ME/CFS RESEARCH ROADMAP WEBINAR SERIES

NERVOUS SYSTEM

OPEN SESSION MEETING

FRIDAY, AUGUST 25, 2023

PROCEEDINGS

DR. VICKY WHITTEMORE: Good morning, everyone. I'm Vicky Whittemore. I'm a program director at the National Institute of Neurological Disorders and Stroke, and a program director who oversees ME/CFS research grants and works together closely with Dr. Koroshetz to coordinate the Trans NIH ME/CFS Working Group. So, it's my pleasure to welcome you to the first in a seven-part series of webinars that are being organized in order to develop a research roadmap for ME/CFS. The seven webinars will take place between now and December, and the deliverable from all of this effort will be a report to the NINDS Advisory Council at their May 2024 meeting. The goal of the research roadmap process is to assess current ME/CFS research and identify gap areas or opportunities to move the field toward translational research, translational studies, and into clinical trials to develop treatments for ME/CFS. The research priorities identified will be used to guide research across the community, not just by the National Institutes of Health. The ME/CFS Research Working Group -- can you please advance my slides? I don't see how to

do that. Thank you. So, the NINDS ME/CFS Working Group of Council is composed of diverse stakeholders, researchers and clinicians, non-profit advocacy organization leaders, and the broader ME/CFS community, including individuals with lived experience, including individuals who have ME/CFS and caregivers. So, the Working Group includes five individuals with lived experience, and we've invited an additional 27 individuals to participate in the various webinar planning groups. These individuals self-nominated through a process that we initiated early this year. And we'd like to thank all of them, all of the individuals of the Working Group of Council, as well as the individuals with lived experience for participating in the development of the research roadmap. So, this webinar is being recorded, and the recording and transcript will be posted for the public. And we'll send out an announcement when that is ready. Once the research priorities are identified across all of the webinars, the Working Group of Council will put them all together into a final report for the council in May of next year. In the meantime, after each of these webinars, we will be posting the research

priorities on a crowdsourcing platform called IdeaScale that we will also send out an announcement about so we can receive additional input and feedback from the community. So, today, the individuals from the research -- and if I could have the next slide, please. The individuals from the Nervous System Webinar Planning Group have joined us, along with the co-chairs of the Working Group of Council, Drs. Maureen Hanson and Cindy Bateman. I'd like to recognize all of the members of this planning group, specifically Jarred Younger, for all the work that you've done to organize this first webinar. I'd also like to thank the NINDS team -- if I could have the next slide, please -- who's worked behind the scenes to get all of this organized and to work with all of these working groups, as well as our Holly Riley and our team at RLA who are providing the logistics and have helped us with all of the organization up to this point. So, everyone's welcome, as you listen to the presentations, to enter your questions into the Q&A by selecting the Q&A button at the bottom of the screen. And we will be taking those questions. So, if you have a question specific to

a speaker, please indicate that, and we'll be taking some questions specific to each speaker right after each of their talks if there's time. And then we'll utilize those questions as well at the very end of the webinar for the longer -- hour-long discussion. So, with that introduction, I'd like to take this opportunity to introduce the chair of the Nervous System Webinar Planning Group and the moderator for today's webinar, Dr. Jarred Younger. Jarred is a professor in the Department of Psychology at the University of Alabama, Birmingham, with secondary appointments in anesthesiology and rheumatology. He's also the director of the Neuro-inflammation, Pain and Fatigue Laboratory and a member of the UAB PAIN Collective. He's currently funded by NIH and the Department of Defense to study new techniques for diagnosing and treating neuro-inflammation. With that, I'll turn it over to you, Jarred. Thank you.

DR. JARRED YOUNGER: Thanks, Vicky. And I am going to just go right in and introduce someone else. That's what we'll be doing most of today. We've got a list of speakers that was really our wish list, and we got our entire wish list and

so, we're very grateful that everyone agreed to talk. These are the people at the top of their field. So, this is going to be a good half day. I could go on and on with acknowledgments for each speaker, but every second I see -- -- whole things they're going to talk about. So, I'm going to very briefly introduce each speaker. And we're going to start with Trisha Fisher, and she's going to give the first talk, which is the lived experience talk. Scientists, we carve a path, and we hope that that ends in a place where we can effectively meet the needs of patients. And so, we are very grateful for the individuals that can articulate the experience of ME/CFS, as very difficult as that is. So, Trisha has, I think, almost 30 years of lived experience with ME/CFS. And there were relentless efforts of her mother and the New Jersey ME/CFS Association. And through their efforts, she was diagnosed at the age of 12 with ME/CFS. And she, much later, continues to meet those challenges. She's a wife, mother of two children, and the treasurer of a New York City-based private equity firm. And she's also a gifted communicator. So, I'm very much looking forward to hearing her perspective

on the future research in ME/CFS. So, I will turn it over to Trisha for the lived experience talk.

TRISHA FISHER: Thank you. My name is Trisha Steefel Fisher. I'm 40 years old. As already said, I'm a wife, a mother, and a full-time finance professional with a daily 3-hour round-trip commute. I'm an ME/CFS patient, and I am one of the lucky ones. I was diagnosed in 1995 after recovering from Epstein-Barr virus. A few weeks of resting and dancing the tri-hourly Tylenol-Advil shuffle, and I was okay. And then one day, I wasn't. For context, in 1995 I was 12 years old. More people were familiar with the term yuppie flu than the diagnosis or even the veracity of chronic fatigue syndrome. There was no Google, and I couldn't ask you. In 1995, during the late-night hours that I was too tired to sleep, which is the most frustrating contradiction of all ME/CFS symptoms, I learned I wasn't dying from watching midnight reruns of "Golden Girls". Season 5 Episode 2 was titled "Sick and Tired". Despite repeatedly receiving a clean bill of health after months of physical and cognitive impairment so debilitating, she had to stop working, Dorothy Zbornak was diagnosed with

chronic fatigue syndrome. And I could have written the episode myself if brain fog didn't make it too hard to find the words and if pain and fatigue didn't keep me from grasping a pen tightly enough to write on the days that I couldn't even lift my arm. I just made the episode more exciting by adding a fainting spell from intermittent hypoglycemia and I just complicated it with postural orthostatic tachycardia. I am lucky that this rheumatologist didn't dismiss my symptoms as growing pain or puberty or anxiety, which happens more than once. I'm lucky I didn't wait months or years for a diagnosis, and I'm lucky to have the opportunity to speak with you today. You are aware of the symptoms that plague ME/CFS patients' central nervous system. But what might be less obvious are the complexities inherent with CNS symptom management for an illness intrinsically linked to the immune system and often further complicated with overlapping comorbidities. Without dwelling on the impact that ME/CFS has had and still has on literally every aspect of my life, and sparing details about that one time the pediatrician asked, "Oh, you still have that?" Or the three

obstetricians who didn't understand why I would rather recover from a planned C-section over the post-exertional malaise of attempting to naturally deliver my 10-pound son, here are some of my experiences with CNS symptom management. The stimulant drug modafinil, 200 milligrams twice daily, with 16 to 20 ounces of coffee an hour later, treats cognitive impairments well enough to get me through a workday. Some days I need more, and other days the exhaustion of busing to work and sitting at my desk is so intense that taking the afternoon dose is a waste of medication. Assuming I'm not too fatigued to remember it. As a young teenager, I stuck with antidepressants for suspected fibromyalgia. Helped with pain, but caused obesity and the long QT wave syndrome, so it was discontinued. My cardiac health returned, thankfully, but I struggled with obesity until fairly recently. Neurontin and similar drugs used for fibromyalgia were not effective. In my late teens, a four-month course of low-dose antibiotics, [unintelligible] and corticosteroids, got in front of the recurrent sinus infections that became bronchitis, if not treated within 24 hours

of first sniffle. Once discontinued, the cycle of recurrent sinus infection bronchitis returned every autumn until a balloon sinuplasty six years ago. A combination of triptans, behind-the-counter Sudafeds, and NSAIDs, treats my pain with light sensitivity migraine. I'm randomly sensitive to smell or touch and sometimes I get a sudden onset of what my husband and I call, the issues which could mean a migraine is coming or a crash. I take three pills ranging from melatonin to Ambien the nights that I can tell a migraine was triggered by fatigue. I take Soma for lower back spasms or for acute stabbing pains in my intercostal muscles. I take narcotics for whole-body pain, and I chart everything. So, I don't accidentally take a dangerous amount of anything. 50 to 100 milligrams of diclofenac potassium help inflammation without causing cognitive impairment. I try to avoid medications that cause cognitive impairment, though I do keep bottles of emergency narcotics in my nightstand next to the emergency prednisones. And I have two vials of injectable Acthar Gel in the fridge as a last resort. Last resort because it's $30,000 a vial and the financial assistance program is so

administratively burdensome. It causes post-exertional malaise. Also in my fridge is Cosentyx for the psoriatic arthritis I was diagnosed with at age 33. A rheumatologist said I'm a classic fibro case until he noticed the scars from the bilateral total knee replacement, I had at 25 for what the surgeon called total side compartmental degeneration that rivaled a retired football player. I started taking a low dose of the immunosuppressant sirolimus in November of '22, and the fog started lifting. There's a difference between fog and fatigue. Half the time, I can push through the fatigue with modafinil, but then I crash, and I need a few days to recover. Fog is different. It's a dissociative jet lag that renders me unable to make simple decisions or find words. Once, sitting on a toilet in my childhood home, I couldn't find the toilet paper, which was on a roll on the toilet paper holder attached to the wall, where it's always been. My fourth day on sirolimus I realized it was working when I got home and didn't need my husband's help to cut the dinner on my plate. I bet most ME/CFS patients gravitate toward finger foods over anything that requires a fork and knife. A

microwavable pizza pocket doesn't require strength or energy or dexterity and it's less likely to cause post-exertional malaise. It's also a lot easier to navigate from inside the fog. I'm lucky because my recent progress is not short-lived, but there are two sides to every coin. I spent three decades too fogged to comprehend this kind of pain. Life outside the fog means I am acutely, profoundly aware of my pain. And chronic pain causes chronic fatigue. I'm lucky, 30 years of trial and error has taught me and most of my family. Because it takes most of my family, including my children who still don't have the mother they deserve, how to balance my symptoms with the rest of my life. And as painful as it is, I'm lucky now more so than ever to apply what I've learned about CNS symptom management from outside the fog. So, please make this your compass when defining the ME/CFS research roadmap. Symptom management in a vacuum is not working. I'm not discounting its importance, but we must treat and prevent the entire cascade of central nervous system disruption to regain any quality of life. I am by no means healthy, but I am the healthiest I've

ever been. I'm 40 years old, a wife, a mother, and since 1995, a full-time ME/CFS patient. And I am one of the lucky ones. Thank you.

DR. YOUNGER: Thank you, Trisha. I don't know if everyone can hear me, I heard that my Zoom was not working very well. I hope it is working better now. If not, I will figure out what to do about that, but it looks better so far. So, thank you very much. I want to introduce the next speaker, Gudrun Lange. And Gudrun is a clinical neuropsychologist. She's at the Pain & Fatigue Study Center in New York. And there is so much clinical and scientific confusion and misconception when it comes to cognition in fibromyalgia or Gulf War illness or ME/CFS or Long COVID. Cognition is a core part of these conditions for sure, but sometimes, it seems to me that it's the least understood. And so, I'm very glad that Gudrun can kind of be the light in all the confusion. Every time I stumble upon a group starting to haphazardly incorporate cognitive measures into what they're doing, I can just gently say, "Hold on a second. There's somebody you should speak to first," and that is Gudrun. So, it's all yours.

DR. GUDRUN LANGE: Thank you very much, Jarred. And thank you very much, Trisha, for your report of the lived experience, because it taps right into what I'm actually going to be talking about today. John, I'm just going to say next slide, and you can go on to the next slide. What I'll be talking about today is the status of current knowledge about cognitive function in ME/CFS and the gaps in knowledge. But it turned out that I'm going to be talking mostly about inclusion of cognitive assessment in clinical trial design. And then finally, I'm going to focus on what we can do to make it better as we go forward. Next slide, please. We have a rich history of neuropsychological research for about 40 years, about 25 of those I've been around. And we know at this point pretty much that it's there. We know its nature. It is mostly focused on complex information processing and efficient information processing, as Trisha was so well describing before. And it is perceived as severe as disabling. In fact, very early on when Jason already estimated that cognitive function is perceived by about 89% of ME/CFS patients. And the deficits they perceive most commonly are

described as memory and concentration problems. And I'll go into that a little bit later. Next slide, please. When I reviewed the literature, it quickly occurred to me that I have written off a lot with this presentation. And I was surprised by the fact that cognitive assessments are not very often used in clinical trial design, despite the fact that we know that they are real, that they are not manifestations of depressive disorder, even though depressive disorder can be comorbid. And I should have also put that they are not manifestations of somatic symptom disorder. They are real. They are not due to poor effort, no motivation to do well on cognitive tasks. They are a real deficit and impact life significantly. Next slide, please. I'm going to briefly talk about patient-reported measures of cognitive dysfunction in ME/CFS. Most measures addressing cognitive function in ME/CFS studies and trials turn out to be patient-reported outcome measures, also called PROs. I have a little list here of patient-reported outcome measures that are often used in clinical trials. The first three are specific to ME/CFS and the rest is not specific to ME/CFS. With the SF-36

pretty much used in most clinical trials, but not as a primary outcome measure, but somewhere in the secondary outcome measure list. What's missing on this list are questionnaires that are specific to cognitive function. And it's not that they're not available, they are. They're normed and standardized. Next slide, please. If you're interested in finding out more information about the use and efficacy parameters of the tools I've just presented, this is a really good resource. It's the "Management of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome Report: Updated Review" that was prepared for the CDC by Pacific Northwest Evidence-based Practice Center and published in April '22. It gives you before and after treatment group comparisons of patient-reported outcome measures that have shown to have not only statistical significance, but also estimates of meaningfulness -- of clinical meaningfulness, which is a measure that has to be taken into account when you report data of clinical trials, and that is often lacking. Assessment of cognitive function is not commonly used as a primary or even co-primary efficacy outcome measure of function. And by function,

what is often meant are categories of mental fatigue, in general, attention concentration memory problems, but not specifically addressing the processes that underlie cognitive dysfunction in ME/CFS. Now, the next slide, please. There is no doubt that the use of patient-reported outcome measures in clinical trials is appropriate if the research question is as follows, "What is an individual's perception or experience of cognitive dysfunction in comparison to a previous timepoint?" This question reflects inquiry into a person's interpretation of what cognitive dysfunction means to them. Cognitive dysfunction is often perceived as catastrophic, as was so well described by Trisha this morning. As complex cognitive processes are affected, that previously ran sort of in the background, automatically. And now, all of a sudden, they have risen to consciousness and the person has to attend to them. And to make this experience a little bit more real life, a lot of our patients describe the changes in their driving activities. Since we were 18 years old, most of us learned how to drive. So, we get in the car, turn on the car, get out of the driveway, and do what we have to

do to get to where we need to get. Not so for ME/CFS patients or persons with ME/CFS. They have to now think about every little step of driving a car. So, the environment becomes much more stressful and impactful on cognitive function. But also, the person in the car sometimes describes that they're talking to themselves saying, "I have to now turn the blinker out. Oh boy, where's the light switch in this car?" even though they've had this car for a long time. Some people are missing the exits that they use every day. Some people even drive by their homes or end up in a different street in a neighborhood that they lived in for a long time. This is highly disturbing and is really, really sad. Next slide, please. Now, we'll turn to the objective assessment of cognitive function in ME/CFS. Importantly, cognitive function, rather the processes underlying cognitive function or cognitive dysfunction, is one of the few symptoms of ME/CFS that can be objectively quantified psychometrically with valid and reliable measures. So, how come we don't see it more often? I reviewed Kim et al.'s recent systematic review of primary outcome measures for ME/CFS in

randomized controlled trials. They considered 500 trials but only 52 passed eligibility criteria for review. About 60% of the RCTs in the analysis used a single primary outcome measure. The rest used two or more co-primary outcome measures. My review of these 52 trials revealed that only 11 RCTs used an objective cognitive measure administered in clinic, face-to-face, as one of the primary outcome measures. A majority of the remaining 41 trials employed patient-reported outcomes, most of them non-specific to ME/CFS. The objective neuropsychological measures used included brief measures that are often not sensitive enough for statistical significance, never mind clinical meaningfulness, a topic that I'll address in the next few slides. The outcome measures that were used were Digit Span forward and backward; Symbol Digit Modalities, which is actually a robust test; Trails A and B, not so much; the Stroop, yes; and the computerized arithmetic task, which I don't know what the norms are. It's one of those lab-specific things that I use to evaluate cognitive function. Next slide, please. The recent "Systematic review and meta-analysis" by Sebaiti et al. makes some very

