there's a market difference and effect size of ethnicity. And when you look at race of these individuals, again, you see that there is health disparities with regards to, when compared to Asians and Caucasians, you find that individuals belonging to the BIPOC communities of Blacks and Native Hawaiians and Pacific Islanders are other category, which are of mixed race are sleeping less than Asians and Caucasians suggesting that there is a health disparity. And what we want to compare this with this is that as we all know, the COVID condition affected the BIPOC community disproportionately greater than the rest of the other populations, suggesting that there may be, as Monica pointed out, that the short sleep duration may be adversely affecting the immune system and increasing susceptibility to infection.

I wanted to refresh the slide that Monica shared with you with regards to how Eric Prather and colleagues at that time in the University of Pittsburgh showed that the amount of self-reported sleep duration was associated with a vaccine or humoral response to the hepatitis vaccine. And you can see a dose response curve there. But I've also wanted to share with you is just that poor sleep quality in the form of insomnia is associated with an attenuated immune response as shown in the right panel, which comes from north Texas work by Danny Taylor and colleague showing that when individual suffer from insomnia, they have a less robust humoral response to the influenza vaccines. So this is to all vaccines, not just hepatitis vaccine. And it's a matter of both the quantity of sleep, but also the quality of sleep that can affect immune response. And so this is a snapshot of how there's a huge amount of geographic variability.

On the left you see the CDCs surveillance data showing that short sleep duration are in these bell states that you can actually see with a darker color signifying a greater proportion of population in those states being sleep deprived or not sleeping the seven to eight hours as Janet earlier alluded to, which is the Goldilocks zone of period of time that people need to sleep as recommended by various consensus status, which derives from various population level data. And on the right side, you see data in January of 2021, which is pre vaccination for the most part, because it was only first responders and healthcare workers who had been vaccinated until that point. And you can see that there's a remarkable similarity across these states with regards to where sleep duration was reduced, how you see that's part of the case rates of COVID pre-vaccination time period seemed to be present prior to post-vaccination due to various states having various vaccination rates. This introduced a certain amount of variance, which I'm going to not be sharing at this point in time.

So what happened during the code pandemic? So again, I'm sharing with you some preliminary unpublished data from the University of Arizona Antibody Surveillance Study. This is again in a sizeable number of population at the U of A, statewide, across the entire state, about 17,000 individuals. You can see that on non-work days people get more sleep as compared to work days. You can see also that in the X axis, it's a time period of May 2020 through October 2020. So this is again on purpose sampling prior to the vaccine becoming available. And you can see that as all of us did our commute times reduced. And you can see that the sleep duration in the general population on work days increased because of reduction in commute times.

Now, again, that could be a preferential bias in individuals of lower socioeconomic class and BIPOC communities who are preferentially more densely represented in that group, that they may be shortening of the air sleep because they did have to commute to work and work in certain essential jobs that required them to actually be at the workplace as opposed to being able to be on Zoom. That's one. The second reason I wanted to show the slide is that the importance of concurrent controls in the RECOVER study. And we are glad that the RECOVER study has concurrent controls because we can't use historical controls when you're looking at non-infected people. When I shared the 17,000 individuals, it's part of a surveillance program, not all of these individuals were infected with SARS-CoV-2. And what I'm going to show you in subsequent slides as data that's suggested for all of these co-variant and confounders, so just age, sex race, median household income and the presence of any and all of these medical conditions.

So folks are familiar with how this SARS-CoV-2 virus gains entry into the cell by binding the H2 and are familiar with the various components of the Spike protein, as well as the fact that there is the nucleocapsid against which antibodies are mounted. This is again, data from the University of Arizona Antibody Surveillance Study by Ripperger [inaudible 00:46:01] lab, which shows that early on when there was disturbing results coming from Spain suggesting that there wasn't going to be immune resilience against this virus, this was the first data that showed that there was indeed neutralizing antibodies present in the form of anti-RBD and anti-S2 that were present that will confer some natural immunity as opposed to anti-nucleocapsid, which is more [inaudible 00:46:28] and doesn't last long. I want to share this slide with you. And I want you to note that when you're looking at these antibody, RBD, S2 are orthogonal antibody, there is the indeterminate zone where it's associated with more non-neutralizing effects, which is essentially cell assay to see if the serial of these individuals can through plaque formation in the laboratory.