important points. They looked at original case control research studies using objective cognitive measures published between January 1988 and February 2019. All studies included in the analysis evaluated ME/CFS adults that were clinically diagnosed with ME/CFS and had no neurological or psychiatric comorbidities and compared them to healthy adults. The group applied rigorous statistical inclusion criteria -- I was happy to see -- that included the following measures under investigation had to have been used at least two studies. Important because it's hard to compare tools across -- assessment tools across studies if they're used in a non-standardized way. The group differences between ME/CFS and controls needed to be of statistical significance at the P 0.05 level and had to be accompanied at least with a moderate weighted effect size of 0.5. So, here I'm going to briefly address why it's important to include an effect size as recommended by the American Psychological Association, by the way. A P-value of 0.05 simply infers that a comparison between group means is applicable to 95% of the sample under study. The calculated effect size between

groups, as defined by Cohen, informs about the magnitude or size of the effect. Does the difference between group means actually have practical or clinical significance? In order to determine that, Cohen defines effect size in the following way. 0.2 is a small effect size, negligible effect size, 0.5 is a medium effect size, and often considered the minimum threshold in treatment trials. An effect size of 0.8 or greater is considered large and rarely robust. Since calculated effect sizes are standardized measures, for instance an effect size of 0.5 equals half a standard deviation, independent of sample size, comparison of effect sizes across trials is possible. Now, there are criticisms of Cohen's effect sizes. But in my book, at least I provided some framework of evaluating clinical meaningfulness. Next slide, please. In the interest of time, I will focus on the gist of the information provided in the next three slides. They all address whether clinically meaningful changes were obtained with traditional neuropsychological measures listed on each of the slides in domains relevant to cognitive dysfunction in ME/CFS. Sebaiti and co-workers

found no differences in performance between ME/CFS and controls on tasks tapping in the domains of overall intellectual function as measured by the NAART and also WAIS or subtests that tap into verbal comprehension. In the executive functions with a focus on impulsivity, ability to conceptualize, and strategize without time constraints, and in what we call instrumental functions, focusing on language skills, visual functions, and computational abilities. In my opinion, these are important negative findings as they affirm that especially intellectual functions without dynamic subcomponents, but acquired over a lifetime, is likely to be normal in the ME/CFS. So, it does not signal -- the likelihood that it signals a neurodegenerative process is fairly low. So, it is important to know about cognitive strengths as it helps to highlight cognitive weaknesses. So, in my opinion, an estimate of overall intellectual function should be included in every clinical trial RCT addressing cognitive function. These tasks are quick, take maybe five minutes of research time, no practice, and can be administered remotely. Next slide, please. Now,

stepping into simple or even complex reaction time and speed of motor movement or coordination provide inconsistent results across studies due to variable effect size across studies. Unfortunately, some of these simple tasks are used as trial outcome measures, and they are not very informative. So, I would not recommend using any of them, especially as a primary outcome measure. Next slide, please. Next slide. Thank you. Sebaiti and co-workers reported robust findings that these moderate effect sizes and low variability across studies in the following domains. Sustained and divided attention with working memory and speed components, reflecting multitasking, which is our number one problem for patients or persons with ME/CFS. The tasks used here are the Continuous Performance Test and the conditions were numbers with and without extraction, as well as the Paced Auditory Serial Attention Test. There are several versions of that test. The one with four conditions is going from very quick interstimulus intervals to longer interstimulus intervals is the one that will give you a medium effect size. Another area of robust findings is another area that is of interest, and

it's affected in ME/CFS, which is processing speed. Symbol-Digit Modalities Test or the WAIS Coding Test is the most commonly used for that. And then we go to the area where memory deficit is perceived. Memory deficit in ME/CFS is most commonly due to difficulties with encoding and learning of novel information and is not the memory problem that we generally see in patients with dementia. So, when patients report that they have a memory problem, they report it because they cannot remember the entire information that they were supposed to absorb but didn't. But you can only recall that information that you encoded. If you didn't encode it, you won't recall it. But they do not forget information. So, that's enough about this. Can you please switch to the next slide? So, I already alluded to some gaps in knowledge that could be possible reasons to account for hesitancy to include objective neuropsychological measures in ME/CFS RCT design, clinical trial design. Two methodological issues are really obvious. First one is that traditional neuropsychological measures used are clinic-based. So, the subjects need to come into the office, must be well enough

to travel. Supervision is necessary to ascertain proper test administration and completion. Another problem is or rather the problem associated with the travel issue is that potential participants may already be exhausted just from the experience of travel to the study site. So, we don't know how that impacts on cognitive function that's measured after their arrival. The second big deal is that investigators often focus on the use of brief measures that may only have small negligible effect sizes, such as the WAIS IV Digit Span Forward and Backward, and thus may not be robust indicators sensitive to clinically meaningful changes in group means due to an intervention or treatment. Therefore, I recommend that investigators shy away from using these measures as primary or co-primary efficacy measures, even though they are perceived as quick, easy, and not tapping in a lot of research time. Next slide, please. At this time, I would like to shine a special light on the participant recruitment issue that I kind of alluded to in the previous slide. Due to the predominance of clinic-administered neuropsychological measures,

researchers are often not able to include patient groups that cannot travel to the research site. Those are homebound or even bedbound ME/CFS patients. An estimated -- about 836,000 to 2.5 million Americans suffer from ME/CFS and about 10 to 25% of these are estimated to be either homebound or bedbound. Patients are rarely, if ever, participating in studies employing objective cognitive evaluation. Even though homebound or bedbound persons with ME/CFS might be the important beneficiaries of pharmacological or non-pharmacological treatment and thus need to be captured in RCTs. Next slide, please. So, how can we move forward to integrate cognitive assessment in clinical trial design? Cognitive batteries now exist that can be administered remotely. They more and more often include practice trials and easy to understand instructions, possibly reducing the workload on study personnel, facilitating use of in-home test administration as well. The COVID pandemic helped us to accelerate development in this area. An increasing number of studies show face-to-face and remote in-home test administration produces equivalent results even for neuropsychological

measures that have shown to produce robust group differences between ME/CFS and healthy controls, those traditional neuropsychological measures I mentioned earlier. Next slide, please. Some of the remotely administered batteries that have been used in ME/CFS samples are listed here. They include the NIH toolbox that has the Flanker test that we evaluated. The Flanker test is a little bit difficult to administer. The Cambridge Neuropsychological Test Automated Battery, computerized reaction time measures, the Continuous Performance Test third version, and the CogState Brief Battery. Next slide, please. Under the leadership of Dr. Unger, the CDC has tried to address some of the questions I posed with the multi-site clinical assessment of ME/CFS, also called MCAM, cognitive substudy of 261 ME/CFS and 165 healthy controls. We are currently preparing the findings for journal submission. The goals of this substudy were to evaluate a brief computerized neuropsychological screening battery that can be administered reliably and repeatedly in clinic and remotely at home after an exercise challenge. We needed a brief computerized cognitive battery that had

been created and used in populations with similar cognitive dysfunction as seen in ME/CFS. And that was a sample of patients with mild traumatic brain injury. The battery also needed to be able to be administered in clinic as well as remotely at home. It needed to be sensitive to cognitive deficits shown to exist between ME/CFS versus healthy control and had to have good test-retest reliability to assess cognitive function following an exercise challenge over time. Next slide, please. Based on our review of the peer-reviewed data in the early 2010s, when we conceptualized that study, we decided to use the computerized cognitive brief battery developed by CogState. The battery includes six standardized short tasks, psychometrically appropriate for detecting cognitive change and within subject designs. Administration is short, 17 minutes plus minus one or two. Our outcome measures were accuracy, performance accuracy, and speed of performance. Next slide, please. I will not go into the details of the study at this time. We will report on it soon in our upcoming paper. The important point I want to make here is that there was no difference in performance accuracy on any

of the CBSB tasks, confirming previous observations by our group and others. We found significant statistical differences, though, on all speeded outcome measures. So, the take-home point right here is speeded outcome measures most likely give you a result of group differences while accuracy outcome measures do not. Next slide, please.

DR. WHITTEMORE: Gudrun, this is Vicky if you could please wrap up in the next minute or two.

DR. LANGE: Okay. I'll try. On the left side of the slide, you see a graph showing the mean latency of a simple reaction time task. We observed small but statistically significant group differences in simple reaction time and used it as a covariate for the other tasks. On the right side of the slide, you see the Groton Maze Learning Task which showed to be very effective in finding differences across groups. Slower is lower and the blue line is the healthy controls. They're doing significantly better to a clinically meaningful degree. Next slide, please. And here's the task. I'm not going to go into it in the interest of time. Next slide, please. At this time, the CogState Brief Battery is

validated for unsupervised at-home use in cognitively normal as well as mild cognitively impaired individuals, and it's not perceived as overly stressful or tiresome. CogState also has been used in other ME/CFS studies with large sample sizes at this point. Next slide, please. So, I'm not going to go over all of these points because of time issues. But we know which areas are affected in ME/CFS in terms of cognitive dysfunction. We have standardized tools sensitive to differences and changes in cognitive dysfunction and need to use them appropriately. Next slide, please. ME/CFS cognitive dysfunction can be assessed remotely, it's cost-effective, and we can reach people that we haven't been able to reach with traditional methods. At this point, I'm going to wrap this up with the last slide. Next slide, please. And I would like to direct your attention to the NINDS Common Data Elements compendium that is available on the internet and has a compendium of neuropsychological measures in there that have shown to be sensitive to differences between groups in ME/CFS. And with this, I'm finishing up. Thank you for your attention. I hope this was informative.

DR. YOUNGER: Thank you. Awesome. I really appreciate it. And we're going to move right along. We had a little bit of time for Q&A, but I know everyone has so much to talk about. So, we're just going to keep on with the talks. And I want to introduce Dr. Rowe. So, Peter's a professor of pediatrics at Johns Hopkins. He's director of the clinic at the Children's Center for Chronic Fatigue. And he's going to be talking about dysautonomia. There are lots of ways I could introduce Peter, but I will say that there are very few people that are willing to tackle ME/CFS and Ehlers-Danlos syndrome and mast cell activation syndrome. And then when you take that group and you reduce it down to the people who are willing to tackle those things in children, that is a rarefied and a heartbreakingly small group. And so, Peter is essential. He quite simply does the things clinically and scientifically that must be done, but no one else does them. And every time I hear of a new interesting research direction, I say, "Wow. Someone is pursuing this." Peter's name always comes up attached to it. So, I'm really excited to hear from Peter.

DR. PETER ROWE: Great. Thank you very much, Jarred. And thanks to you and Vicky for the invitation to present today. I think I can make these slides go ahead. So, I wanted to start off with Jarred's direction, which was what do we know about dysautonomia in ME/CFS? What do we need to know? And then how do we move forward with clinical trials? So, we've known for a long time that orthostatic stress can provoke the cardinal symptoms of this illness, including fatigue, but also including exercise intolerance, cognitive dysfunction, and PEM. And in fact, the first recognition that this might be the case, that I've been able to find, was published in 1940 by McLean and Allen. And they said they'd given the name of orthostatic tachycardia to a syndrome characterized by an excessive acceleration of the heart when the patients change from the recumbent to the erect posture, orthostatic exhaustion, blurring of vision, weakness on exercise, and syncopal episodes may occur. And they said this is a syndrome which seems identical with effort syndrome, irritable heart, or neurocirculatory asthenia, which were the names at the time for what we now call

ME/CFS. All they had available to treat patients was a high intake of sodium chloride, up to 16 grams a day, and then this elevation of the head of the bed that we see in the upper right. They went on four years later to publish another paper entitled -- subtitled "Defects in the Return of Venous Blood to the Heart." So, they understood in 1944, what we now call preload failure or inadequate venous return to the heart as being a key feature in the pathophysiology of these symptoms. Unfortunately, this work was essentially buried or ignored. We didn't know about it. But when we found out about it afterwards, we started looking at patients with ME/CFS in the mid-early 1990s. And in one of our studies, we had 23 ME/CFS patients who were a mix of adolescents and adults, 14 controls. This was a pilot study that was intended to help us estimate the sample size for a randomized trial of Florinef. But what we found was striking. That is all of the patients who we put on a 70-degree head up tilt had an increase in their fatigue and lightheadedness. They had warmth and nausea, whereas the controls were simply bored. We also learned very quickly that patients were unwell

for several days after the tilt test. But we introduced an intervention which was to give them two liters of normal saline intravenously, and so, that prevented the post-tilt exacerbation of symptoms and made them leave their clinic feeling better than when they came in. This work has been extended and done more elegantly by a number of other groups. Since then -- this is a slide from Julia Newton's group in the United Kingdom showing that if you measure a variety of autonomic symptoms using the COMPASS questionnaire, ME/CFS patients are clearly and significantly different than controls. Not only that, the degree of autonomic dysfunction on the COMPASS correlates with the Fatigue Impact Scale. We know that orthostatic stress can impair neurocognitive function. And so, one of the studies that helped really bring this out was done by Marvin Medow and Julian Stewart, who have been important investigators in orthostatic intolerance. And they showed that as you gradually increase the angle of the tilt test, ME/CFS patients had different problems on the n-back tests. And as you increase the complexity of the n-back test, they made more errors and had

slower reaction time. So, we often hear about cognitive dysfunction as an independent symptom, but it clearly can also be aggravated by orthostatic stress. I want to highlight a few papers from Linda van Campen and Frans Vissers' data in the Netherlands. I've helped with some of the writing of these papers, but the data are entirely theirs, and they deserve the bulk of the credit for this. But they looked at the influence of the tilt test on cognitive scores using a 2-back and a 3-back test. And as you can see here, after the tilt test, immediately afterwards, patients had worse scores on their cognitive studies. They also looked at what happened seven days after the tilt test. And you can see that ME/CFS patients had worse scores on numeric rating scales for concentration. So, this is suggestive of orthostatic intolerance being capable of provoking PEM. The symptoms of orthostatic intolerance are largely due to reductions in blood volume and venous return to the heart when people are upright. And that leads, importantly, to underperfusion of the cerebral circulation. So, if we look at this slide that summarizes the main pathophysiologic

influences on patients, they have an increase in the amount of pooling, possibly due to a defect in vasoconstriction. There's about a 10% reduction in intravascular volume in people with orthostatic intolerance. And these two things combine so that when they stand or are in an upright tilt position, they get a marked reduction in cerebral blood flow. And as David Goldstein at the NIH has termed it, they get an increase in the sympatho-adrenal response, a big increase in catecholamines and adrenal release. And that can lead to a variety of different phenotypes of orthostatic intolerance at the bottom. One is classical orthostatic hypotension, delayed orthostatic hypotension that occurs beyond the three-minute point, what we termed neurally mediated or reflex hypotension, postural tachycardia syndrome. And then some people can have low orthostatic intolerance with a normal heart rate and blood pressure response. When we tried to measure the cerebral blood flow differences, we initially began with transcranial Doppler, but could not identify differences between healthy controls and patients during a head-up tilt test. And I know that Dr. Novak, who

will be speaking later, has had better success with this technique. But van Campen and Visser came up with a method of measuring brain blood flow by putting their Doppler probe on each internal carotid artery for about 20 or 30 seconds, and then each vertebral artery for the same amount of time. So, that over a two- or three-minute time span, they can tell you by adding the flow through those vessels, the total cerebral blood inflow. We obviously don't know what is happening within the brain, but that's the amount of blood that is pumped to the brain. Their studies have shown that you don't really need to be an ultrasonographer to see the difference between the flow through each vessel when the person is supine and then when they're standing. And I want to show you the data from their very large study of adults with ME/CFS during head-up tilt testing using this technique. This study enrolled 429 adults, which was more -- almost as many as the cumulative published research that we had summarized in the Institute of Medicine report. So, this is a very large study, notice that 28% of these patients after 30 minutes upright met criteria for POTS, 14 had

delayed orthostatic hypotension. But the vast majority of adults had a normal heart rate and blood pressure response, 58%. And they might have been at risk for being told there's nothing wrong. But when you employ the cerebral blood flow measures, here are the key findings from that paper. Healthy controls have a 7% reduction in brain blood flow compared to their supine values after they've been up for 30 minutes. In contrast, the entire group of ME/CFS patients had a 26% reduction in brain blood flow. So, I think you could reasonably conclude that it's no wonder that these patients have difficulty with processing, as Gudrun was talking about, thinking of the right words and concentrating. When you break that group of all patients down, 90% of them had objectively measured reductions in brain blood flow, significantly different than controls. The ones with POTS or delayed orthostatic hypertension were worse. But the group that could have been dismissed as normal, the ones who had a normal blood pressure and heart rate response, nonetheless had a 24% reduction in brain blood flow, over threefold greater than what you'd see in healthy controls.

When these patients were all measured supine, the ME/CFS and healthy control groups did not differ with regard to their brain blood flow. This slide from that paper shows that midway through the tilt at the 10th minute, the degree of cerebral blood flow reduction was correlating well with the number of orthostatic symptoms that patients reported. This paper is more recent from their work. They looked at a group of people with normal heart rate and blood pressure responses to the tilt, and they were interested in looking at the relationship between heart rate and stroke volume. And that relationship should mean that if stroke volume goes down, we compensate with an increase in heart rate. And so, in healthy controls, you see the 95th percentile prediction interval here with the blue line of the slope of the change in stroke volume and the change in heart rate. What you see in the gray area is that close to 40% of adults with ME/CFS, typically the ones with more severe symptoms, were unable to elevate their heart rate during the tilt test in accordance with the drop in stroke volume, suggesting and consistent with what's called chronotropic incompetence, something that had

previously been identified during exercise testing of people with ME/CFS. But we think this was the first demonstration of this during orthostatic testing. What else do we know? We know that the risk factors for orthostatic intolerance can include seemingly disparate conditions. And Trisha was talking about a number of other comorbid problems. And I think we have come to appreciate that mast cell activation, joint hypermobility, vascular compression syndromes, and in a small subset, neuroanatomic problems can accompany the autonomic nervous system dysfunctions. Here's an example of one of the large studies comparing the hypermobile Dutch ME/CFS patients with the ones who didn't have hypermobility. And notice that the ones who are hypermobile had a far greater reduction in brain blood flow, regardless of the type of hemodynamic response they had to the tilt test. This slide is meant to illustrate some of these other conditions we know from the work at Vanderbilt by Cyndya Shibao and Italo Biaggioni, that postural tachycardia syndrome can occur in people with mast cell activation disorders. In the Theoharides' review paper in the New England

Journal, you can see, I think, here that many people with mast cell activation have cardiovascular problems, and many of the other symptoms of mast cell activation can mimic those of ME/CFS. On the bottom left is a patient we had cared for at Johns Hopkins who had some rather refractory symptoms of POTS and ME/CFS. We found later when her reflexes became abnormal that she had congenital narrowing of the cervical spinal canal and a disc bulge at C6-7, which her spine surgeon replaced. And within less than six months, her ME/CFS symptoms had resolved, her POTS had disappeared, and she's now well into her eighth year of follow-up having had previously refractory ME/CFS symptoms, now entirely healthy. And on the bottom right is some work from Steven Smith and his colleagues pointing out that abnormalities that involve vascular compression, in this case, May-Thurner syndrome, where you get the right iliac artery compressing the left common iliac vein, that these problems are associated with POTS and that treatment of these pelvic venous abnormalities can be associated with a nice improvement in overall symptoms, including the chronic pelvic pain. We also know

from the past that orthostatic intolerance is one of the most treatable components of ME/CFS. This was a slide from one of our earlier papers showing that with open treatment, not blinded therapy, but open treatment of orthostatic and colonoscopies with Florinef, midodrine, beta blockers and others, the patients who had volunteered for this study came in with a wellness score of 35 out of 100, where 100 means optimal health, zero means dying. And over four months of therapy, they increased to a mean of 70. And this is in many ways, still the data that we find clinically, perhaps a little bit better now that we have medications like ivabradine and a couple of others that can be used to treat orthostatic intolerance. Well, what do we still need to know about dysautonomia in ME/CFS? Well, we need better diagnostic tools, including, as the Common Data Elements Committee concluded, that we have to have better questionnaires that focus on orthostatic intolerance. We need, as anyone who's had a tilt test knows, more easily available, less taxing, and less expensive orthostatic tests. And I think it's important to have a better ability to identify both for

clinical care and for research studies, the patients who have refractory dysautonomia as a result of other structural problems that probably would not respond to medication intervention. These include cervical stenosis, cranial cervical instability, and some of these vascular compression problems. We need to know more about mechanisms. We don't know what initiates the circulatory dysfunction and whether it's one thing or multiple hits. So, it could be that the autonomic nervous system disturbance is a consequence of something else at a more central level with downstream autonomic and circulatory control dysfunction. We don't know how infection triggers dysautonomia. We need to look at the genetic factors that influence its development, since there's a heritable component to ME/CFS. And it may be that the dysautonomia is triggered by a peripheral phenomenon. This is a slide on the mechanisms of POTS from a paper that was published after an NIH meeting. So, connective tissue laxity may play a role in part by making the vessels more compliant and allowing more blood to pool when the venous hydrostatic pressure is higher. There is interest in what

role small fiber neuropathy plays in leading to excessive pooling. There's been a lot of interest in whether antibodies, autoimmune antibodies that can block the ability to vasoconstrict, or can activate cardiac receptors, increasing tachycardia. There might be a variety of reasons for central sympathetic activation, including neuroinflammation, possibly with mast cell activation. Cardiovascular deconditioning is more controversial. It's been thought to be the case as the part of the pathophysiology for ME/CFS in the past. But there's nice work from van Campen and Visser suggesting that the CPET results have nothing to do with the cerebral blood flow, raising the question of whether deconditioning plays a big role or not. And then we know there is an inadequate aldosterone response to standing that's poorly understood. So, these are among the mechanisms that need to be looked at. I think we also need to ask what other symptoms are impacted by the circulatory dysfunction. So, there's a lot of interest in neuroinflammation. But could it be the result of this suboptimal blood flow that we see in ME/CFS patients when they're upright? Is there some kind of perfusion, reperfusion injury

that is taking place? And then separately, we often think about immune dysfunction in this illness independently. But anything that elevates catecholamines in orthostatic intolerance will provoke adverse sympathetic immune interactions. We know that each of the lymph nodes has sympathetic innervation, and could these problems be secondary to the orthostatic intolerance, but nonetheless affect viral reactivation or responses to infection. And along those lines, I wanted to show two models for how symptoms might occur. This is from Klaus Wirth and Carmen Scheibenbogen in Germany. So, with upright posture, you get a reduced preload, orthostatic stress, increased sympathetic activity, which leads to tachycardia, but also to vasoconstriction. And they posit that this leads to underperfusion of skeletal muscles and a variety of vasodilators spilling out of there, also reducing renal function, and leading to various pain and other symptoms. And then this slide is a reminder that a variety of problems with increased sympathetic activation can have immediate impact on a variety of immune functions, including factors that might affect

the enteric nervous system. And so, I think more work needs to be done to connect these two areas, both the immune system and the autonomic dysfunction. We also need better treatments. We have a number of treatments that have been available in treating orthostatic intolerance, but very few studies that have looked at these comprehensively in people with ME/CFS. So, this includes vasoconstrictors, volume expanders, and drugs that control heart rate or catecholamine release or effect. But in addition, I think we need to think about whether you can address the dysautonomia using therapies used, for example, for mast cell activation syndrome, notably famotidine or cromolyn. I mentioned cromolyn because we've been treating a young man from Maine who has been refractory to all of the medications on the previous slide but had a lot of facial erythema and cutaneous erythema. And when we put him on cromolyn, he started being able to sit upright rather than being forced to lie down all day. My colleagues at Hopkins, to mention another example, Malcolm Brock and his Belgian colleague, Frank Bosmans, are looking at the combination of hyperhidrosis in POTS, in whom

they found that a proportion of these patients have a defect in a mutation in one of the sodium channels. And when they use sodium channel blockers like guanfacine, they can see improvements in symptoms and overall function. So, this leads to some mechanism-based treatments that might be fruitful. I want to end with a couple of points about the challenge of heterogeneity in ME/CFS. And originally, I had presented this at the FDA conference, I think, 10 years ago. But it's important to recognize that flares in comorbid illnesses can occur during randomized trials, and they have the potential to obscure the treatment effect of the drug under study. So, that heterogeneity can be reduced by careful subject selection, clear case definitions, eligibility criteria, especially for these subsets, and studies of single agents for ME/CFS and orthostatic intolerance will need large sample sizes. So, I think, ultimately, that's going to necessitate a much greater devotion of resources to clinical trials, including support for clinical trial networks, so that we can do these studies efficiently and in a timely manner for the people who are desperate

for help. Some of the strategies that can be employed include stratification to address duration of illness, since that seems to be a factor in at least the chronotropic incompetence, different pathophysiologic subsets. One thought is that we should have run-in periods for treating some of the comorbid disorders and controlling them as much as we can. Or another strategy is to identify the people who respond to the drug understudy, then remove people from that medication and only randomize the responders. People have thought about randomized trials of withdrawing the ostensibly effective therapies. Crossover designs might be effective for some conditions and as might end-of-one trials. So, I'm going to stop there and thank those who've made our work possible over the last three decades, including a number of philanthropic groups for whom we're quite grateful. So, I'll stop there, Jarred, and hope that we've recaptured some time.

DR. YOUNGER: Thank you very much. Excellent, Dr. Rowe. We're going to continue on. I see all the questions that are being asked. I know we're keeping those, and so, I imagine there's a couple

of different venues, maybe the discussion at the end or maybe offline, get a chance to answer these questions. Thanks, Dr. Rowe. I'm going to go right on and introduce Dr. Bergquist. So, Jonas is a full chair professor at Uppsala University in Sweden. He's in the Department of Analytic Chemistry and Neurochemistry. And I had a hard time, and I always have a hard time, trying to mentally encapsulate Jonas' work. He has a remarkably diverse expertise set. He does basic science and clinical science and clinical practice. Body, brain, there's really no place that's off-limits. There's no level of magnification that's off-limits. I think any research modality is considered. And so, the field is very fortunate. He's decided to bring all that to bear on battling ME/CFS. I'm very -- -- talk about kind of this, kind of more spinal elements to the central nervous system contributions to ME/CFS.

DR. JONAS BERGQUIST: Thank you, Jarred. Can you hear me?

DR. YOUNGER: Yes, sounds good.

DR. BERGQUIST: Yes. Okay. Great. Can I control my slides or is it John who is

controlling them? I don't know. I can maybe -- I am trying to hit the bottom. Yeah. Okay. Now I know how to do that. Great. So, I'm very happy to be with you this morning, I guess, at your place. It's soon evening here. We are running a neurology meeting here right now. So, I'm just snuck out from that meeting to give this short presentation. And as Jarred said, I've been working with quite many different applications, mostly in neurodegenerative diseases, in neurology, and also trying to find out good biomarkers for many different disorders that hits the body but also mostly the brain. But I do some other things also. But then 10 years ago approximately, I was really -- got really interested in working with ME/CFS. And I've been since then focusing really on trying to help as much as we can do from Uppsala in Sweden and together with our colleagues and around the world with different centers of collaboration. So, I'll just go into what I'm going to speak about today is the neuroinflammatory role of ME/CFS that we are focusing on in Uppsala and together with colleagues. And I will give you a brief update on where we are there. But just to get started, as

the questions were sent out from Jarred, and I was to address some things. And this is my interpretation of what we know today. We know that most cases of ME/CFS have a post-viral fatigue event. We know that they have reports on different infections, but quite often, Epstein-Barr virus infection, mononucleosis, or influenza type of infections. We know that most women are affected by disease. About 60%, 70% are women in our patient cohorts. We know that there are demonstrated changes, both in the central, but also in the autonomic nervous system. We know that the metabolism and especially the energy metabolism are affected with, for instance, elevated lactate levels, already after mild exertions. And right now, we are using that as a part of the diagnostic tools. So, we ask our patients when we see them to perform a mild exertion. It could be a step test, or it can be also a mild ergometer bike test for them or a mental test even. And then we measure lactate levels, and we can see that they are elevated. And they stay elevated in most patients for a longer time than in our healthy controls. We also see that there are immune phenotype differences

and also on the functional parts. And we know that there are incidences of autoimmunity or over-representation, generally, of autoimmune disorders in these patients. But we have also monitored autoantibodies following what Carmen Scheibenbogen in Berlin is doing. So, we see also the same type of autoantibodies against beta-adrenergic and muscarinic receptors, for instance. Then there are many reports. I haven't worked so much on this myself, but gut microbiome changes and problems with intestinal tract comorbidities and so on is quite commonly reported on, as you all know. And then nowadays also we see that there is quite strong evidence on gene expression that changes and epigenetic changes also. For instance, after provocation, you can see that there are variabilities in expression. So, unfortunately, there's a lot of things we still don't know. I hope I can move my slide. Yeah. So, what is the exact mechanism that causes all these symptoms? Still a mystery. What leads to all the changes we see in all the different organs? So, it's really a multi-system disorder, as you know. What links those changes and what is the initial triggers? I mean, we are

focusing on the post-viral idea, but we know also that trauma and other incidences could well be initiator or trigger. Of course, we would like to know if there is a common explanatory model for all these symptoms. So, we could target that with therapy of some kind. And I will maybe, if I have time, just end up with what we are doing right now on the therapy side. And of course, one of my tasks is to find good biomarkers, diagnostic, predictive, or even preventive if possible. Because that would of course be, in the end of the day, what we are really searching for, to make sure that people are not affected by this disease. So, we are focusing, with my background in neurology, on the brain, and the human brain is of course extremely complex. And we think, of course, the brain is the most important target when it comes to studies on these neurological diseases. But as you already heard this morning, and so, of course, all the other systems and organs in the body are affected to a large extent. So, of course, we need a complement of competencies, and we need to collaborate a lot around this multisystem organ disease. So, one of our favorite targets when it comes to studying

the neurological system, central nervous system, is, of course, to look at the cerebrospinal fluid. And although it's an invasive sampling technique, we really see a lot of specific biochemical markers and differences in this liquid that surrounds the central nervous system. And I think many of you know, but I just want to say that the liquid is produced in the central part here of the brain in the region called corneal plexus. We produce about 500, 600 milliliters per day in an adult person. And that volume is resorbed several times. So, we actually replace that volume all about 20, 30 milliliters per hour during our day. And this liquid, of course, has a mechanical function. It protects the brain from bouncing around in the skull, but also, it flushes the brain and transports away products that is released from the central nervous system. And of course, if we can measure the molecules that are present in this liquid, we could also then see the chemical picture of the central nervous system without going in and taking a biopsy of the brain tissue. So, it's a liquid biopsy, you could say, in some sense. Yes. I think you also know that you draw this liquid

through a lumbar puncture in the lower back region with the patient in the recumbent position. You can also, at some point, take a supraspinal fluid from the ventricles in the neck region. But the most common one is the lumbar puncture way. And as you see on this slide here, you go enter between the spines and you'd enter the dorsal sac, which is a region where you can penetrate into the tissue without harming any nervous tissue. So, you then have a possibility to draw this liquid. And in a human adult, we take approximately 10 milliliters per sampling. And in younger individuals, we have taken about 3 milliliters. And the reason for that we draw this volume is not that we really need that big volume for our analysis. We use -- for some analysis, we can take 10 microliters. But we want to make sure that we get a representative sample of the volume. So, there is a gradient from the central part of the brain into the lower back region. So, in order to cover that gradient, we typically take 10 milliliters then. And let's see if I can move my slides here. Yes. And you get some local anesthesia, and you get -- yeah. Sometimes you have to put a blood patch to stop leaking here.