And you can see that if you have low titers in the indeterminate zone shown in blue, that means you are non-neutralizing antibodies, as opposed to a robust neutralizing antibody presence if you are in the orange territory. And that was the threshold that was identified in this particular study. So what I wanted to show you is the relationship between both sleep dysfunction or dysregulation and immune dysregulation. What I show you here is sleep during non-work days and the humoral antibody response. You can see that out of the 17,000 individuals, only about 500 individuals at that point in time, between the deciles of time and the X axis may of 2020 to October of 2020 pre-vaccination who were zero positive clearly are in the indeterminate zone. And you can see that when we look at a function of time and that the individuals with immune dysregulation with the inadequate response of their humoral antibodies are also the ones who are sleeping more during non-work days.

Now, individuals who are clearly positive versus people who are clearly negative, as you know, the false positive and false negative rates were low in the south [inaudible 00:48:13] Antibody testing, there was no difference there. And so when you do group means, adjusting for time and all of the other cover rates I showed you before is shown in the middle panel in yellow and green. There's a greater amount of sleep or greater amount

of perhaps sleep drive or sleepiness in these individuals, as opposed to people who are clearly positive, who don't seem to have the sleep dysregulation. Similar data was also found on workdays and for the sake of privacy, I will not be sharing those slides with you, but this is as yet unpublished data.

But we realized that there was a large amount of confidence intervals on the previous slide so we decided to look at time as shown by Janet with regard to how we recommend seven to eight hours as being optimal, that people who sleep less than six hours or more than nine hours are associated with greater risk for cardiovascular mortality, as well as all-cause mortality in population studies. And we decided to look at this antibody titers, which is shown as log values here. And you can see that there is this classic in red U-shape appearance showing a biphasic response, suggesting that you want to be in the optimal seven to eight hour zone. But if you sleep too little, which it's a short sleep duration, or if you sleep deprive yourself, or if you sleep excessively, you're more likely to have a non-neutralizing amount of antibodies in your sera and therefore more likely to have some persistent immune dysfunction. Now, whether that ties in or how it ties in with our immunity, how it ties in with viral reservoirs, that those are very interesting questions, but this suggests such an association.

I wanted to share on the circadian rhythm aspect. So when we talk about gene environment interaction, the amount of sunlight or the presence of light or dark actually informs through eyes' retinal input into the optical radiations to the suprachiasmatic nucleus gene transcription in the suprachiasmatic nucleus, which is the master clock that regularly sleep. And so if someone were to go into a dark cave, this means the cave experiments, you essentially undergo delayed phase shift. Now, why is that important? Well, when you have the cellular missionary, every cell in the human body has the clock genes which keeps circadian time. And we know based on preclinical research that it's the hours of 06:00 where there's an exuberant NF kappa B response, which is tied to clock and bema 1 regulated NF kappa B response. In other words, the same insult with lipopolysaccharide or Cecal Ligation and puncture experiments in mice, you will have a more pro-inflammatory response tying in sleep and the circadian rhythms and the immune response and inflammation very closely to each other.

And so this is data I'm sharing with you in regards to the circadian rhythmicity of individuals who are admitted to a hospital and survived critical illness. This is pre-COVID data. And you can see there is a phase delay in their circadian rhythm patterns. There's both a phase delay shown on the left side, where there's a delayed phase of people who are sicker shown in orange lines, as opposed to people who are less sick shown in blue lines. And there's also reduction in the amplitude or the vigor of the response. And so this ties again to the cellular machinery, it's a bidirectional relationship. If there's circadian dysregulation, such as jet lag, you can actually create a pro-inflammatory milieu that's been shown in preclinical experiments. Similarly, if you have a pro-inflammatory milieu that can actually cause delayed sleep phase. And delayed sleep phase in epidemiological studies has been shown to be associated with mortality.

So the question is, can you tinker with this phase delay? And can you improve the phase delay? Can you tinker with the sleep duration and improve sleep duration, optimize sleep duration rather, and would you reduce

inflammation and would you improve outcomes? Those are the tantalizing questions, but we need to study this in individuals with PASC in a comprehensive manner, just in the RECOVER study. Whereas really notable was the linear association between advanced age and the risk for developing SARS-CoV-2 infection and severe life-threatening COVID disease. And so a lot of individuals have done work on CD8 positive cells and cellular senescence that actually confers that risk. And this is actually work showing how poor sleep quality shown in the X axis is associated with the reduction in telomere length of CD8+ positive cells suggesting that there's a close association with aging.