But it's nothing really dramatic about the lumbar puncture. And we have a very, very nicely standardized procedure for drawing our samples. And there are very few patients that have any complications afterwards. It's less than 1% of the patients that report on slight headache or post-puncture issues. So, we are pretty confident with this kind of sampling. Well, looking at cerebrospinal fluid is not nothing new. One person who have done that early on was this fellow, Jöns Jacob Berzelius. He was actually a student from Uppsala, at some point, but he moved to Stockholm and was also very much active in creating the Royal Academy of Science in Stockholm. As you know, they are responsible for heading or for giving out the Nobel Prize every year. But Jöns here was also a very keen clinical chemist, you could say. So, he wrote the first clinical chemistry book that we had in Sweden and one of the earliest ones in the world actually. It's called "Lectures in Animal Chemistry". And in that book, you can actually get an idea of how kind -- what kind of analysis they did on cerebrospinal fluid. They called it brain water at that time. And unfortunately, this is in old

Swedish, but in the blue box there it says in Swedish that, "This liquid is typically clear or a bit yellowish, and sometimes it could even be a bit green." I have not seen any patients giving us green cerebrospinal fluid. "It has a sweet, salty taste." They were not using the kind of sophisticated analytical instruments that we do today. We typically use more spectrometry instead. But he also declared that there is a content of biomolecules, for instance, proteins. And he was actually the person in the world who actually gave the molecule protein its name, protein, from the Greek. So, he tells here that if you treat this volume or liquid with alcohol, for instance, you can have precipitation of these molecules that are proteins, and you can analyze them further. So, why we think it's very important to look at cerebrospinal fluid typically when it comes to ME/CFS is, of course, that as we know in many other neurological diseases, there could be patterns of interest from biomolecules like antibodies. Let's say in multiple sclerosis, we know there is oligoclonal antibodies produced centrally in the brain. And then we can measure them as oligoclonal bands in

the cerebrospinal fluid, but also looking at specific molecules like immune-related molecules like cytokines or other inflammatory markers. And of course, then that could give us an insight in if there is a neuroinflammation going on in the disease. And as I mentioned, I mean, this is one way of looking at the central nervous system without getting a biopsy. And it's a good complement to what we can measure in circulation in blood and also what we can see with imaging techniques, for instance. So, all in all, we think this is one way to look at ME/CFS patients and then hopefully then find the underlying mechanism related to the pathogenesis. And one target that we are looking at is then the neuroinflammation. And as you may know, central nervous system, like a few other organs in the body, are so-called immunoprivileged. So, they are normally having a rather low immune activation. And the reason for that is that we don't really want that inflammation or big immunological reactions in certain organs like the brain, because that causes also damages and scar tissue formation. But when there is an inflammation going on, we get an activation of

the innate immune system. We get activation of astrocytes or microglia. So, they get actually immune competent, and they can start producing some of these signal molecules that we see in the immune system like cytokines. And of course, if we are unlucky and we have a new inflammation, we will have then damage on the neurons and the degradation of the neurons and myelin sheaths around the neurons, for instance, that leads to neuronal loss in the end. And in ME/CFS patients specifically, we don't think that the new inflammation activity is very high as compared to many other diseases, as I will go into a bit. But we know, based on our findings, that there is a low-grade activation of the immune system in many of our patients. And that may lead to some of the consequences and also lead to symptoms like brain fog or headache and pain-related disorders. So, what we do typically with our patients we draw blood samples, of course, most available, and also well-established detection for different molecules that are activated in circulation. We try to draw as many lumbar punctures as possible from our patients. We do sample cleanup and preparation. And then we typically use high

resolution mass spectrometry doing proteomics and metabolomics in connection with rather advanced bioinformatics and pathway analysis, et cetera, to decipher what is the difference between healthy controls, contrast groups, and our patients of interest, in this case ME/CFS. So, during the last years, we have been working quite a lot with related diseases also just to get some comparison or contrast groups. So, for instance, post-COVID patients, and in this case, a paper we published a few years ago, in 2020, where we actually could detect that the virus went all the way into the central part of the brain and could be detected in cerebrospinal fluid. And we monitored then ongoing inflammation, and we also measured quite a lot of brain inflammation related damage molecules in circulation and in cerebrospinal fluid. Luckily, this kind of severe infections that happens in the brain are very rare when it comes to COVID infections. So, we haven't seen that many patients with this kind of dramatic inflammation, luckily. But it can apparently happen in a few patients. And just to let you know, this patient survived this infection, took a long time, and now is in rehab

since a few years, and is doing pretty well, actually, after even this kind of dramatic inflammation, as you see on this MRI picture. Another paper that was just published, we have looked at herpes simplex encephalitis patients. As you may know, it's herpes virus type 1 that causes, in a few cases, infections that go into the central nervous system and also causes inflammation and brain damages in patients. In this study, we had the opportunity to collect around in Sweden together with many of our infection clinics. Patients managed to follow them over time in -- well, for us, a rather large cohort, about 50 patients. And just to show some of the data, we had the opportunity to collect from patients over time. So, we had three lumbar punctures done in acute phase in after 20 days and approximately after 100, 120 days. And what we saw were correlations with the inflammatory protein markers with some of the more important things, for instance, treatment of related phenomena, lesions that we found on the MRT, and also activity of anti-NMDA receptor IgG molecules, so autoantibodies against NMDA receptors. And as one of the examples of the

findings, we found that apolipoprotein A1, for instance, correlates with this acute seropositiveness of autoantibodies against NMDA receptors. And we know that the presence of these antibodies is also related to neurocognitive issues and problems and could lead to long-term consequences for the patients. When it comes to our ME/CFS patients then, we started looking at patients with ME, yeah, about 10 years ago now. And this was the first collaboration with Steve Schutzer and Ben Natelson that many of you know. And we then collected cerebrospinal fluid from ME patients and compared them with post-treatment Lyme disease patients and then with healthy controls from me. And we could then see that there was a general upregulation of inflammatory markers. We saw differences and similarities between ME patients and the post-treatment Lyme disease patients. We just recently had a sort of follow-up paper accepted in Annals in Medicine, also with Steven, with a comparison of CSF, cerebrospinal fluid proteome, in ME/CFS patients and fibromyalgia patients. As you know, many ME patients have comorbidity with fibromyalgia. And maybe to our surprise, or -- yeah. I'm not sure

what we thought from the beginning, but we did not see any big differences between ME/CFS and the fibromyalgia patients when it comes to the CSF proteome. We think that maybe the fibromyalgia has more inflammatory reactions in the periphery while the ME/CFS patients have this low-grade inflammation centrally, and maybe that is reflecting the data more. So, if you have ME or if you have ME with fibromyalgia, it's not that big difference when it comes to the proteome in the cerebrospinal fluid. An ongoing study, this is unpublished data. This is a collaboration that we have done now in Sweden together with the Bragée Clinic, Björn Bragée, and others at the clinic. And we have now done finished the collection of the samples actually. So, we have done lumbar punctures, we have done pressure measurements during puncture, and we have done sampling of blood and saliva in ME patients and matching healthy controls. And this has also been followed up with MRI and analysis of brain structures in the neck region and centrally. And as you see on this picture here, you see that one thing that we also looked at was the intracranial pressure, and you can do that in different ways.

You can do it during the lumbar puncture, but you can also use the ONSD with the ultrasound measures. So, you can look at the optic nerve dimension and you can see if that's a bit compressed, that could also be related to elevated CSF pressure, intracranial pressure. And actually, there was a rather high incidence of elevated CSF pressure measures in our ME patients. So, that is an interesting finding that we are following up on. And let's see how I'm doing in time here. We have now done the proteome of our patient sets. This is just one picture or one example of how the data looks like in 61 ME patients. We have added on our post-COVID patients, around 40, and healthy controls. And as you can see, when it comes to the proteome in CSF, we have a rather big overlap between our ME group and our COVID group, while we then have a separate clustering of our controls. And I think this is something that we are very keen on understanding and also see what is going on on the inflammatory market specifically in the COVID and ME/CFS group. So, pilot clinical trials, as I mentioned, is started and ongoing. And we are now setting up a trial together with David Systrom's

group at Harvard. And this is going to be a combination of low-dose naltrexone and mestinon in combination. And they are very excited, and this will be launched very, very soon now. About 160 patients will be included. We also have some other trials in pipeline, and we are now waiting for -- yeah -- funding and setting up the practicalities around it. But there will be some more anti-inflammatory treatment trials, and there will also be some antiviral treatment trials starting rather soon. And I will just end with the medical imaging ideas that we are also conducting. Won't give any details here, but we are now setting up another method also for measuring activation of immune cells in the central nervous system. And this is going to be a very good complement to our lumbar puncture measurements and our biomolecular screening. So, I think this is something we really look forward to getting going. By that, I think my time is out. I want to just thank our patients and their controls that on very altruistic basis are volunteering to help us with our studies. And we are so happy that we have this tight connection and collaboration with patients. And of course,

our funders, Open Medicine Foundation and Linda Tannenbaum, I want to mention her specifically, fantastic person to work with, and all the other supporters of our research. So, with that, thank you for your interest. And I'm not sure if we are going to take questions or not, Jarred, you tell me.

DR. YOUNGER: Very good question. Let me answer that. So, yeah. We got a little far behind, and we have to take a break. So, we're going to take a break, but there's some very interesting questions in the Q&A section of Zoom. And so, I'm going to encourage all the speakers up to this point to check that and look for the questions that were directed to you and see if you can give an answer via the Q&A window. So, let me know if you can't find that. But thank you very much. We're going to go right into the break. And let's do it for -- let's do a 10-minute break. And then we have -- I'll talk after the break and Dr. Mullington will talk as well. And then we'll have another break. So, we've got some chances for future breaks. But let's do -- let's come back at 11:50 a.m. So, does that sound right? That's a little bit over -- yeah. That's a

little bit over 10 minutes. So, let's do 11:50 a.m., so about 13 minutes and we will resume.

[Short Break]

DR. WHITTEMORE: Okay. I think it's time to get started again. I just want to make a couple of announcements before I introduce Jarred. So, we've gotten a lot of questions about when the recording will be available and where. So, the RLA team will need to do a little editing of the recording and to develop a transcript that needs to be 508 compliant. So, that will take one to two weeks. But we will send out an announcement as soon as that, both the recording of the webinar as well as the transcript, are available. That will be placed on the website. And so, all of that information will be sent out to everyone. So, secondly, I have to say lesson learned for this first webinar is that we need to allow more time for each speaker so that there's time after each one for four specific questions for that speaker. We're very sorry that that has not happened. But we will try to address as many of the overarching questions in the discussion. So, again, the main goal of this webinar is to inform the Research Roadmap Working Group of Council

about research priorities. And so, it's challenging and probably not appropriate for us to answer questions that are specific to your case or to your symptoms. But we've asked the speakers to address some of the questions in the Q&A if they are available and can do that. But we'll try to have an overarching discussion at the end of the webinar and try to address any outstanding questions after the webinar if there are still some questions that we don't get to or topics we aren't addressing. But with that, I would like to introduce Dr. Younger, Jarred, who has been a really excellent partner working with us on many fronts in ME/CFS, let alone in tackling his own research projects. He's really led our effort together with Beth Sullivan in the development of the ME/CFS common data elements. And we were in the process of finalizing a revision of the core common data elements, so watch for that, that's coming soon. But also, in really leading the effort to organize this webinar. So, with that, I will introduce Jarred, who will talk to you about his research and neuroimaging and neuroinflammation. Thank you, Jarred.

DR. YOUNGER: Thanks, Vicky. Let me see if the controls open up. I think I see them. See if it works, looks good. Okay. So, I'm going to take 20 minutes. I've got a little alarm that's going to yell at me if I go over. And I want to describe the role of neuroimaging in moving forward to effective treatments in ME/CFS. There are an amazing amount of things we could cover. So, I'm just going to pick and choose some things to highlight from new research and recent research that's been published. I want to catch up on a little bit of what I've been doing and show you some brand new results. And then I want to tie that to some other contemporary results. And then I need to answer these three roadmap questions. What do we know with neuroimaging and ME/CFS? What do we need to know? And how do we get to effective treatments? So, for background, I want to give you the 30-second short story. That is -- and I don't have a pointer, so I'm going to kind of verbally direct you around these slides. The cumulative neuroimaging work supports the idea that ME/CFS involves abnormal inflammation in the brain. The basic concept here is that microglia are the immune support cells of

your brain. They're everywhere that neurons in your brain are. And on the left, that kind of in the purple color, you can see they have a resting state. That's their normal state, but they can be pushed into an active pro-inflammatory state that's in red, where they're pumping up pro-inflammatory cytokines. And these make you feel sick. They make you feel fatigued. They interfere with cognition, but they can also be pushed into an anti-inflammatory state where they calm down inflammation. And they can also move between these states -- between any of these two states, and they can do that very rapidly. In fact, there's movies of them changing from one form to another in less than 30 seconds, so it's very fast. And they do this all the time, reacting to the environment. But there appears to be some circumstances where the microglia are stuck in the pro-inflammatory position. We don't know all the reasons why. We know that a massive viral or bacterial hit can do that, particularly if the virus or bacteria invaded the brain, or if someone has multiple immune hits too close together, it kind of traumatizes the immune system. And so, the microglia stay in their

activated state, like they're always on guard. And that's what we think, that's the hypothesis of what we think is happening with ME/CFS. So, how do we prove that or how do we look at that? So, one method is positron emission tomography or PET, and we use a radioligand called DPA-714. And it's a ligand for receptors with a radio isotope attached to it that gives off the signal we can detect. So, we can inject this, see where the ligand goes, and then pick it up with the PET scanner. It turns out that microglia express a receptor called the translocator protein receptor only if they're in the pro-inflammatory activated state. And so, if we inject a ligand for that, it will only stay where the microglia are pro-inflammatory and activated. And so, that's what we use to know if there's inflammation in the brain, if there's microglia activation in the brain. This picture right here is a normal person with no evidence of neuroinflammation. And this technique, it works really well. It's been used in Alzheimer's, multiple sclerosis, and traumatic brain injury, very, very informative. And we're running this right now with an NINDS R01 in ME/CFS. I may talk

about that a little bit more briefly later, but we'll probably finish that in December of this year. So, I don't have results for the ME/CFS yet, but we just published the same thing in fibromyalgia, and about half of those participants also meet chronic fatigue syndrome criteria. And so, there's definitely going to be some overlap between what we saw in fibromyalgia and what we see in ME/CFS. And there are many similarities between fibromyalgia and ME/CFS. There's fibro fog instead of post-exertional malaise, and there's -- I'm sorry, like fibro flares, and there's fibro fog instead of brain fog. There's a lot of overlap. We don't know that they're 100% for sure the same thing. I wouldn't say that, but there's clearly some overlap. So, we published this study. This was funded by the American Fibromyalgia Syndrome Association, and the primary author is Christina Mueller. And basically, we found that about half of the brain in fibromyalgia, individuals with pain and fatigue, shows this microglia activation. There's inflammation happening all throughout the brain. These check marks on the left are the brain regions where the microglia were abnormally

activated in the patients. And just to show you, this is a program called -- I think it's pronounced Neurotorin or Neurotorion. But it's a program you can actually access and it just allows you to drive through the brain. This is a top view looking to the left where that arrow is pointed. We did not see any abnormal microglia activation in the front parts of the brain. So, the prefrontal cortex here, which is where your personality is and your thinking, no evidence of inflammation there. No evidence in the premotor cortex, that's where you kind of plan your motor response, that looked fine. In the precentral or the precentral gyrus where you execute your movements, we did not see any evidence of microglia activation there. But after that point, everything was inflamed, everything had microglia activation. It started with the somatosensory cortices, and this is where you perceive all your body sensations. It's where they come in for you to actually feel them, all the information throughout the body, all the different sensations. There was lots of microglia activation in that region. Then in the parietal lobe, huge section right behind that, also lots

of activated microglia. And this is where pain and touch and everything else is integrated, all your body senses. So, it makes a lot of sense that this would be activated. I do want to note real quick that our colleagues at Griffith University, they released a paper not too long ago showing these exact same regions had brain volume increase, which means there was more matter in the somatosensory cortex and the parietal lobe. And that volume increase was associated with greater fatigue severity. And I think a lot of people think that's counterintuitive. They think, "Well, it's got to be hurting your neurons if you have ME/CFS or killing off the neurons and so, there should be less. Why is there more?" And I think one reason that may be the case is because the MRI that detects brain matter cannot distinguish between microglia and neurons. They look exactly the same on MRI scans. This is microglia in green that's holding on to two neurons. It actually grabs onto them. So, they're right next to each other. And when you do a volumetric scan, you don't know if you're looking at neurons or microglia. So, the fact that there's more brain matter in these