And what is more is that this work by Peluso and colleagues shows that there's a reduction in T cell assays, Nucleocapsid specific interferon Gama production by CD8 T cells, which are terminally differentiated. As well as CD8 T cells expressing CD107a, which is a marker of degranulation. So just saying that there is a close tie in with the relationship of PASC and the CD8+ T cells. And we know that those are relationship between CD8+ T cells and their senescence and the sleep pad duration, as well as sleep quality. So this is another way in which mechanistically we can actually perhaps improve T cell viability. So I wanted to end with this penultimate slide by saying that the disease burden is high and the RECOVER study plans to do home sleep studies, polysomnography, as well as questionnaires who looked at the multiple facets of sleep and put them into four distinct phenotypes.

And the beauty about this is that there are imminently efficacious, treatments that are available to CPAP or dental device for sleep apnea, wakefulness promoting agents for the excess of data and sleepiness and CBTi, sedative-hypnotics for insomnia and melatonin and light therapy for circadian rhythms, as well as there's some data on modafinil, armodafinil and ritalin on fatigue that can actually help with all of these symptomatology.

So in summary sleep duration is associated with an attenuated humoral response. We don't know the directionality, whether it's the abnormal sleep duration that causes the attenuation of the humoral response or vice versa. And of course, we don't know about residual confounding and reverse causation, which needs to be answered by intervention based mechanistic studies. Sleep problems are highly prevalent in this population. It's a highly, highly patient centered outcome. Patients complain about PASC and fatigue and sleep problems. We need to bring treatments to the four. It's eminently treatable. We have effective therapies that are already available. But we need to do better phenotyping so that we can practice physician medicine based approaches, bring in the right treatment to the right patient at the right time. And we also need to have concurrent controls. I'm glad that the RECOVER study has concurrent controls because there was an effect of time during the pandemic on sleep duration and we need to adjust for that. And with that, I will hand it off this Susan Redline and thank you for your attention.

## **Dr. Susan Redline**

Okay. Thank you very much. I'm particularly pleased now in the formal presentations with the discussion of sleep apnea, which is a specific disease, which however disturbs sleep in ways that you've already heard about

in terms of the disruption of sleep and even overlaps insomnia, to some extent although uniquely exposes the patient to intermittent hypoxemia. So what I'm going to do is to briefly introduce you to some key aspects of sleep apnea I believe relevant to COVID 19 in PASC, talk about the distribution of sleep apnea and the population and the overlap with PASC related risk factors. And then I'm going to review some of the mechanisms that link sleep apnea to some of the cardiometabolic and neurocognitive dysfunction and disorders that also are relevant to PASC. I will briefly talk about some data or about sleep apnea and COVID-19 associations. And then I'm going to leave you with the notion that there may be a bidirectional association between sleep apnea and COVID, and that sleep apnea may even moderate and amplify risk for PASC. And then I'll tell you a bit about measuring sleep apnea in recover and briefly some research opportunities.

So obstructive sleep apnea is a very common condition. It occurs in up to 60% of the adult population. It is characterized by intermittent obstruction of the upper airway as you can see here into the interfering with breathing because of these discrete periods of airway obstruction known as apneas and hypopneas, that result in hypoxemia, frequent awakenings and sleep disruption, turbulence of airflow or snoring, and then daytime sleepiness and fatigue. It is a disease that's manifests by a complex number of risk factors, including anatomic factors such as excessive fat or soft tissue that compromises the airway space as well as alterations in brain stem and other aspects of ventilatory control. And as I will mention, there's emerging data that inflammation may also increase risk of developing sleep apnea through effects on brainstem centers, as well as local airway responses. We define disease... As you can see here, this is a little snippet from a home sleep apnea study by these periods of apneas and hypopneas and that's an overall index with an index greater than five thought to be minimal sleep apnea and greater than 15, moderate.

Now, as I mentioned, sleep apnea is very common and very under-recognized in the population. So this is data we collected in over 14,000, mostly young adults, mean age of about 40 years from Hispanic community health study, from four sectors across the USA and six different Hispanic, Latino backgrounds. And as you can see, the overall prevalence of at least mild sleep apnea, an AHI greater than five was 25% with a somewhat higher prevalence than men in the light green compared to women. Even moderate sleep apnea was found in 10% of the population, again, somewhat higher in men than women. And interestingly, of those with moderate to severe sleep apnea, only 1.5% had reported a diagnosis of sleep apnea before we monitor them. You could also see that sleep apnea prevalence, here in blue is men and red in females, increases with age and the gender gap narrows with age. Sleep apnea also has some variation with race and ethnicity. And here we're looking at data from an older cohort, the Multiethnic Study of Atherosclerosis, which had a mean age of 67 years of the age and included white, Black, Hispanic, and Chinese American participants.