regions where we're finding microglia activation could suggest that the microglia are aggregating in those regions or they're proliferating, and they're actually multiplying. And we know that interleukin-1 beta and tumor necrosis factor-alpha can make microglia proliferate. And we know that ME/CFS -- multiple studies have shown that ME/CFS involves interleukin-1 beta and TNF-alpha. So, I think this is a very interesting possibility in why we're seeing these results with PET or with MRI. And I've got to move on to some other things. It just continues. It goes back to the occipital lobe. The occipital lobe had huge amounts of activated microglia. The precuneus, which is a really important site for the emotional response to body sensations and pain, all of them showed microglia activation. So, there's just, again, about half of the brain showing inflammation. And I just want to mention that my colleague, Marco Loggia, he did a study very much like this with a different PET radioligand and found very similar results to what we found in somatosensory cortex, precuneus. So, I just want to say that there's convergence among the different groups. The question now is,

what does it look like in pure ME/CFS, not fibromyalgia who may also meet ME/CFS criteria? So, when we run this or when we finish running this, it's going to be, is the pattern the same as fibromyalgia? That's going to be incredibly informative. Is it a different pattern? That will also be informative. Or do we not find any differences between ME/CFS and healthy? That will be incredibly informative as well. And I do need to note that a Dutch group in 2021 found no differences between ME/CFS and healthy controls in a small pilot. I think it was like 10 and 10 people when they looked at TSPO, but they used a radioligand called PK-11195 that is an older, less specific radioligand. And I'm really glad they ran that study. I think it's important to look at. However, to answer the question, definitively, we need to use one of the second generation radioligands like DPA-714 or PBR28, and it needs to involve more people before we can say there's no difference between the two. So, we will be talking more about that closer to the end of the year. So, let's shift over quickly to a different imaging approach, MRI and magnetic resonance spectroscopy. Probably the most

repeated neuroimaging finding in ME/CFS in the history of research using neuroimaging in ME/CFS is elevated lactate. There are several papers showing lactate all throughout the brain in ME/CFS. Some of these papers were done by Benjamin Natelson, who's on the panel today. And this uses spectroscopy that can measure different chemicals in the brain. And we use a technique that allows us to measure it throughout the brain. And again, I can't point to it, but you can see it says lactate. Lactate is usually small to non-measurable. That's generally what you want. If you have lactate that's measurable in your brain, it suggests that something is wrong, that the cells are not getting the energy that they need. The same thing happens in your muscles. When you overwork them, they're going to demand more energy than your oxygen, than your blood supply can provide. And so, you get an increase of lactate. Same thing in the brain. It could be that there's extreme hyperactivity, such neuroinflammation. It could be a mitochondrial dysfunction, could be an oxygen perfusion problem. It could be oxidative stress, insufficient glucose. There's a lot of potential

causes, but it's telling us something is awry in the brain, and it's generally seen as part of the neuroinflammatory response. This picture here at the top is a group of healthy individuals. The bottom is ME/CFS with just lots more lactate throughout the brain. My graduate student, Indonesia Jordan, provided some first analyses this week and with our NINDS trial using this scan where we can get lactate and other things. And I have not had enough time to scrutinize everything and look at everything. So, I just wanted to show one quick slide from these analyses. I'll be looking at these more in depth over the next week. And all I wanted to say is that we've previously reported that ME/CFS involves lots of lactate. And this really shows this again, the zero group to the left are healthy controls and on the right are ME/CFS participants. And what I want to point out here is that while there are lots of ME/CFS individuals who do not have abnormal lactate, you do clearly see that group extending higher on the one column where there is significantly higher lactate. And this is about 20 or so percent of the ME/CFS sample. These individuals have clearly

abnormal lactate that is almost definitely going to be driving symptoms. So, it's all looking very good. I really look forward to digging into these data and preparing our first reports from this. So, let's get into the roadmap questions and tell you -- and we'll talk more about these things in the discussion. First thing is, what do we know with neuroimaging? And I'm just going to pop them all up so they're -- so it's faster. Here's what we know. There's been neuroimaging work in ME/CFS for about 20 years, MRI and fMRI. And we know that the structure and function of the ME/CFS brain is abnormal. But for most of the time, we didn't know why. Number two, more recently, we have the tools to show us how and that indicates brain inflammation. Number three, not all individuals with ME/CFS show the severe inflammation. So, there are critical subgroups and it may be that treating them will require different medicines, different approaches. There's high lactate is probably the most consistent indicator of fatigue in ME/CFS. At number five, I want to note that the inflammation we see, while it can be completely debilitating, there's no evidence that it is high level

emergent neuroinflammation like bacterial meningitis, which can kill you, or sepsis, which can kill you, it does not look like that. The levels are not high enough. I think I've only one time seen severe, severe, severe neuroinflammation, and that was somebody with traumatic brain injury. So, it's not, again, emergency issue level inflammation. It's more of a low to moderate level. It just never goes away is the issue. And then also I have seen no evidence that there is neurodegeneration. So, it's not like multiple sclerosis or Alzheimer's or Parkinson's disease where there's something negative happening to the neurons themselves. All right. Other questions, what do we need to know? Big thing I need to know is, what is the origin of inflammation? We know we can see that the brain is inflamed. We don't know exactly why. Is there a virus in there, a bacterium? Is this a problem with the vasculature? Is it an autoimmune problem? And you can see this list. There are multiple things. My research leads me to believe that it is an abnormality in the microglia themselves. But unless we rule out these other things, there might be other reasons why the

brain is inflamed. And that leads into the next question we need to know. The answer to it is the brain inflammation we're looking at, is that the enemy or is that actually trying to help us? We know there's inflammation. It's very likely that that inflammation is the cause of the symptoms. Because the microglia that are producing these pro-inflammatory cytokines are literally hanging on to the neurons that drive your perception of fatigue. They're right there. So, they're probably what's causing the symptoms. But is the inflammation -- is that microglia the problem? Is something wrong with them or are they trying to deal with a problem? Is there an infection somewhere or an abnormality that the inflammation is trying to correct? Or is it like a misguided friend that's trying to help, but it's causing more problems than helping? It would be really nice to figure that out. So, how do we move forward particularly with clinical trials, and then I will be done. I'm really big in clinical trials now. I think it is where we should be putting most of our resources now. We have enough evidence to show that there's brain inflammation in ME/CFS. So, we need to get forward with the

clinical trials. We're not 100% sure, but we have enough evidence to go on to the next step. It's true, we see microglia activation, we see lactate. I can think of alternative explanations for what we see, and you heard one of them today. It could be, it's possible that there's an oxygen perfusion problem. And because there's not enough oxygen getting to the brain, the microglia are freaking out, and then you have a buildup of lactate. That is possible. We test cerebral perfusion, but everyone's lying down in a scanner. And that could be a condition where we don't see the perfusion problem. So, yes. We could try to get a grant to get like a sitting or a standing MRI to see if we see perfusion problems or try to do a tilt test provocation like what Dr. Rowe would use, and that would be super informative. But that could take about five years to run, and I think we really need to drive forward with clinical trials in parallel with interesting things like that. So, we've got to move forward with the clinical trials. We need to focus on repurposed medications. We need to do parallel testing. We need to test multiple high-priority drugs in parallel, and then advance the

ones that look the most promising to the next level and try to quickly get to the things that work. And we need to have consensus criteria for all these clinical trials. So, they all use the same criteria so we can directly compare the results. And again, I hope we'll talk about this in the discussion. Got a couple more slides and I'm done. In terms of what to try, I have a massive list of things I would love to see tested in ME/CFS. This is only a fraction of that list. I just filled up one slide. I started this a few years ago and now it's grown huge. The white compounds are available via prescription for humans. The blue is not available for human use yet. And the green are botanicals, which anyone could pick up. I do want to say very clearly that I'm not suggesting any of these things for ME/CFS right now or for anything else. These are candidates for researchers to consider for future clinical trials. There's just so many that have great basic science evidence. I mean, it would take a lifetime to test even a fraction of these. But we need to prioritize these and then just start testing them if we want to come up with the answer. I wish I could talk about -- yeah.

There's so many of these I would love to talk about, but maybe they'll come up later in the discussion. But there's no shortage of really good options to test. Last thing, in terms of prioritizing the treatments, I believe I've got four criteria to rank what their priority is for me testing. They have to be available for human use now. They have to have shown evidence for modulating microglia activity. They have to have shown a study where there was clinical benefit, either to a human or to an animal model. And there can't be any evidence of serious adverse events. I don't want to cause more problems. And there are plenty of agents that meet those criteria. Very last thing, in terms of neuroimaging and ME/CFS, I really want to see neuroimaging incorporated with clinical trials, especially if they're targeting the immune system and brain inflammation. That was mentioned earlier today. And it's just -- it's so nice. This is just a random example from the literature. This is somebody with ME/CFS who did hyperbaric oxygen treatment, and they had all this hypometabolism. That's all that activity on the left. But after 50 sessions of the hyperbaric

treatment, the symptoms were resolved, as was the hypometabolism in the brain. This is just an end of one study. I just wanted to show how helpful it is when you can show someone improve, but you can also do the brain scans to show why they improved. And I think that's really important. So, I better end it there. The questions, I think, to keep on time, we'll just roll along with the presentations. I will keep an eye -- this is my mascot by the way. This is from our leukocyte tracking study, infiltrating the blood brain barrier that I will be talking about some other point in the future when we collect more data. But I will look in the Q&A window and I will answer any questions that are thrown my way. And I appreciate everyone. And with our progression of events, I'm actually introducing the next speaker. So, I think I will if everything's good with the Zoom, I will just go right into introducing Dr. Mullington. So, Janet Mullington, PhD is a or the sleep expert. She's at Harvard University's Division of Sleep Medicine, professor of neurology. And the work she does is critically important in this field. I mean, she's looking at sleep and health and

psychological states and the really complex relationships between these things and probing into the physiological substrates of healthy and unhealthy sleep. And more recently, she's used this expertise to help her understanding of Long COVID. I believe that sleep is one of the most underappreciated areas of improvement, and it's unfortunate that so few people get a chance to see a sleep expert. I wish we could take Janet's knowledge and just put it in everyone's brain, and that would be so helpful. But until we can do that, we will settle for these really good webinar talks. So, I want to turn it over to Janet.

DR. JANET MULLINGTON: Thank you very much, Jarred. And thank you for all the work you did in organizing this, along with Vicky and others. Thank you very much. And I'm very delighted to be here. I can advance my slides -- let's do so. As Jarred said, Janet Mullington -- oops, sorry. Okay. So, non-restorative sleep is an important part of ME/CFS and it's a bit of an oxymoron, isn't it? I mean, sleep should be restorative. I'm going to begin with talking a little bit about the problem, the challenge that we face in

understanding non-restorative sleep. Then I'm going to talk about some studies that have been particularly inspirational for me and for my group, and show you a little bit of preliminary data, talk about a new study that we're starting, and then move to what I think are opportunities and gaps needed to better understand the role of non-restorative sleep in ME/CFS. So, non-restorative sleep, as I mentioned, is the hallmark. Sleep deficiency, though we know, causes performance and cognitive impairment. This has been known for decades and based on research initially in healthy volunteers under controlled experimental conditions where we can really carefully select participants who are healthy sleepers and then we can deprive them of different amounts of sleep and different timing of sleep and wakefulness and really look at the effects on performance and good outcomes. And we see that there's a really clear effect of insufficient sleep on performance. And as you stay awake, what this is showing here in the center of the screen, sleep pressure increases throughout the day. And if you stay up later, there's more sleep pressure. That if you stay up

very late, you actually may not be able to sleep as long as if you go to bed at your regular time because of circadian factors. So, these are diurnal 24-hour factors that also have an influence on your wake and sleep. And when we experimentally sleep-deprive people, they experience brain fog at times, or cognitive impairments that involve some of what we've heard about in terms of slowed reaction time and difficulty in functioning at optimal levels. So, we would like to get to markers of non-restorative sleep. And a marker of non-restorative sleep can really help us track the progression and evaluate treatment efficacy. And EEG is really an established method for looking at brain function, and newer methods are incorporating EEG and including often a transcranial magnetic stimulation, transcranial direct current stimulation, and sometimes, as we've heard elegantly, MR imaging as well. And one of the questions is, can we come up with a brain signature of fatigue that can be extracted from the EEG? And I just want to take a moment and explain what it is we're looking at when we monitor the sleep of individuals. There's EEG

electrodes that are applied prior to sleep, and then the individual sleeps while somebody else is monitoring the EEG. The gold cup electrodes typically are applied to the scalp by the eyes. So, that we can monitor rapid eye movements during REM sleep and EMG, which muscle tone typically on the chin electrodes. And it is shown here to decrease during the night and particularly during REM sleep, which is marked by muscle atonia. And here you can see the cycling through the night with individuals dropping into stage one, stage two, stage three, and stage three and four are what we now call NREM, non-REM sleep, deep non-REM sleep. And these cycles typically in healthy individuals cycle four or five times through the night of seven or eight hours of sleep. And EEG is also measured, of course, clinically during the day. And EEG spectral coherence data has been analyzed from patients with ME/CFS. And this is an inspirational study for me because they were able -- these investigators, Tony Komaroff and other collaborators at Children's Hospital, Frank Duffy, analyzed EEG data from hundreds of patients and found that they were able to

discriminate using discriminant function analysis of the EEG coherence, which is essentially looking at the correlation between different regions, and the correspondence of the electrical activity coming from different regions of the brain. And they were able to investigate the patterns and differences between people who had no ME/CFS and had healthy function and people who had ME/CFS. And I think this is very promising. I think it is one area that really should be followed up with. Another area that was particularly inspirational for us was looking at the EEG during wake again, but before a night of sleep and after a night of sleep. And this group from Mexico, led by Maria Corsi-Cabrera, looked at the EEG in evening before bed and then compared it to the morning EEG in healthy controls that you see on the left and in people with primary insomnia. It's not ME/CFS, it's insomnia. But insomnia as you know, is severe sleep deficiency. So, they looked particularly at -- well, they looked at all of the spectra but found in beta and gamma a real difference between people who had healthy sleep and people who were suffering from chronic insomnia in terms of their

ability to dampen beta activity and gamma activity. These are fast frequency electrical activity patterns. The sleep period helped people to decrease this activity. And those who had trouble sleeping were not able to decrease. So, this is comparing pre- and post-bed EEG. What about ME/CFS? Well, this is probably my favorite study. This is a twin study. These investigators studied twins who were discordant for ME/CFS and looked at their ability to reverse the slow wave or sleep pressure that is experienced before bed in the night, in the evening. The healthy twin was able to do what we expect to reduce this slow wave activity, this delta power across the night so that they are discharging all of that sleep pressure, if you will, for the slow wave sleep. But the chronic fatigue twin was not able to do so. So, they started out with a lower level of accumulated pressure in slow wave activity, and they were not able to get it down to the level that it was supposed to be at in the morning. So, this implies a real problem with the homeostatic function of sleep. So, this is just to remind you, sleep pressure builds up with wakefulness. And then this curve here that I didn't speak

about before, really is modeling what we see here, the decrease of the sleep pressure across the night. And so, there's something wrong with the homeostatic function in ME/CFS, is what I think. So, we think that the post-viral hypothesis is a very appealing one from many perspectives. And currently, we have an opportunity to learn a lot. This is showing you just some data from the clinic, and these are three people who are studied who have severe hypersomnia. That means that they're very tired. They also can complain of insomnia at the same time and sometimes these may alternate. And here what you see is the gray is showing you wakefulness and this is showing you lights out. It's taking a while to fall asleep. If you look at the third row here of data, you see a lot of back and forth trying to get to sleep. And then what you see is finally consolidated sleep. And then this is a multiple sleep latency test following it, where we examine what is going on in the sleep pressures across the day and can look for things like REM onset, which you see right in the middle panel. Here you see, again, a lot of fragmentation of sleep. And then in the

naps, you actually see REM onset in two naps. You can see it over here as well. In the top histogram panel, you see, again, a lot of fragmentation of sleep and less sleep onset actually is only occurring in two of the naps, briefly in the final one. So, these patterns help us to understand what's going on with sleep fragmentation and sleep pressure. But particularly surprising is the presence of REM onset, which is a characteristic of narcolepsy. But REM onset during daytime sleep can also be seen in some severe sleep deprivation in following -- sorry, severe restriction of sleep over a long period of time. I misspoke, not deprivation per se, not in an experimental way. So, we have been studying Long COVID participants, and we have a database in collaboration with colleagues at the Mass General Hospital -- excuse me -- Donna Felsenstein and Ken Sassower, and we have been looking at the EEG of these three different groups. And one of the things in terms of the clinical comorbidities that stands out really is the prevalence of sleep and circadian disturbances in the ME/CFS patients. We also see them in Long COVID. And we

have also been looking at the sleep architecture in these different groups. So, the control on Long COVID is from an experimental study or a research protocol, and the ME/CFS are from clinical cases. So, they're not exactly identical conditions. And we also know that REM sleep latency here is showing a difference, but the long sleep rate -- sorry, REM sleep onset latency is often associated with medication. Anti-depressant medication can reduce REM sleep amount. And we also found fragmentation and arousal increased in the Long COVID and ME/CFS patients. Another area that I think warrants further investigation is the finding of blunted cortisol in morning fasting cortisol. A group has recently reported in --

DR. WHITTEMORE: You're suddenly muted, Janet.

DR. MULLIGAN: Am I muted now?

DR. YOUNGER: No. We hear you now. Thank you.

DR. MULLIGAN: Okay. Great. Sorry about that. So, there's been a report that in Long COVID, there's a lower morning cortisol level. And there have been inconsistent results in ME/CFS. And I think that one of the things we need to do is

compare by looking at 24-hour cortisol, because cortisol has a strong circadian effect. So, I think that's an interesting area that needs to be pursued. And another area of inspiration or another inspirational study was that of Davis and colleagues. And they characterized carefully the symptoms and the timing of the onset of symptoms in Long COVID. And fatigue, of course, comes out early and stays persistently in Long COVID. But other sleeping symptoms also show up early and stay prevalent. And one of the other things that was quite interesting that came out of this for sleep researchers was to see reports of vivid dreams, nightmares, and lucid dreams. And this kind of phenomenon may give a clue about REM sleep. And we have seen a couple of reports coming out looking at REM without atonia. And this can be precursor to REM behavior disorder, which may be prodromal for neurodegenerative disease. So, I think this is also an important place to focus some attention in the research on EEG, looking at the fragmentation or the maintenance of atonia during REM sleep. So, we're currently doing a study looking at ME/CFS and Long COVID patients. We are getting this study

and examining cortisol and melatonin, as well as some inflammatory mediators, cytokines and resolvins, mediators that dampen inflammation. And this is just showing you the protocol that we're beginning. And we have EEG here, a sample. So, we'll be able to look at, as Corsi-Cabrera did here, look at the EEG before sleep and after sleep to investigate the homeostatic process through the night. And then we will begin hourly blood draws. As we're doing that, as I said, we will measure melatonin to look at the circadian integrity of the system, but also the cortisol. So, we'll be able to see if there are amplitude and phase differences. And then we will do multiple sleep latency tests so we can look at sleep pressure during the day. And then after another night of sleep, the hourly blood draw ends, and there will be cerebrospinal fluid sample in the morning so that we can look at mediators in the cerebrospinal fluid, including orexin. So, orexin is a peptide used in the brain, and it is really important for state boundary control. So, we will be able to look at that as well. That's never been investigated in ME/CFS or Long COVID. So, we're also looking at

the spectral qualities in EEG. Spectral qualities have been looked at in ME/CFS, and there hasn't been a lot found. And that's, I think, because the methods were limited. This is an example in Long COVID, but we are going to be looking at ME/CFS and Long COVID in our preliminary data and in our currently enrolling study. And this allows us to look across the night and to look really at the disruption. So, we'll be able to look at the fragmentation and spindle quality as well as alpha, delta, and other patterns in the EEG that may help us to understand the sleep deficiency. I think it's really important to understand the switching between states, and particularly the switching that occurs to move the brain from a deep restful state into a brief and quick arousal and wakefulness. And in this histogram up above, you can see a lot of switching between these states. This shows you wake REM in one and two and three, and three being the deep sleep, and then wake, you can see the amount of wakefulness that occurs here. Most of it's brief, and very often people aren't even aware of the fact that they're waking up at night. But this process of going back and forth, I think it's important to

understand the burden of that on the homeostatic process.

CHLOE JONES: Thank you. And Janet, we have just about one minute.

DR. MULLIGAN: Okay. I'm almost finished.

MS. JONES: Okay.

DR. MULLIGAN: And this is how case studies work. And this shows just big data and how it can help us understand the individuals within a broader context. And so, we are looking at the ME/CFS and Long COVID EEG in a broader context that's defined by this big data. And I think we really have an opportunity to move beyond the sleep architecture with some of these big data and machine learning methods. And I think that twin studies of Long COVID would also be helpful to understand and to be able to help us compare with genetic control and really understand the biology. I think we need to also follow Long COVID cohorts for a longer period of time and be able to look at the interventions. And I think we have to share the data so that I think if there are data that are collected, we really need registries so that we can mine the data for future purposes. And with that, I would just like

to acknowledge and thank our collaborators in ME/CFS and Long COVID and acknowledge the patient-led research collaborative, the Open Medicine Foundation, grateful for participation also of our patient collaborators, Beth Pollack and Leticia Soares, and also collaborators at MGH and across the country as well. Thank you very much.

DR. YOUNGER: Thank you, Janet. I saw some interesting questions coming through the Q&A window, so take a look and take a crack at those. And let's do another little break, 10 minutes. Let's come back at 12:50 p.m., 10 minutes from now. And then we're going to have Novak speak, and then we're going to do our panel discussion to wrap things up. So, still some good things coming up. So, I will see everyone in 10 minutes.

[Short Break]

DR. PETER NOVAK: Hi, Jarred. How are you?

DR. YOUNGER: Hello. All right. Let me -- we are here at 12:50 p.m.

DR. NOVAK: I will share my screen, or I will use your screen?

DR. YOUNGER: I think they're going to set that up in just a second. I'll introduce you, and

I bet everything will magically pop up. But if it doesn't, we will figure it out.

DR. NOVAK: Okay.

DR. YOUNGER: So, we have Peter Novak. He's going to be talking about peripheral nervous system and ME/CFS, maybe a couple other things. He's from Brigham and Women's Hospital, Department of Neurology. Dr. Novak does decidedly integrative clinical research. He ties together these really dispersed elements that kind of don't reveal their story until they're brought together. And he has one of my top 10 papers from last year, which is that mast cell disorders are associated with decreased cerebral blood flow and small fiber neuropathy. I'm like, "You've got mast cell disorder, you've got cerebral blood flow, you've got small fiber neuropathy." That's one of the best titles I've ever seen, how you get three such exciting things tied together so cohesively. So, I think that's really a testament to kind of his visionary scientific approach. I'm really looking forward to this one. And so, I'll let Novak take it away. And let's see if the technical aspect works. There should be a little

gray box somewhere where you can advance your slides. I think you got it.

DR. NOVAK: Can I start?

DR. YOUNGER: [affirmative]

DR. NOVAK: Thank you, Jarred. You are very generous. And so, I'm Peter Novak. I'm running an autonomic lab at the Brigham and Women's Hospital. And thank you very much for sharing this opportunity or giving the opportunity to share our experience with the autonomia chronic fatigue syndrome, which is a very complicated and, at the same time, intermingled topic. And I will talk about a very small part of the chronic fatigue syndrome, which is our review of the peripheral nervous system. So, I will talk about small fiber and related dysautonomia, then I will talk about association of chronic fatigue syndrome with small fiber neuropathy, of course, Long COVID, and at the end, a few implications for chronic fatigue. I will try to be concise, and Dr. Rowe will be mentioned, or review a lot of interesting autonomic issues. I will try to not to do -- avoid the overlap. So, first, peripheral nervous system consists of the large and small fibers. Large fibers are typically

myelinated, they are more than 50 microns. They control the movement muscles. Small fibers, they are small because they are like typically less than five microns, and they are non-myelinated, or have one less level of myelin. They are very slow conducting. And they might be affected in chronic fatigue syndrome, although the implication of this involvement is not clear yet. So, and small fibers, they are divided into autonomic or proper, and sensory, afferent. So, sensory afferent fibers, they modulate touch, chronic pain. Typically, it's burning sensation. Sensory modulation is transmitted by small fibers. So, autonomic fibers, they are motor, they are efferent, so they innervate all organs. So, typical characteristic of autonomic fibers is that you don't feel them. So, end damage of autonomic fibers manifest as end-organ dysfunction. So, we call it umbrella dysautonomia. So, for example, damage of the autonomic fibers going to the GI system cause gastro-parasitic GI fibers in general, abnormalities innervating the heart cause tachycardia or fatigue hypotension, and so forth, et cetera. So, autonomic fibers are divided in

sympathetic, parasympathetic, and enteric. So, the autonomic system is also can be divided into the central part. It mainly consists of the brainstem reticular formation and the hypothalamus. Hypothalamus is very important because it is called the first ganglion which controls the autonomic nervous system. And according to one very old theory, hypothalamus is the cause why people with chronic fatigue syndrome, they always feel like brain foggy. They don't feel good. It is like having constant flu-like symptoms. And we go to the one theory from last century, hypothalamus will switch to this sick mode and maintain this feeling of the sickness. It was never proven, but it's one of the very interesting theories. Sympathetic system is widespread modulate on the peripheral organs. But probably the most important from the context of chronic fatigue syndrome is because the parasympathetic system -- I will talk a little bit later -- is the inference portion of the autoimmune reflex. It mediates autoimmunity and their whole energy fiber. And by dysfunction of the parasympathetic nerves, we might see abnormality in autoimmunity. Small fibers are

very easy to detect with small fiber neuropathy. Diagnosis is very established. It is easy using skin biopsy. It is minimal invasive procedure. So, just two- or three-millimeter biopsy is typically done at distal or proximal legs. And we are simply counting the number of fibers sensory and deeper autonomic. Very easy to do. So, how is small fiber neuropathy related to chronic fatigue syndrome? So, prevalence is very high. According to one study, actually this comes from David Systrom's study you mentioned before, one-third patients with chronic fatigue syndrome are diagnosed having small fiber neuropathy, using the simple experiment for a gene of 9.5, which is one neuronal marker. And the prevalence is very similar, for example, in fibromyalgia or postural orthotactic tachycardia syndrome. However, the prevalence of the chronic fatigue syndromes and small fiber neuropathy might be much higher. One problem with skin biopsies is we do it only in peripheral spots. So, it is a good reason to assume that CFS is autoimmune or inflammatory condition. So, in this situation, we see frequently abnormalities also proximal, not only distal sites. And one example would be, for

example, dyspnea. Dyspnea or shortness of breath is extremely common in chronic fatigue syndrome offering a [unintelligible]. And those patients with dyspnea, they have normal lung function. And we think that small fiber neuropathy actually underlines pathological substrate for dyspnea in chronic fatigue syndrome, and also in Long COVID, a part of Long COVID. So, implications of small fiber neuropathy and CFS is still unknown. But in theory, based on the physiological studies, dysautonomia due to small fiber neuropathy can contribute to orthostatic intolerance and cerebral hypoperfusion. I will show an example of this later. And dysregulation of parasympathetic small fibers, which mediate inflammatory efferent reflex, may contribute to autoimmunity. Now a few words about the POTS. It's a bit of an overlap, POTS and small fiber neuropathy. Because small fiber neuropathy can cause the POTS and vice versa. And historically, both studies were separated from the small fiber neuropathy studies. But nevertheless, like up to 20% in general about the chronic fatigue syndrome, they have POTS. And still, we show that this percentage might be even much higher in the

adults and up to 50% especially chronic fatigue syndrome, they do satisfy criteria for the POTS. A few words about what POTS is. So, it is a chronic intolerance or chronic orthotactic intolerance condition affecting mainly women, age less than 50. It's very common affecting up to 3 million people in United States. It is a heterogeneous condition. So, it is neuropathic or metabolic secondary to the conditioning or psychological problems. But we think about 20 to 50% of POTS could be due to neuropathy. And also, both are received frequently by infection. So, also immunity still might play a role. And diagnostic criteria include combination of chronic orthostatic intolerance and excessive tachycardia. POTS is easy to diagnose by simply standing up and measure the heart rate. The best test is, of course, the tilt test, which also gives you more details about the POTS. And this is how it's both looked on the tilt test. On the left side, you'll see the control, right side is the POTS. From the top down, you'll see the heart rate, continuous heart rate and blood pressure, end-tidal CO2 and cerebral blood flow, lower rates from the middle of the heart rate obtained

by ultrasound. So, this is classic textbook example for the POTS. The blood pressure is stable during the tilt. There is excessive tachycardia during the tilt, more than 30 beats per minute. At the same time, the person is hyperventilated to see the decrease of the end-tidal CO2 and the decrease of the blood flow. This is important to note that decrease of the cerebral blood flow in POTS in most people due to hyperventilation by hypocapnia, hypocapnia causing vasoconstriction of the cerebral artery. And also --

DR. WHITTEMORE: Dr. Novak, excuse me, people are complaining about muffled sound that it's difficult to hear you.

DR. NOVAK: I can be closer to the microphone.

DR. WHITTEMORE: Yeah. Thank you.

DR. NOVAK: Better now?

DR. WHITTEMORE: Try it, and we'll see. Thank you.

DR. NOVAK: Yeah, sorry for that. So, patients with POTS, they experience orthostatic intolerance because they are hyperperfusing the brain. And they're hyperperfusing the brain not

because of the tachycardia, but because of a decrease of the cerebral blood flow due to hyperventilation, because hypocapnia can induce a cerebral vasoconstriction. So, this is an example. And also, Stewart compared chronic fatigue syndrome with the POTS in adolescents. And he again found depressed baroreflex, which means that people have decreased parasympathetic functions or abnormal parasympathetic functions. And I mentioned before, parasympathetic functions causing abnormal autoimmune responses. And also, what was very interesting that dysautonomia in orthostatic intolerance in POTS and in chronic fatigue was similar. So, going back to POTS, patient with POTS, they do have orthostatic intolerance and mild but widespread dysautonomia. Normally, clinical is restrictive with dysautonomia. So, now a few words about the Long COVID. So, Long COVID is post-infectious disorder, and it's become clear from the beginning, there are extensive similarities between Long COVID and chronic fatigue syndrome. So, it is hoped that we can extrapolate knowledge from Long COVID to chronic fatigue syndrome. Long COVID is probably the most studied infection in

the history of humankind. So, looking at the multiple similarities between Long COVID and CFS, for example, 10% of COVID-19 patients develop chronic illness, consistent with chronic fatigue syndrome. The same 10% of patients with acute infection, they will develop a Long COVID. And from these patients, half of them, half of Long COVID patients develop symptoms consistent with chronic fatigue syndrome, going on objective testing. So, it is reasonable to assume that there are some common mechanisms leading to Long COVID as well as the chronic fatigue syndrome. There are multiple similarities between Long COVID and chronic fatigue syndrome. For example, duration of the disease, chronic fatigue, post-exertional malaise, very characteristic for CFS, right? It's also seen typically in Long COVID patients. There are similarities in neurocognitive, psychiatric domains, neuroendocrine, and, of course, in autonomic manifestation and immune. From autonomic manifestation, it is orthostatic intolerance, cardiovascular abnormalities, chest pain, palpitations. Also, I'd like to mention the dyspnea, respiratory abnormalities are extremely

common in both Long COVID and chronic fatigue syndrome. Even the people have normal -- most people have normal lung function, and we believe dyspnea is due to small fiber neuropathy as mentioned before. However, even Long COVID is not that simple condition, it is really multi-system syndrome affecting cerebrovascular, small fiber neuropathy, dysautonomia, hypocapnia, dyspnea, and about 20% Long COVID patients satisfy criteria for the POTS. This is our study. We compare POTS without Long COVID and then Long COVID or POTS patients. And they're very similar in terms that they are widespread, but mild dysautonomia. And I would spend some time over here because Dr. Rowe mentioned that chronic fatigue syndrome is associated with decrease of the cerebral blood flow. And there are two or three very nice studies from Dr. Van Campen and Dr. Rowe, both co-authors, which show the patient with chronic fatigue syndrome, they do have decreased cerebral blood flow. There are three conditions in decreased blood flow and stable blood pressure, which is POTS, postural tachycardia syndrome. Again, blood flow is caused by hyperventilation. There is hypocapnic cerebral

hypoperfusion, which is the same condition as the POTS in the middle of the picture. In this condition, patients are hyperventilating, which is causing decreased blood flow, but the tachycardia is absent. So, we think this HYCH and POTS are spectrum of the same disorder. What's very interesting in most of the Long COVID patients, there's still decrease of the cerebral blood flow. But those patients, they don't hyperventilate, and they are not tachycardic and they don't have orthostatic hypotension. So, in other words, they have decreased cerebral blood flow because of their stiff vessel. When they are standing up, you have to have this compensatory vessel dilation to keep the peripheral nervous system stable. So, this compensatory response is missing in some of the Long COVID patients. And again, it is overlap with Long COVID and chronic fatigue syndrome. And most, about 20% will have this pattern on the autonomic testing. Even Long COVID is not heterogeneous, simply it is heterogeneous condition and there are different theories about what is causing Long COVID, including autoimmunity and this virus activation might be probably the most studied. But