I first want to point out that 66% of this community based cohort across the U.S. had moderate to severe sleep apnea when studied with an in-home polysomnogram. And moreover, if we look at the clinical disorder we call sleep apnea syndrome, an elevated AHI and an elevated sleepiness scale here called the Epworth Sleepiness

Scale, we see that 10% of the population had met this definition with the highest prevalence in Blacks. And again, a small minority of these participants were diagnosed, especially in the minority groups. Now we're interested in sleep apnea because in some ways, and as I'll show you, it impacts multiple aspects of physiology leading to multiple manifestations that in some ways even mimics many of the outcomes we're interested in, in PASC. So sleep apnea, as I will show you, has been clearly associated with the development of cognitive deficits and accelerated cognitive decline.

In fact, brain fog, I first heard in association with sleep apnea. It increases risk of accidents and injuries as well as increases risk of multiple cardiovascular cerebral, vascular, and metabolic diseases, as well as leads to premature mortality. And there is some growing data that in fact, sleep apnea is associated with about a 30-60% increased risk of COVID 19 related mortality. Now, what might account for multiple adverse physiologic responses? And I think we have to first recognize that sleep apnea exposes a person to these nightly repetitive stresses where the airway closes and opens and where breathing and gas exchange is interrupted. So here you could see with obstructive apneas, you have really occlusion of the airway increased respiratory effort, you have these Muller maneuvers really exposing the heart to mock swings in intrathoracic pressure. Hypoxia and hypercapnia apneas may even last as long as a minute before the person resumes their breathing.

When they do resume breathing, it's usually as a result of an arousal from the growing CO2 levels and hypoxia and their sleep will move from a deep to a light stage or even wakefulness. And often there will be an autonomic response's sympathetic surge, and that will cause breathing to resume with saturations to then go up. But during this whole cycle, there'll be mocked fluctuations and blood pressure, heart rate variation, hyperventilation, and the cycle continues. Now in relationship to COVID and PASC I also wanted to show you really the list of more maybe molecular changes that may happen as a result of these cyclical changes. So for example, recurrent hypoxia and re-oxygenation. And here by the way, is a little snippet from an overnight oximeter. And you could see that oxygen saturation overnight, and we have eight hours of data. And this is someone with severe sleep apnea where saturations are 90, 96%, the beginning of the night where you could see this mocked, almost saw tooth pattern with these really, really deep desaturations. And these, in fact, the deepest desaturations occur periodically, they happen to be occurring in REM sleep.

And these desaturations and desaturations promote fluxes of free radicals, oxidative stress, inducing, endothelial expression, and suppressing nitrogen oxide leading to local vasoconstriction. And most importantly, endothelial damage, which as you know, has been reported in COVID and it may be part of the post-like syndrome. But there's a myriad of exposure. So in addition to the hypoxemia, hypercapnia arousal, you have the intrathoracic swings and pressure. And in aggregate these exposures do cause this sympathetic nervous system overdrive. And here's a very old slide from Rin Sommers showing really sympathetic, peripheral sympathetic outflow, being monitored in a controlled participant, and here's someone with sleep apnea during the day. So you get the overdrive and it persists during the day, and that could lead to systemic and pulmonary vasoconstriction. And then

with these exposures, inflammation as well as platelet aggregation, again, endothelial dysfunction, and these mock fluctuations in blood flow leading to problems of coronary perfusion, increased oxygen demand, cardiac ischemia, and electrical instability.

And here's a little schematic showing really the causal direction by which these exposures, in this case the intrathoracic pressure swings, the intimate and gas exchange problems and the sleep fragmentation, really may be leading through main pathways for cardiovascular and metabolic health through these pathways of sympathetic activation, inflammation, endothelial dysfunction, as well as metabolic pathways that include insulin and leptin resistance, lipolysis, impaired lipoprotein clearance leading to a wide range of cardiovascular metabolic problems. But in addition, these exposures also lead to multiple other issues, including daytime sleepiness, poor quality of life, reduced work performance, memory and cognition problems, mood disorders, and even growing evidence of increase for certain cancers.

Now, let me show you just a few studies to reinforce the magnitude of these associations. So here again is a study from the Hispanic community health study. And this was based on over 11,000 people who were free of hypertension and diabetes when they were first studied with a sleep study and then followed for approximately six years. And in the top half of the slide, you could see what the overall odds is, adjusted for multiple potential confounders of developing new hypertension in the overall cohort in men in orange and in green in women. And you could see that overall, the odds was about 60% likelihood of developing hypertension. Likewise, there was an increase in this somewhat weaker, but a significant increase about 25% of developing diabetes. Doc Cy, had just talked about insomnia and I just want to point out that insomnia also was associated with increased risk of incident hypertension. And we know that sleep apnea, cardiovascular disease closely aggregate.

So here is a slide showing the overall range of the prevalence of sleep apnea, depending on most conservative to more liberal definitions. And you could see whether it's Coronary heart disease, stroke, heart failure, and arrhythmias. Overall, the prevalences are about 50%. But moreover in multiple longitudinal studies, we've identified significant associations between sleep apnea and not only the incident hypertension of diabetes, but incident stroke, coronary artery disease, heart failure, and mortality with overall hazard ratios ranging from 40% to stroke, which was amongst the strongest to almost 300%. Now, observational data, always raised questions about causality, but here are what I think is nice data emphasizing the temporal associations, even with acute apneas adversely affecting the heart.

Here are two very different studies. So here on the left is what's called the case crossover study we did in the Sleep on Health Study, where within individuals, we modeled the likelihood of having either atrial or ventricular arrhythmia after an apnea relative to after a period of normal breathing. And what we found was that in fact, in any given individual, apnea itself was 18 times more likely to trigger an apnea than a comparable period with normal breathing. And that really gave very, very nice temporal association and really predicted that there would be one excess serious arrhythmia for about every thousand hours of sleep. And here's some more recent

data that used some very novel approaches of using implantable cardiac monitoring to assess both sleep apnea and a-fib burden simultaneously. And if you look at the overall severity of sleep apnea on the top, you could see, this is a given person, the nights that there were more apnea, there was more a-fib activity.

I also wanted to share with you that potential outcomes of sleep apnea as a potential risk factor for subclinical pulmonary fibrosis are even interstitial lung disease. And although this is a fairly new area, my colleague, Dr. Lederer has put forth this idea that sleep apnea through these current molar maneuvers may itself generate alveolar epithelial cell injury, release various proteins and inflammatory mediators that affect the lung integrity resulting in remodeling and then lung fibrosis. And we have begun to confirm some of these hypotheses in large scale studies. So here's some data again from the MESA study. And what you could see here, in fact that if you look at the association of obstructive sleep apnea with two measures of subclinical interstitial lung disease made by CT of the lungs using a measure called high attenuation areas or interstitial lung abnormalities, you could see that there is, even after adjusting the smoking and BMI, significant associations, when you get to moderate sleep apnea with these findings. And I emphasize this because there's quite a lot of interest in COVID's long term effects on lung fibrosis.

And I now want to just also not forget to review a little of the data about cognitive decline and sleep apnea. So one of the seminal studies was published from a study of older women, the study of osteoporotic fractures. And this was a modest cohort studied over four years, but really even a simple measure of the frequency of oxygen desaturation, ODI was associated at a moderate level, a 70% increase risk of developing mild cognitive impairment or dementia. And in fact, this study along with numerous other studies were included in a meta-analysis where the overall odds of developing MCI or dementia related to moderate sleep apnea was estimated at about 1.25. And this was a very nice figure from Dr. Mullins who really mapped also many of the EEG characteristic changes that you'll get in sleep disordered breathing as what's often seen in dementia and aging biomarkers.

But what is really interesting is sleep apnea can be treated with a mechanical stent known as CPAP. And in fact, many of these EEG markers of dementia and accelerated aging that are abnormal in sleep apnea versus controls, do reverse with CPAP. And we've heard a lot this last hour about inflammation, and I did want to note that inflammation is also part of that pathogenesis relating to sleep apnea with mortality. And here's a measure where we found that hypoxemia was associated with about a 30% increase risk of mortality in this older cohort of men called the MrOs cohort. But we also saw that hypoxemia was associated with numerous peripheral markers of inflammation clean IL-6 and CRP and interferon. And in fact, when we do a mediation analysis, it's really through a pathway of inflammation that hypoxemia in a large pot contributes to excessive mortality. But what's also interesting... And this has worked for my colleague Tianyi Wong using data from multiple cohorts. In fact, he used data from the three big [inaudible 01:13:48] health professional cohorts, as well as MESA.