clinically, we know that Long COVID is heterogeneous. Some patients have predominant chronic disorders, some with dysautonomia, some chronic fatigue. And it was very interesting that a recent study actually confirmed a Long COVID heterogeneity on molecular levels. For example, this study, for example, this group did show that based on the proteomics profiling, there are five clusters of the Long COVID and only two clusters are autoimmune or inflammatory, which is very consistent with our clinical observations. So, even Long COVID patients, they vary. So, again, there's a heterogeneity with Long COVID. So, how is dysautonomia and small fiber neuropathy relevant to chronic heart fatigue syndrome? So, pro-inflammatory responses are controlled by evolutionary, very old neural circuits. We call them inflammatory reflex. And autonomic nervous system is an important part of inflammatory reflex. This is a short summary of this. So, afferent sensory fibers, they detect molecular products of inflammation, injury, or infection. They send information to the brain, to hypothalamus, and to the brainstem, and efferent motor neural arc, which comes mainly from

parasympathetic system modulates immune response. It's very interesting that the main transmitter is the acetylcholine, we call it also the coordinate system, and the most or highest concentration of acetylcholine is in the spleen. So, spleen plays an important role in autoimmunity, although it is not clear exactly what does it mean. So, the cholinergic anti-inflammatory pathway transmitted by the vagus or parasympathetic nervous system then modulates inflammatory responses and mainly by influencing TNF, IL-1b or IL-6, the main inflammatory markers, but there are plenty of others. So, in summary, how peripheral nervous system is related to chronic fatigue syndrome? So, small fibers, particularly autonomic fibers are frequently involved in chronic fatigue. They can cause a variety of the manifestation including POTS or postural tachycardia syndrome or simply autonomic small fiber neuropathy. Parasympathetic dysfunction, which is frequently seen in chronic fatigue syndrome, can mediate autoimmunity. And POTS-like syndrome can result in cerebral hypoperfusion because there is a decreased cerebral blood flow. I would like to stress that

we observe decreased blood flow which is indirect marker of hypoperfusion. We don't have any good test for this to measure hypoperfusion directly. And also based on these studies or based on how it's easy to get small fiber neuropathy diagnosis or dysautonomia, those variables can be used as a biomarker in some studies, but only probably in subset of chronic fatigue syndrome. And I will finish here. Thank you.

DR. YOUNGER: Thank you, Dr. Novak. Our next session, we've got some time set aside to do a discussion and kind of address these questions, things that have come up in a larger level or higher level. And I believe, Vicky, if you take things over, I think this is going to be open to all the speakers and all the workgroup members to go through these major questions. So, Vicky, I will let you have it.

DR. WHITTEMORE: Yeah. Thank you. So, I'd ask all the speakers and anyone who would like to turn their cameras on to participate in this conversation. And I believe we have some slides with questions. RLA, if they could please put those up. So, our three questions back to what do we know, what do we still need to know, and how

do we move forward with clinical trials, I think are the overarching questions. And I think there was a second slide, the next slide, that really are additional discussion questions for us to ask. So, really thinking about all of the excellent talks we've heard this morning. So, thank you, everyone. It's a lot of information and a lot to think about and put together. And I saw a comment in the Q&A about how do we begin to put this all together. And I think that is what makes ME/CFS so complex, is that we don't necessarily understand the underlying pathophysiology, and it impacts so many different systems. But I think if we can begin to think about what are the critical studies that we need to do now to be able to move to clinical trials. And I'll start there and see if any of the panelists or the speakers or any of the other members who are on would like to comment on that.

DR. YOUNGER: Hey, Vicky.

DR. WHITTEMORE: Yeah.

DR. YOUNGER: I see all the speakers. I don't see anyone else. Are they actually on here and I can't see them?

DR. WHITTEMORE: Yes.

DR. YOUNGER: Okay. Great. Cool.

DR. NOVAK: So, I think the most important thing is to start with biomarkers. So, if you do any clinical trials, you have to have some way how to measure the response. And the idea is to measure it in quantitative, fully objective way. So, for example, in our studies --

DR. WHITTEMORE: It's still a little difficult to hear you.

DR. NOVAK: Yeah. Sorry. I might try to switch another computer but I'm going to get closer to the microphone here. Can you hear me now better?

DR. WHITTEMORE: That's better.

DR. NOVAK: Yes. So, the main issue is they're trying to figure out if they have a very similar clinical trial. The most important thing is to define the biomarkers, which should be ideally fully quantitative objective, because we want to measure the response. And so, in our studies, we are still using clinical definition, for example, postural tachycardia syndrome or chronic fatigue or Long COVID, which are kind of wide definition, wide and heterogeneous. They are not only the abnormalities. For example, we have

a Long COVID subset. Some of this actually, such like a chronic fatigue, but let's say we are looking for the abnormal skin biopsy with abnormal inflammatory markers. So, we call it inflammatory neuropathy. And we can trace the progression or regression over the time. One example, let's call this patient also, for example, satisfy criteria for Long COVID, for chronic fatigue, or for mild cognitive deficit. By that way, we are avoiding the controversies with how to measure the response.

DR. WHITTEMORE: Thank you. Anyone else want to comment? Yeah. Go ahead, Janet.

DR. MULLINGTON: I think that there's a lot of data that has been collected that we could benefit from making sure that we have really good harmonization and repositories available because just looking at that twin study, for instance, getting a hold of that EEG, that's a treasure. And being able to apply new data analytics, methods to old data can be really cost saving and very informative and provide a shortcut because then we can move on to validating instead of collecting data from the beginning. So, I think

those are really important to include going forward.

DR. WHITTEMORE: Yeah, just to comment there. So, the Data Management Coordinating Center, that we have funded for now going into the sixth year, has developed a data repository called mapMECFS where data can be shared. It started out with data being curated from publications and now investigators can also upload data to that data platform. There currently aren't EEGs stored there. But I know of EEG data platforms where the data could be shared, and clinical data could be shared in mapMECFS and linked. So, I think that's something that we can really look at going forward.

DR. MULLINGTON: Great. That would be fantastic. Thank you.

DR. WHITTEMORE: Yeah. Anyone else want to comment?

DR. LEONARD JASON: Vicky, just a couple quick comments, if you can hear me okay.

DR. WHITTEMORE: Yes.

DR. JASON: One thing that I came away from in terms of a paradigm shifting idea is that there really are very different types of

subgroups, and that one treatment for one group might not be effective for another. So, I think that's a paradigm shifting idea that probably we're going to need to have large enough samples so that we can really begin to parse the subgroups that might be affected or not. And the other thing that I come away with this is where would including the patients might be critical? Because in a sense, community-based samples might be somewhat different from clinic-based samples.

MS. FISHER: Now, do you consider subgroups to be based on pre-existing or overlapping comorbidities or based on symptom presentation regardless of comorbidity?

DR. JASON: I think they're both important.

DR. WHITTEMORE: Yeah. No. Those are both excellent points. And I was thinking about that when Jarred showed the data from the lactate. And if you have this population subgroup that has very high lactate, are they different and how are they different from the individuals who do not? And would they, I mean, why would you treat the two groups of individuals with ME/CFS the same if there's a difference in how the lactate presentation. So, I don't know. I'll come back to

you for you to speculate on that. But I think the other point you make also, Lenny, is also really quite important about where you collect, where you recruit the study participants from. And we actually have provided some biospecimens to a researcher in Switzerland who's looking at chemokines. And he has very different results depending on which two of the sites. We initially provided biospecimens from two sites that were involved in the CFI ME/CFS study, and he got very different results based on where those individuals were recruited from. So, we're now sending additional biospecimens to him to look at that further. But I think that's also something that may be critically important. So, I'll come to you, Jarred, about, I mean, I know you just got that lactate data. But just thinking about it, would you treat all of the individuals with ME/CFS the same or sort of parcel out those individuals that specifically show high lactate?

DR. YOUNGER: I would probably treat them different. When I look at that, I think of just the amazing heterogeneous presentation of people infected with SARS-CoV-2. You've got the same virus, but just an incredible range of

pathologies. People with just kind of respiratory damage peripherally, some people that look like they have blood-brain barrier infiltration and central immune system problems and everything in between. And you would treat those people differently. Sometimes you've got to target the virus. Sometimes it's other peripheral stuff. Sometimes it's not the virus. It's some kind of long-lasting issue. I mean, you all know there's so many different things just with this one virus. And so, something like ME/CFS that's not due to a particular virus, there can be all sorts of levels of problems and pathologies under that. And so, yes. We would treat those very differently. And I think people who show signs of what was very moderate, at least inflammation, are going to be treated differently than individuals who don't show that. Absolutely.

DR. WHITTEMORE: Gudrun, I see you have your hand up.

DR. LANGE: I agree with Lenny and with Jarred. There is great heterogeneity, and that is why I think, coming from a behavioral perspective, we have to hook it up with behavioral measures that mean something in terms

of how do I feel, what does it do for me. I think all these talks today were fantastic. But where's the behavioral indication of what it actually does? And to Jarred's point, if you hook it up with a, let's say a cognitive outcome measure, a self-report and a psychological measure, you can see the symptom load and the cognitive load. They may go in different directions, but maybe you can come up with an idea about what kind of phenotype there is for cognitive dysfunction. And also, what Peter was saying this morning about the interaction between OI and cognitive function. I'm aware of that study. I think they did a great job. And actually, I calculated the effect size, my favorite, and it's a good one. So -- but I think that whatever medication or treatments are given to people with other ME/CFS symptoms in addition to, let's say, in my case, cognitive dysfunction, that needs to be captured. Because it could be possible that -- and I'm not making this up -- mast cell medication is also beneficial for cognitive dysfunction. And I think we need to capture that. And so, far, that has not been done.

DR. WHITTEMORE: No. I absolutely agree with that, Gudrun. When we were at the Invest in ME Conference in the UK, a Norwegian group presented a new instrument that is designed specifically for individuals with ME/CFS to measure and look at impact on function of daily living, that looks to me like it's going to be an excellent tool. It doesn't really get at cognition, I don't think, but really how do your symptoms impact your function, and that then could be used in a clinical trial to look at improvement in those functions of daily living. But I think those are critically important points, absolutely. Peter, you have your hand up.

DR. ROWE: Yeah. Just a slightly different take on this, Vicky. One of the things that has run through the question-and-answer period is people are saying that they can't find doctors. They can't get cared for because they're so complicated. And I think one of the issues that we have to address with clinical trials is we don't have enough clinics around the country dealing with this problem in order to run a clinical trial, even if we had a really good intervention. And so, I think there's got to be

something like, and I've said this before, the kind of effort that's been put forward for HIV in the beginning to have these clinical trials networks to pay investigators who will be not only investigating but invested in the problem. And I think without that, we've got a very little chance of getting these trials off the ground. I think with a big clinical trial network, we could have multiple studies going on at the same time looking at, say, a high lactate group, a group that has a lot of mast cell activation, and really advance things through these trials much more rapidly. But without the people doing this, I think we're in trouble.

DR. WHITTEMORE: No. I think that's a very important point, and I see lots of people agreeing with you, Peter. NINDS has a clinical trial network called NeuroNEXT that has sites across the country that is a potential network that we could look at deploying clinical trials for ME/CFS through in collaboration with ME/CFS investigators and clinicians without having to set up a whole new clinical trial network. But that's clearly something that I think that we should look at.

DR. ROWE: And maybe we can link up with some of the Long COVID clinics that are being funded as well as another option.

DR. WHITTEMORE: Yes. That's another obvious potential option. Jarred? You're muted, Jarred.

DR. YOUNGER: I never make that mistake. I just want to say I totally agree with Peter. This is exactly where I think we need to go with the clinical trial network. Again, I use the COVID-19 response as kind of a model. I mean, just the way everyone just started running these kinds of impromptu clinical trials everywhere, probably a lot of them without IRB approval and stuff, just physicians and clinics, just running clinical trials, getting that information up to the next level and then testing those and coming up with best practices. And things happen so fast. I don't think we should do it exactly like that. But I really think we need to identify, prioritize, as I showed, there's so many different agents that could work. There're so many options to get a lot of pilots going simultaneously in parallel, have those tested in the network, and having, again, consistent outcomes. Again, so they can be directly compared

and then identify which things have a signal, again with pilot studies, maybe just 50 people or so, just to see if there's something interesting and then move that up to the next level. And I think if we really want to get something into the hands of patients, that's the fastest way. The serial stuff that we've been doing, where you spend 10 years testing one treatment, and then if it bombs, then you just wasted 10 years and you start another one. We just don't have time for that anymore. So, we really have to make things more parallel and get multiple things tested simultaneously.

DR. WHITTEMORE: No, absolutely. And this really requires, I think, a lot of careful thought because you don't -- I think we need a way to really carefully phenotype the patient so we're putting them into the right trials. Otherwise, we're going to continue to have failed trials simply because you're putting people potentially in the wrong subgroup into a trial. So, I think it's those things need to be put in place before I think we can do that, but it's where we need to start. Absolutely. Gudrun, you

had your hand up. And I see Benjamin, but Gudrun, go ahead.

DR. LANGE: Yeah. I couldn't agree more with Jarred. I think time has run out, with respect, to fiddle around for these long-protracted initiatives that take years and years, actually decades. This is my third decade in the field. Do I need to say more? So, patients are suffering. And so, maybe we can just start sort of like Jarred was suggesting, in an informal way. What do we already have? Are there any measures in all of these 540 studies that some people have identified in the systematic review that we can harmonize with and do it in a more informal fashion just to get started? Because if we don't get started, we don't get finished. So, that's my five cents.

DR. WHITTEMORE: No. I absolutely agree. And I think you're going to hear some really interesting things that could be potential biomarkers in some of the upcoming webinars. So, I think coming full circle, once we've heard from the metabolomics and the immune system groups, it's going to be really important to bring this discussion all back together to really say,

"Okay. Here's what we've heard. Here's really -- ." And that's what this research roadmap is intended to do, is to say, "Okay. Here's what we have, here's how we can move this forward in the best and most efficient way." So, Benjamin, you had your hand up.

DR. BENJAMIN NATELSON: Two things, and one is, one of the problems we're going to have to keep in mind with any therapeutic trials is that patients are on medicines, and most of the drug-related therapeutic trials in fibromyalgia require patients to come off medicines, and that in and of itself is a problem. But we're going to have to try to come up with treatments that can be done while the patient is on the best clinical management possible. That's number one. Number two, I was really unaware until Gudrun's talk that there is a neuropsych vehicle out there called this Groton Maze Test that she showed that one can do. And I don't know, seven to 10 minutes, that picks up the disability manifested by many CFS patients. And had I been aware of it, I've recently put in for a device trial where I'm going to be using heart rate variability as a dependent variable for improvement, but I

certainly would have included the Groton. And I think we need to think about including that in future studies.

DR. WHITTEMORE: Thank you. Trisha, I see you have your hand up.

MS. FISHER: On the subject of data collection, on the subject of using the information we might already have to see maybe how to phenotype and group people together.

DR. WHITTEMORE: Your audio is a little wonky. Can you say that again, Trisha? Sorry. Or maybe it was just on my end.

MS. FISHER: Sure. From the patient's perspective, I would suggest asking when collecting your sample population, who of those populations are using a smartwatch. Because if you go into their smartwatch, you can find out how many times or how many medications they've forgotten to take. Because I'm telling you they're wearing their watch to remind them. Because I do, and I forgot it today and I'm in trouble now. Things like that, very easy things that are measurable that do exist. I mean, we're talking about maybe some really cool technology that already exists. Patients don't have access

to it, but they do have smartwatches. They do have schedules. They do have Google calendars. Because if they don't, their families wouldn't know when to drive them to the doctor. Things like that.

DR. WHITTEMORE: Very good point. Thank you, Trisha. Gudrun, do you have your hand up again?

DR. LANGE: Yes.

DR. WHITTEMORE: Yeah. Go ahead.

DR. LANGE: There was a question in the chat about whether there are any qualified data collection tools in the FDA data bank. I checked that out when I was preparing for this talk, there is only one tool, and that's the PROMIS Fatigue Toolkit. So, we have a lot of really, really seasoned smart people who are working in questionnaire development and so on and so forth. Why can't some of these people be tasked to help develop tools that can be qualified data collection tools. So, that when we finally get to a randomized clinical trial, we can actually take these tools, put them in, and move forward without much delay. I'm thinking of Lenny or David Cella, people of that caliber.

DR. WHITTEMORE: That's a good point. But it's my understanding that instruments used in clinical trials don't all have to be FDA-approved if they're validated tools. Am I wrong about that?

DR. LANGE: No. That's true. But if it goes into an FDA -- into the FDA for indication approval, it's much quicker if you have a tool that's already approved by the FDA. But you make a good point. We should work on validating ME/CFS-specific tools.

DR. WHITTEMORE: We're validating tools that exist in an ME/CFS population.

DR. LANGE: Exactly.

DR. WHITTEMORE: So, when I was recently at the IACFS Conference and talked about the common data elements and that we were using the PROMIS, someone stood up and said that they found that that was not at all helpful, actually, for their clinical trial that they were conducting. And that actually the Chalder was much more effective. I'm not familiar with this tool, but I know Jarred -- I was talking with Jarred about this and Jarred indicated that it's a tool that he's been using. So, I think, again, thinking

about validating these tools and having data in the population before you then go into a clinical trial is really valuable.

DR. LANGE: Yeah. I agree.

DR. WHITTEMORE: I don't know. Jarred, if you want to comment on that.

DR. YOUNGER: Yeah. It's with those tools in particular, it just depends on what the use is. So, there's no one good tool for every situation. I think PROMIS fatigue is great for just a basic one-time assessment of a participant in the study, but not really good for ME/CFS longitudinal or clinical trial work. So, if you want to do a pre-post, that is probably not the tool I would choose. I would much rather pick something like the Chalder fatigue scale for a pre-post. So, yeah. I think there'll be a -- our core one-time assessment, and then there should be a suite of established tools specifically for clinical trials that you get baseline in at one or more points after the treatment. And that's kind of hard to put together. A lot of these cognitive scales, there's one form or maybe two forms, but you can't get the same cognitive test. A lot of them again and again and again, or

there's practice effects and people get used to them. So, it's very tough, but it's something important we need to do.

DR. LANGE: But it's doable.

DR. YOUNGER: [affirmative] Yeah.

DR. LANGE: And efforts are moving forward of especially validating remotely these tools, whether you use them on laptops, iPads, there are differences and similarities across populations. But I mean, it can be done. But it's an effort, and that's an effort that needs to be funded somehow. But it can be done, hopefully, without so much red tape that it's never going to get there. And we're using a million tools that we can't compare over the studies that we all do. And it's getting us nowhere.

DR. WHITTEMORE: I absolutely agree with that. Cindy had a comment in the chat. Cindy Bateman, do you want to turn your camera on and your audio?

DR. CINDY BATEMAN: Sure. I mean, this is a little bit of a follow-up to Peter's comment about low diagnosis rate, which makes it really hard to do clinical trials. And I think if we could have an effort to provide basic diagnostic

tools to clinicians broadly, then the number of patients would go up, the number of patients diagnosed. And that really isn't -- those things are really not available to the general clinical population. And if they were pushed out, just say simple ways to validate the Institute of Medicine criteria, for example. So, many clinicians don't do orthostatic testing, they don't do cognitive testing, and they don't know what to look for in sleep studies. And we do have -- we've made some progress on how to measure post-exertional malaise with good questionnaires from Lenny. But it wouldn't take very long, I think, from our group of experts to come up with a small panel and then do a few small studies to validate, and then really widely push that out to clinicians.

DR. WHITTEMORE: Thank you, Cindy. Yeah. Lots of people are agreeing with that comment. And I invite anyone else on the panel to turn your cameras on and join the conversation or ask questions. Jarred, do you have a comment?

DR. YOUNGER: Yeah. Related to an earlier -- I was just interested with our panel. The question came up of is there something that divides up your population? Is there something

that creates subgroups? And I know some people on here have some interesting things that -- including Cindy, I'm sure. I don't know what it is, but I'm sure she has some things that divide up the sample and create meaningful subgroups. Just as an example, if I run 100 people with ME/CFS, there's a subgroup that has just weird elevated C-reactive protein. But it's not high enough to cause any physicians to be alarmed, but it's around that kind of 10 level. No one knows what to do with it, but something's going on in a little higher erythrocyte segmentation rate. And so, about a third of them look like they have some kind of systemic kind of inflammatory thing. It's just not diagnosable using contemporary methods. So, I know there's subgroups. I was just wondering if anyone else has subgroups that they've identified. And a PEM is an excellent one. I mean, that's definite. But what else people have seen that create meaningful subgroups?

DR. NATELSON: For CFS, we've used onset, sudden versus gradual. And we've also done some work with the presence or absence of psychiatric comorbidity. That's been helpful. I think the

sudden versus gradual was very useful. And now, of course, with Long COVID, we have all sudden and we have short illness duration. And that's why I agree with Peter Novak that we're going to learn from Long COVID about ME/CFS.

DR. WHITTEMORE: Yeah. If I can add, data coming out of the ME/CFS collaborative centers, Maureen Hanson's group as well as Durya Unnit-Mats' [spelled phonetically] group, really shows the difference between male and female, which we've known for a while. But also, from Durya's data, differences that have been seen in the shorter duration illness of individuals with ME/CFS versus those with longer illness. And so, I think those are critical as well as age of the study participants. Those are all things that need to be taken into account that I think in a lot of earlier studies weren't at all accounted for and everyone was just thrown into one group. So, those are all variables that I think we need to really be conscious of and think about as these studies are designed and the data reported out. So, Lenny, you had your hand up and then Janet.

DR. JASON: I would agree that duration, onset, even severity really help us kind of differentiate patients, as well as some of the biological biomarkers that were discussed during the session today. These are all great. Just to go back to an earlier question about kind of PEM versus fatigue. It's really interesting that if you look at a lot of the Long COVID questionnaires that have been developed, psychometric properties of them are relatively rare. So, actually trying to develop some kind of guidelines for which types of questionnaires to use that have some validation is something that these field needs as well as the Long COVID ones. Take fatigue, for example. A lot of the questionnaires really deal with just onset. And things like frequency and severity are just often not looked at, even in the big recover study that's going on, although they're making some changes there. But let's just take fatigue, for example. Just as an example, a person could have high levels of fatigue that they said over the last six months they've had fatigue. But that doesn't differentiate major depressive disorder from a person with ME/CFS. So, if you look at the

severity of the fatigue, that's when you get the differentiation, as well as PEM. If you look at the severity of PEM, you get the differentiation. But just occurrence measures are not going to differentiate these critical subgroups. So, just something to think about.

DR. WHITTEMORE: Thank you, Lenny. Janet?

DR. MULLINGTON: Yeah. I was going to say some of the things Lenny just said. But I would also like to just sort of underscore the opportunity that we have with Long COVID to really understand and to be able to get the time synchronization of that important beginning and have some ability to look at comorbidities from that perspective as well. Because as we found with comparing ME/CFS and Long COVID, the ME/CFS patients are much more complicated in a sense because they have been -- and they take -- they're on more medications. They've had a longer course and have been through more to get a diagnosis. I think that it's important that we take the opportunity to understand the evolution of this kind of post- sequelae in this syndrome. So, I'm hoping that we can really benefit from a

lot of the initiatives that are going on for Long COVID.

DR. WHITTEMORE: One other plug I'll make for a future webinar in this series is the Genetic Susceptibility Genomics webinar. Oved Amitay, from Solve ME/CFS Initiative, and I have had several very interesting conversations with people from Precision Life and other groups doing genetic studies that the data actually doesn't exist now yet but is being analyzed and will be presented at that November 1st webinar. And because I do believe that there is not one underlying cause of ME/CFS, but there may be different causes or different underlying pathologies that all lead to the symptomatology we see in ME/CFS. And so, as many of you know, I'm sure, there's a very large genetic study that's being supported in the UK, where they're recruiting 20,000 individuals to do genetic GWAS genomic studies. And they're going to be -- what they've shared with us is that they'll be prepared to present some of the preliminary data from the first 4,500 patients at the webinar in November. So, I think taken from that perspective

as well, to really look at the underlying potential genetic variability that we're seeing will be critically important as well. Other comments from anyone? Anyone on the panel or any of the other speakers?

DR. LISA ENGEL: I actually just wanted to add something, Vicky, if I could.

DR. WHITTEMORE: Sure. Yeah. Sure.

DR. ENGEL: Facilitating mobile clinical trials would be, I think, a huge advantage if we could somehow get that rolling and facilitate more of that for the more severe population, especially.

DR. YOUNGER: I second that. When possible, I really like -- so I've started to do decentralized clinical trials wherever possible, and I know some others have as well. We basically use -- maybe I'm not supposed to mention companies. There are companies that go to your house. They will do the blood draws. You don't have to go anywhere. You can ship treatments to their house. They can do all the self-report stuff, symptom reports on their phone or computer or something. And so, in a lot of cases, there are ways. And we're actually running trials right

now. So, I think people should consider whether it's possible. And that just gives access to people who are not even close to a university, never in their life have they ever been able to participate in a trial, or they don't have the mobility to do so. And so, I've been really pleased. We just started. I've been really pleased so far in that process. So, I want to do more of it.

DR. WHITTEMORE: Yeah. And I actually think that's a somewhat fortunate consequence of the pandemic is that I know that for some of the NIH-funded clinical trials, they really had to shift in order to be able to do distance involvement and sending people out to the homes or doing online assessments, when possible, rather than having people come into a clinical site. So, I think that's really been an interesting shift in the way we think about and our ability to do clinical trials in that way. Gudrun?

DR. LANGE: I agree with that. And I mean, in clinical practice, I almost exclusively do that. So, there is now an increasing amount of data about equivalency between measures and like a host of tools that you can use. To Lenny's point,

that PEM and effort may split the groups, from an anecdotal perspective, I use a tool, and I'm not going to say the name now either, clinically, that has several measures and is a psychometrically validated tool. But one of the measures is stamina. And so, the task is a complex information processing task, and again, that measures stamina while they're doing it. There is a subgroup of people that come in with relatively low gas in the tank, so to speak, but they run out of gas almost immediately. Those people are generally in bed about nine days after the cognitive testing, because I keep track of that. When that doesn't happen and the stamina doesn't go down so quickly, there is much more resilience and people, they're back on their back three, four days. So, yeah. There's a group that is really, really efficiency depleted. And thank you, Jarred, for the one line on your slide that you don't see neurodegenerative disorder in ME/CFS, I second that.

DR. YOUNGER: Yeah. Good point. It's a common misconception that people with ME/CFS their brain is just being destroyed left and right. And really, I have seen not a scrap of evidence to

suggest that that's occurring, as opposed to many other conditions. So, yeah.

DR. LANGE: Likewise.

DR. WHITTEMORE: Yes, Lenny.

DR. JASON: I just wanted to kind of mention, as Jarred has said, that we have been, for the last couple of years, been using a network of phlebotomists. And there's several different companies that provide this service, reasonably priced. So, it is available to investigators now. And we've been pretty satisfied with being able to get good blood samples as long as they're not sent in on Friday because then you have the weekend and that's not a good thing. So, the other thing I wanted to mention is that there's an issue of how we're recording our data in terms of some of the lifestyle issues that people are involved in. So, for example, PEM, its relationship to pacing is very, very important, as is many other symptoms. So, I think we need to understand that a number of patients are doing things that make them actually able to not be as symptomatic because they're very careful. They're pacing, they're watching themselves. And that experience, and even the biology, might be

somewhat different when they're not pacing. Certainly, the symptoms are incredibly different for those folks who are pacing versus those folks who aren't when we look at PEM. So, I just think we need to sort of be thinking about the ecology of the different things that people are doing when we look at the measures.

DR. WHITTEMORE: Thank you. Very important points. Chloe?

MS. JONES: Hi. I kind of wanted to pose a question to the panelists regarding their interest in focusing on more multi-treatment protocols for clinical trials. Specifically, yeah, I'm thinking about how this is only the nervous system webinar. And even with the nervous system webinar, we have all these different delineations. And then we know that's really heterogeneous population group there's all these subgroups even with these single phenotypes, you want single people like Trisha. It's always a multi-treatment protocol that people are adopting to manage their symptoms. I'm worried that maybe this prioritizing parsimony is maybe delaying some real progress in trying to identify singular treatment for this singular symptom or this

singular treatment for a singular subgroup that is maybe the best by the margin of few percentage points. I'm curious if researchers would be interested and instead of trying this kind of multi-treatment protocols, trying to help the largest pool of people that they can thinking like this should hopefully address or abate symptoms for a lot of people. And then we can kind of delineate from there which treatments are best for which subgroups. I realize it comes with its own challenges and a lot of collaborations involve. But I'm curious about kind of taking that perspective on as sort of siloing -- starting with improvement generally then siloing from there rather than trying to build up these very separated research efforts. Curious if anyone had any thoughts about that kind of approach.

DR WHITTEMORE: The question, Peter, do you want to take that on?

DR. ROWE: Yeah. I like that idea because when I look at our patients and we've tried to address as many of the comorbid conditions as we can, they're often on 10 or 12 medications. And I've thought for a while, especially when the CBT

theory was more prominent, I would have loved to have had a randomized trial where people feed me patients that I can treat my way and somebody else is doing it with CBT and compare the outcomes. And you could certainly adapt that to suggest how about the people who treat orthostatic intolerance versus, say, the way that the Stanford group has focused on antiviral therapies? Who comes out ahead? How about mast cells? How about people who look at the biomechanical strains? There are a lot of potential ways of taking what Chloe was mentioning and making very practical studies.

DR. WHITTEMORE: Anyone else want to comment?

MS. FISHER: I just want to say that I appreciate that question very, very much because it is really the spirit of what I was getting at. Cast the wide net as wide as you can so that what remains is acute symptom management on an as-needed basis.

MS. JONES: Thanks for your thoughts.

DR. WHITTEMORE: You had your hand up before, Trisha. Did you have a different point you wanted to make?

MS. FISHER: Ironically, I don't remember.

DR. WHITTEMORE: You can just put your hand up when you do.

MS. FISHER: Thank you.

DR. WHITTEMORE: All right. We're coming to the end of our session here. We just have a few minutes left. Any last comments from anyone?

DR. JASON: Vicky, can I just make just a quick comment about the last --

DR WHITTEMORE: Sure, sure.

DR. JASON: -- comment said that Peter suggested, as well as a couple of others? If you look at tobacco research, for a person to quit smoking, which is number one public health problem in this country in terms of mortality, usually takes seven or eight efforts for a person to be successful. One's trying multiple different things to basically get off this addiction. I think with basically the same idea could be that a failure for a particular biomedical medication is really insight if something's not working for that person. But they might need three or four different things. If we can track those people over time as to what's working, what's not working, then we're going to basically catch up

with some of the other fields that have been around for 50 or 60 years.

DR. WHITTEMORE: Absolutely.

MS. FISHER: I remember now. Sorry.

DR. WHITTEMORE: Yeah, go ahead.

MS. FISHER: It had to do with basically echoing and reinforcing the sentiment of the statement that was made about pacing. I believe that, through my own story, through folks that only know me superficially, that don't know what I do every day, they would have no knowledge or any understating of what I go through every day. And that's 100% attributable to pacing and to the support provided by the people around me who pick up the slack when pacing doesn't do it.

DR. WHITTEMORE: Okay. Very good. So, if you could take the slide down so I could share my screen, please. Thank you. So, I'm going to just very quickly wrap up here and give my great thanks to all of the speakers for your incredibly thoughtful and thorough presentations, to Jarred and the Working --webinar planning group for putting this webinar together. I just wanted to tell everyone that the best way for you to get any announcements about upcoming webinars, about

when we post the recordings, all of that is to go to this link on the NIH website. So, NIH.gov/mecfs and sign up for the email list serv there. That way, you'll be sure to get all the announcements that come out about this webinar series going forward. So, the next webinar will be focused on the immune system and will take place -- we haven't set the date yet, but we're looking at dates in late September or early October. But we'll get that date out to you as soon as possible. So, with that, I'll call this webinar to a close. And again, my great thanks to all the speakers and the panelists, and my special thanks to all of you who have joined to listen in on the webinar, your incredibly thoughtful questions and comments. And we really appreciate everyone taking the time today to be with us for this webinar on the nervous system and hope to see you for the next webinar on the immune system. So, thank you very much.

(Meeting adjourned)