And he looked at how CRP levels measured, in some cases, 20 years earlier, predicted new onset of sleep apnea. And he found, in fact that CRP not only predicted sleep apnea, but particularly sleep apnea associated with

sleepiness. And so, one thing I'd like to mention is sleep apnea does result in increase in inflammation, but inflammation may also increase sleep apnea. And there is emerging data, it's very limited that patients with recovering from COVID with persistent symptoms, that there's about a seven to 10% prevalence of newly diagnosed sleep apnea. And maybe there is this by directional association we need to care about. So now let me turn a little bit more briefly to COVID-19 and sleep apnea. And this was courtesy of my colleague, Brian Cade, who's been working with the Mass General Brigham Biobank to really understand this issue. And to begin with, again, is that sleep apnea aggregates with many of the COVID and past related risk factors, including older age, minority ethnicity, hypertension, diabetes, and especially obesity, as you can see by this word cloud from the Biobank.

Now, Dr. Cade, early in the pandemic published an article in the American Journal of Respiratory Critical Care Medicine using data from MGB Biobank, showing an association of sleep apnea as a risk factor of COVID-19 related death or ICU admission. And he's recently updated this in unpublished work and further looked at sex differences. So he analyzed data in about 30,000 or 31,000 individuals who had documented COVID-19 infection. There was an overall 10% prevalence of sleep apnea, and he used a very stringent definition for sleep apnea. And what you could see here in model three, which has been adjusted for demographics and obesity, that in fact, there is a significant association varying for about 40-60% of death. But interestingly, there is a stronger association in women than in men. And similarly, if we look at death mechanical ventilation, ICU admission, we see a significant association, but it's really driven by the woman.

And this is interesting because women also more typically have a type of sleep apnea that may overlap with aspects of insomnia. So there's some really interesting research opportunities there. And now I like to sort of express my gratitude to the NIH for really their interest in including sleep apnea in the RECOVER cohort. And I just wanted to point out that right now we are working at really organizing the procedures to do home-based sleep studies among individuals who trigger based on poor sleep quality and reported snoring, or have some levels of desaturations. And we've chosen a very robust and very easy to use portable sleep unit that will give us some very nice information on breathing by measuring oxymetry, overnight ECG, nasal flow and respiratory effort, and allowing us to measure some very standard measures of sleep apnea, as well as some advanced metrics. And there'll also be some further triggers that will allow a subgroup of these patients to also be studied in laboratory where we could also get that really detailed EEG and leg movement data, and further understand some of the neurophysiologic aspects of their sleep disorder.

So in summary, I tried to provide a little bit of an overview of emphasizing how common sleep apnea is in the population. I didn't tell you this, but it's particularly prevalent in minority children, as well as the syndrome associated with sleep apnea, particularly in African Americans, as well as in Asian Americans. It aggregates with COVID-19 risk factors, it's associated with increased mortality and morbidity with COVID-19, especially in women. There are some limited data that there is new onset of sleep apnea as in the post-COVID or the post-like period.

And I've spent a lot of time really emphasizing the multiple physiologic effects that sleep apnea can expose individuals to that may increase this susceptibility to pass or modify other COVID-19 outcomes. Some of which may have significant sex and gender differences. And in this particular schema, I really try to show how sleep apnea does interconnect with auto autonomic nervous system and release to sleepiness resulting in hypoxemia, affects the lungs, the heart and inflammation.

And in fact, I do believe that this addition of sleep apnea will give us a window to really understanding the roles of hypoxemia and sleep fragmentation as risks for PASC, as well as amplifying risk factors and hopefully identifying not only causal pathways, but even targets for intervention. And I thank everyone, especially my colleague Brian Cade who's led a lot of COVID work.

### Claire Quiner

Wonderful. We're going to jump right into participant Q&A. We'll start out with a question for Dr. Redline. Dr. Redline, can you tell us how long cases further exacerbated the alcohol substance mediated inflammatory response resulting in sleep disturbances and or disorders?

### Dr. Susan Redline

Oh, I am so sorry. The first part didn't come... Did you ask me about alcohol disorders?

### Claire Quiner

Right? Correct. How long COVID case exacerbated the alcohol substance mediated inflammatory response.

### Dr. Susan Redline

Yeah, there isn't a lot of data on this, but we do know that sleep disorder breathing because of the effects on mood and cognition may increase the opportunity for substance use disorders. And I think what's important is that alcohol, as well as other substances may make sleep apnea and sleep worse. So we can really get in these vicious cycles where disturbed sleep may promote substance use disorders and those substances themselves may exacerbate sleep causing this upward spire that we really need to get at.

### Claire Quiner

Wonderful. Thank you. We now have a question for Dr. Parthasarathy. Do you think that increased sleep pressure seen during acute infection may be a functional change to promote humoral response and conversion to

positive RBD?

## Dr. Sairam Parthasarathy

Yeah, no, that's a complex question. I do think there increase in sleep pressure, as I elicited in my talk may be associated with attenuated humoral response. But I do think that the process goes to some of the work that my colleagues, both Dr. Haack and Eric Prather have done in and with regards to how sleep adversely affects T cell and B cell functioning. So with regards to how this excess of sleep pressure causes the immune dysfunction, I think it's maybe the other way around in terms of the infection causing the dysregulated T cell, B cell function, which is then affecting. So it's a situation of reverse causation is what my personal thinking is. But of course we don't have the data to prove that other than the preclinical experiment data that Dr. Haack shared as well as others have published on... Now connecting that to RBD, that's a tough stretch. I think both Monica and Janet are working in the autoimmune space. They can probably comment on that.

Monica and Janet, did you want to comment on whether you believe there's autoimmune mediated neural damage that may be responsible for the cases of RBD that have observed, or is this just a manifestation of neuro-degeneration? Or is not a situation of neuro-degeneration? But go ahead.

## Dr. Monika Haack

Yeah, I think we need more research in this area. So what is right now clear is, and I mentioned this, that insomnia is an independent risk factor for many autoimmune diseases. So there's something going on with sleep disturbances that is involving autoimmune processes and there is work done on auto reactive T cells, but we still need more work in this area. And I think this is very important given the associations that have been reported in epidemiology on insomnia auto autoimmune diseases.

## Dr. Janet Mullington

And I think certainly the bi-directionality is coming through in all of this work that sleep is affected by pain and by sleep apnea and other disrupting factors. And sleep, the immune factor may be a little bit homeostatic in its response, potentially to increase sleep and slow wave sleep when the immune system actually needs it. So there may be this bi-directionality that's actually functional. So I think the research opportunities are there, it's very interesting work. Thank you.

## Claire Quiner

Wonderful. And one final question. If anyone on the panel could sum up this very complex question in one minute, could you expand on how social, sex and gender disparities may impact or contribute to PASC

symptoms?

## Dr. Sairam Parthasarathy

I guess I'll take that one. In a very complex manner, but all of them is actually in an adverse direction. And one of the most studied areas in terms of how we can do interventions to promote health and wellbeing in individuals who are affected by health disparities is social determinants of health. And as we all know, social determinants of health is actually the main determinant of mortality and longevity in the United States, unfortunately. And so there's so much of work we can do in genetics, autoimmune and immunity, but the bigger girl in the room is social determinants of health. So unpacking that question, I would say that they all trend in the adverse direction in terms of ability to test, ability to get vaccinated, especially for also individuals who are difficult to reach populations, such as individuals with disability and not just individuals who are in a health disparate setting because of adverse social determinants of health settings.

And so that is something that healthcare systems as part of President Biden's initiative needs to address structurally in order to address those social determinants of health, to truly improve the health of the nation with the knowledge derived by the RECOVER study and the clinical trials that overlay on top of the RECOVER study. We still need to do some effective interventions in SDOH, as it's called, in order to actually bend that health disparity curve, which we are still a bit ways from doing. But I believe there are going to be investments in that based on what we see with the most recent national code program and the president's memorandum that was literally released late last week.

## Claire Quiner

Well, thank you just a few closing remarks. Thank you so much to our wonderful presenters today, as well as a thank you to our audience for attending the seminar and engaging with us on the Q&A. As a reminder, a recording of this will be available on [recovercovid.org](https://recovercovid.org) within a few weeks. We'll also be posting a Q&A document that has responses to the questions that we received today, including those that we did not have time to address. Now, moving on to future sessions, our three seminars are held on the second and fourth Tuesday of the month from 12-1:30 PM Eastern Time. We have some exciting topics coming up and we hope to see you in future sessions. Thank you everyone, have a great day.

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